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Antimicrobial Resistance in the Americas: Magnitude and Containment of the Problem

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Pan American Health Organization

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PROLOGUE

At the beginning of the 20th century, infectious and parasitic diseases were the leading cause of morbidity and mortality for the planet's population, especially children. Acute infectious diseases, such as tuberculosis, diarrhea, meningitis, and acute respiratory infections produced morbidity and mortality rates that would be unthinkable today. Furthermore, chronic diseases of infectious origin, such as syphilis and rheumatic fever, also generated high levels of morbidity and mortality.

After World War I, the populations of what are now developed countries experienced a rapid improvement in their standard of living. Coupled with an increase in life expectancy, this was a sign that there were ways of preventing, controlling, or at least reducing the impact of communicable diseases on human health. Better socioeconomic conditions and the rapid implementation of public health measures further improved the health of the population, especially after World War II. The discovery of antibiotics, the miracle cure that would make it possible to save people once condemned to death almost without exception, led even pessimists to assume that the era of infectious diseases was coming to an end. Unfortunately, these assumptions proved wrong. Resistance to one or more antibiotics is becoming increasingly common. This is as true for species which 30 years ago were synonymous with susceptibility—for example, *Streptococcus pneumoniae*—and which in vitro are currently resistant to the antibiotics to which they once succumbed, as for other species, such as *Enterococcus faecium* or *Staphylococcus* spp., which, when resistant to vancomycin, leave very few therapeutical options for their effective control. The appearance of resistant enterococci in several countries of the Americas and of staphylococci with similar characteristics in Guatemala, Japan, the United Kingdom, and the United States of America is a warning to health authorities worldwide.

Although infectious diseases are caused by specific etiologic agents, their origin, evolution, and outcome depend on an increasingly complex biological, social, and economic situation. In 1993, life expectancy at birth in the world's least developed countries was 43 years, while in the developed countries it was 73. Even by the year 2000, life expectancy at birth in 45 of the poorest developing countries was not expected to reach 60 years. In 1993, an estimated 51 million people died worldwide; communicable diseases were responsible for 20 million of these deaths and 16 million, or 80% of them, occurred in developing countries. Infectious diseases, while a worldwide problem, are more frequent where poverty and related factors prevail; malnutrition, lack of running water and latrines, illiteracy, and overcrowding. While higher morbidity and mortality are found in the most disadvantaged groups, no one is exempt, especially now that travel and trade foster close contact between hosts and unknown etiologic agents. The hosts, in turn, can serve as a vehicle for these exotic etiologic agents to come into contact with unexposed populations.

A World Bank study conducted in 1990 and published in 1993 attempted to measure the burden of disease from a series of health problems that, individually or as a group,

weighed on the world. This measure took into account the losses stemming from premature death (defined as the difference between the age at which death occurred and the age at which death would have occurred in a population of low mortality) and the impact of the losses caused by poor health. The impact of the loss of healthy life was quantified for the health problems on the list of 109 cause groups in the International Classification of Diseases, Ninth Revision, and for approximately 95% of the potential causes of disability. The burden of disease was quantified in units of disability-adjusted life years (DALY). The relative burden for specific diseases or groups of diseases was calculated by comparing the DALY for different diseases or groups of diseases. Thus, acute respiratory diseases, diarrheal diseases, vaccine-preventable diseases, malaria, tuberculosis, and sexually-transmitted infections (including HIV) are the leading causes of mortality in developing countries. The first two, acute respiratory diseases and diarrheal diseases, are the leading cause of mortality in children under 5 years of age worldwide. Together, all of the aforementioned diseases produce a burden of 518 million DALY. Of that total, 52% corresponds to acute respiratory diseases and diarrheal diseases, which to a large extent are of bacterial etiology. In 1993, acute respiratory diseases were responsible for the death of 4.3 million individuals throughout the world, among them 2.7 million children under 5 years of age. Diarrheal diseases caused the death of 2.9 million individuals, 2.5 million of whom were children under 4 years.

The assumption in the 1940s and 1950s that the next millennium would bring a world virtually free of infectious disease remained an unfulfilled prophecy. This was partly because economic progress did not spread fast enough or to a sufficient depth, and because certain diseases emerged whose etiologic agents were not recognized as such or were unknown (for example, AIDS and Lyme disease). In addition, the drugs used in the treatment of some infectious diseases became ineffective. Thus, since the 1960s many pathogens have become resistant to several classes of antimicrobial drugs; this became a factor of clinical, epidemiological, and socioeconomic importance, since infections due to resistant microorganisms can be especially difficult and expensive to treat.

Antimicrobial resistance stems from the selection of species with inherent resistance during exposure to antibacterial drugs, or from the appearance of resistant variants among sensitive strains. The resistance of previously susceptible species can come about through mutation and can be transmitted vertically within a single species, or it may be the result of the horizontal acquisition of genetic material from other bacteria. The latter can occur as a result of the action of plasmids, transposons, or the direct capture of DNA, as in the case of certain bacteria (pneumococci).

The excessive and inappropriate use of antibiotics is probably the most significant factor in the development of resistance to these drugs. Intensive use in the community is usually attributable to the fact that in some countries antibiotics are sold without prescription (even against the law) or else, over the counter. Hence, the importance of the recent decision by Chilean health authorities reaffirming the requirement that antibiotics be sold under prescription only. Although fewer prescriptions are written in hospitals, their use of antibiotics is greater. Furthermore, it is in hospitals where almost 30% of the antibiotics are used to prevent surgical infections. Nosocomial infections with mono- or multiresistant microorganisms bode ill for patients who are immunocompromised, debilitated, or elderly. However, even outside these situations, hospital infections are not only difficult to treat, they delay the discharge of the patient and significantly increase the cost of care. Further, if the patient becomes a carrier, the transmission of resistant organisms to the community is facilitated.

If the use of antibiotics to control existing or potential infections in humans is excessive, their use in veterinary medicine for mass treatment, prophylaxis, and growth enhancement constitutes an even greater excess.

The Pan American Health Organization/World Health Organization (PAHO/WHO) intensified its activities in the field of antibiotic resistance in the 1980s, when, in collaboration with Dr. Thomas O'Brian of Brigham and Women's Hospital of Boston, Massachusetts, United States of America, it conducted a survey on activities taking place in the countries concerning the monitoring of resistance to antibiotics. As a result, improvements in infrastructure of the laboratories working in this area and in the training of their staff were achieved. In 1995, due to the regional alert on the importance of emerging and reemerging diseases, including those originating in resistance to antibiotics, and as a result of new mandates issued by the Governing Bodies, the PAHO intensified its activities in this area. Prior to that, it had provided support for the development of a surveillance system that made it possible to study the distribution of the prevalent serotypes and determine the susceptibility to antibiotics of 4,018 isolates of *Streptococcus pneumoniae*, a pathogen responsible for invasive disease, pneumonia, and meningitis in children under 5 years of age that causes significant morbidity and mortality. This network, in which 70 hospitals in 30 cities and 6 countries (Argentina, Brazil, Colombia, Chile, Mexico and Uruguay) originally participated and which has financial support from the Canadian International Development Agency, is being expanded to other countries and now also includes the results for isolates of *Haemophilus influenzae* and *Neisseria meningitidis*.

Work subsequently began on the development of a network to monitor the antibiotic susceptibility of the genera *Salmonella*, *Shigella*, and *Vibrio cholerae*, major etiologic agents of diarrheal diseases that sometimes require treatment with antibiotics. Review of the scientific literature dating back to the 1950s shows that in most countries the results of tests to determine the susceptibility of *Salmonella* and *Shigella* strains to antibiotics included only a limited number of specimens and that there was usually no relationship between the microbiological findings and the temporal, spatial, and population context. Hence, strengthening this network fills a significant void. The consequences of this deficiency go beyond the individual medical problems of children and adults, since the emergence of epidemics of the diseases of the aforementioned etiology give the problem a clear public health dimension. Furthermore, potential transmission through food contamination, sometimes at the very source, by infected livestock, also turns an individual medical problem into an epidemiological one with economic and social consequences. To illustrate, it is worth citing the appearance of *Salmonella typhimurium* DT 104 resistant to five antibiotics, in Europe, especially the United Kingdom, and subsequently the United States. The cholera epidemic is another example. This disease resurfaced in the Americas in 1991 after an absence of 90 years, and in Peru alone resulted in losses of US$ 700 million.

The surveillance network for etiologic agents of enteric disease was implemented in 1996 with the participation of the reference laboratories of eight countries of the Region: Argentina, Brazil, Chile, Colombia, Costa Rica, Mexico, Peru, and Venezuela. The participating countries reasonably concluded that, in order to have confidence in the results obtained, it would be necessary to improve quality control of each laboratory's internal practices and implement a system that would permit periodic performance evaluation. The Laboratory Centre for Disease Control of Canada agreed to act as the organizing laboratory for the system. Subsequently, the laboratories of five Caribbean countries (Ba-

hamas, Barbados, Jamaica, Saint Lucia, and Trinidad and Tobago) joined the network. The information for all these countries, up to 1998, is available on the PAHO Web page. Another six Latin American countries joined the network in 1998: Bolivia, Cuba, Ecuador, El Salvador, Nicaragua, and Paraguay. In five of these countries, with support from the U.S. Agency for International Development, surveillance activities were expanded to cover other bacterial agents found in the community and in sentinel hospitals. A new organizing laboratory was also added: Argentina's National Administration for Laboratories and Health Institutes is in charge of evaluating the performance of these five countries for bacteria other than *Salmonella, Shigella,* and *Vibrio cholerae.*

There are operational differences between the surveillance network for *Streptococcus pneumoniae* and *Haemophilus influenzae* and the one for enteric bacteria. In the former, the national reference center receives the isolates already identified by each national laboratory and processes them for serotyping and determining their sensitivity to antibiotics. In the network, the national reference center is the head of the national network that produces information on both the identification of the species isolated and its sensitivity to antibiotics. The national reference center also supervises quality control practices in each network laboratory and is responsible for conducting performance evaluations.

Promotion of surveillance activities and their findings must be the basis for country actions to prevent the appearance of resistance to antibiotics. Thus, it will be necessary to have information on the policies and practices in place in the different countries; to disseminate this analyzed information so as to show the risk posed by the appearance of resistance, and its economic consequences; to seek partners among the different sectors, in order to disseminate information on successful prevention practices; and, finally, to take steps to facilitate the rational use of antibiotics. All these aspects of resistance to antibiotics, from its prevalence and medical significance to policy implications and the rational use of these drugs, were discussed at the conference held in Caraballeda, Venezuela, in November 1998. Some of the papers presented at that event were published in a special issue of the *Pan American Journal of Infectious Diseases* (Supplement 1, volume 3, May 1999), a publication of the Pan American Association of Infectious Diseases, which collaborated with PAHO/WHO in the organization of the conference. A selection of those articles is included in this publication. Also included are other articles that were specially requested in order to cover subject-matter areas which were not dealt with sufficiently during the conference, as well as to obtain information from several countries that were not represented there.

We hope that the findings described in the chapters that follow will help to show the magnitude of the problem, promote surveillance activities, and point the way for the application of preventive measures that will facilitate the rational use of antibiotics on humans and animals.

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SECTION ONE

SURVEILLANCE OF BACTERIAL RESISTANCE TO ANTIMICROBIAL AGENTS

CHOOSING A TESTING METHOD FOR SURVEILLANCE OF ANTIMICROBIAL-RESISTANT BACTERIA

Fred C. Tenover[1] and M. Jasmine Mohammed[1]

Bacteria that are resistant to antimicrobial agents are continuing to develop and spread worldwide. To track the spread of resistant organisms, it is imperative to have accurate, reliable, reproducible, and cost-effective methods of antimicrobial susceptibility testing available in clinical microbiology laboratories. No single method can detect all mechanisms of resistance. Thus, multiple methods, including a number of novel screening tests, must be used, particularly to detect bacterial strains with new antimicrobial resistance mechanisms. Laboratory-quality control programs are crucial to producing accurate results, and external proficiency testing programs are needed to ensure that the susceptibility testing methods currently used in clinical laboratories will detect organisms with critical antimicrobial resistance mechanisms.

INTRODUCTION

Antimicrobial resistance in bacteria has become a global problem *(1, 2)*. While clinical microbiology laboratories have always tested isolates of bacteria for susceptibility to antimicrobial agents to help guide antimicrobial chemotherapy *(3)*, laboratories now have a broader mission that includes monitoring the susceptibility patterns of microorganisms for the emergence of new resistance patterns. Antimicrobial resistance clearly impacts the therapy of the individual patient, and also affects the community at large, i.e., empiric therapy for certain infectious illnesses must be altered when resistance levels compromise the effectiveness of commonly used agents *(4, 5)*. Decisions to change empiric therapy, however, should only be made after a review of current antimicrobial susceptibility data from the pathogens in question. This means that laboratories must optimize their methods to ensure that the susceptibility testing data they generate are accurate.

CHOOSING A TESTING METHOD

The antimicrobial susceptibility testing method selected for routine use must be reliable, reproducible, accurate, and cost effective. It is possible to generate highly reproducible data that are inaccurate if, for example, the wrong growth medium is used for testing.

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Failing to include 2% NaCl in agar-based methods when performing staphylococcal susceptibility testing will likely yield reproducible but inaccurate results *(6)*. Likewise, some methods may be highly accurate and reproducible, but too costly to perform routinely in the laboratory. Thus, the choice of a susceptibility testing method must consider a variety of factors.

Using standardized methods of testing and interpretation, such as those developed by the National Committee for Clinical Laboratory Standards in the United States of America *(7, 8)*, makes surveillance easier on a regional or national level, since laboratories using a common method of testing can interchange their data more readily *(9)*. It also encourages the development of regional surveillance networks where smaller laboratories may seek assistance from larger reference laboratories, especially when testing fastidious organisms or unusual antimicrobial agents *(9)*. Use of a common software package to store and analyze data, such as the WHONET program *(10)*, also enhances regional surveillance.

Other factors that influence the choice of a susceptibility testing method include shelf-life of reagents, ease of use, ease of interpretation, and the types of organisms to be tested. Not all methods are applicable to all organism groups. Fastidious organisms, such as *Streptococcus pneumoniae, Haemophilus influenzae,* and *Neisseria gonorrhoeae,* must be tested using a different method than that used for staphylococci and enteric bacilli.

ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS

The methods commonly used in microbiology laboratories around the world include disk diffusion, broth microdilution, agar dilution, agar gradient dilution (E-test), and several automated and semi-automated methods *(3, 11)*. Dilution methods, including those that are automated, usually report results in minimal inhibitory concentrations, or MICs, in addition to the qualitative interpretative categories of susceptible, intermediate, and resistant. Disk diffusion tests yield only qualitative results, although some of the instruments that record the diameters of the zones of inhibition from disk diffusion plates can extrapolate MIC values. Genetic methods, such as DNA probes and polymerase chain reaction, can be used to detect DNA sequences associated with antimicrobial resistance genes; however, such methods are usually limited to reference laboratories *(12)*. Genetic methods can be very helpful for arbitrating borderline MIC results or detecting resistance genes in organisms directly in clinical specimens, but such assays are expensive to perform and difficult to control due to contamination of reagents with previously amplified material.

Some newer mechanisms of antimicrobial resistance, such as extended-spectrum beta-lactamases in *Klebsiella pneumoniae* and *Escherichia coli,* or high-level aminoglycoside resistance in enterococci, are not reliably detected by the usual disk diffusion or MIC methods *(13–15)*. Thus, it has been necessary to develop novel screening methods for specific organism-antimicrobial agent combinations to increase the sensitivity of detecting these resistance phenotypes. Several of these screening tests are presented in Table 1.

Many commercial MIC methods test a very narrow range of antimicrobial agent dilutions, often only the two or three dilutions that bracket the MIC breakpoints for that drug. These "breakpoint panels" *(11)* increase the number of antimicrobial agents that a laboratory can test and report, but it is often difficult to know whether the panels are providing accurate results, since the quality control organisms tested do not produce results that fall within those 2 to 3 concentrations, thus giving off-scale values. This can pose problems when unusual results (such as high MIC values) are obtained with clinical isolates, since it is not clear if the test is performing appropriately. This often necessitates testing the organism by a second method, which delays the report to the clinician.

DRAWBACKS TO THE DISK DIFFUSION METHOD

Disk diffusion has been the primary antimicrobial susceptibility testing method for many clinical laboratories around the world for years *(16)*. Unfortunately, as resistance has become more widespread, several critical drawbacks to this test have been discovered. First, disk diffusion cannot be used to test pneumococci against beta-lactam drugs, such as penicillin and cefotaxime *(7, 17, 18)*. Second, disk diffusion does not detect glycopeptide resistance in staphylococci *(19)*. Finally, the results of disk diffusion testing correlate poorly with the results of MIC tests with *Stenotrophomonas maltophilia* and *Burkholderia cepacia (20)*. The alternate method often used for these organism-drug combinations is agar gradient dilution, but given its cost, testing is usually limited to only a few antimicrobial agents. Nonetheless, the method has been shown in numerous studies to generate results comparable to those produced by the broth microdilution reference method *(21, 22)*.

To enhance the utility of disk diffusion testing, which remains the most cost-effective method of susceptibility testing, several screening tests based on disk diffusion principles have been developed *(7, 8)*. Many of the tests in Table 1, such as the 1 μg oxacillin disk screening test for β-lactam resistance in pneumococci, are used worldwide as a cost-effective means of limiting the number of penicillin MIC tests that the laboratory is required to perform. Similarly, the brain heart infusion vancomycin agar screen test, which can be used for detecting glycopeptide resistance in both enterococci and staphylococci, is cost effective and simple to perform *(19)*.

QUALITY CONTROL AND PROFICIENCY TESTING

Quality control testing ensures that the reagents, media, and technologist conducting the test are performing in a predictable and reliable fashion. For susceptibility testing, several bacterial strains with highly stable and well-described antimicrobial susceptibility patterns are tested. The results of testing must fall within defined limits before the results for clinical isolates (or unknowns) can be released. Quality control is often ignored by laboratorians who consider it either too time-consuming, too expensive to perform, or both. Yet, quality control testing is at the heart of good surveillance data, and should be mandatory before such data are used to guide changes in empiric antimicrobial chemotherapy.

Proficiency testing is an ongoing system of testing strains from an external source to determine the ability of a laboratory to identify and determine the antimicrobial resistance profile of challenge strains of bacteria. Profi-

TABLE 1. Screening tests for key antimicrobial resistance profiles

Organism	Antimicrobial agent	Method
Staphylococcus aureus	Oxacillin	Mueller-Hinton (MH) agar*
	Vancomycin	Brain Heart Infusion (BHI) agar†
Coagulase-negative staphylococci	Vancomycin	BHI agar†
Enterococci	Vancomycin	BHI agar†
	Gentamicin	BHI agar containing 500 μg/ml
		BHI broth containing 500 μg/ml
		120g disk on MH agar
	Streptomycin	BHI agar containing 2000 μg/ml
		BHI broth containing 1000 μg/ml
		300g disk on MH agar
Streptococcus pneumoniae	Penicillin	Oxacillin disk (1 μg)

*The oxacillin agar screen test *(7)* is performed by inoculating a standardized amount of staphylococci onto a plate of Mueller-Hinton agar containing 6 μg/ml of oxacillin and 4% NaCl and incubating the plate for 24 h at 35 °C.

†The vancomycin agar screen test *(7, 19)* is performed by inoculating a standardized amount of enterococci onto a plate of brain heart infusion agar containing 6 μg/ml of vancomycin and incubating the plate for 24 h at 35 °C.

ciency testing programs can help laboratories determine whether their current testing methods are sufficiently sensitive to detect emerging antimicrobial resistance mechanisms. Strains should be tested using routine methods and the activity should be viewed as an educational experience. The results of the WHONET QC/PT (quality control/proficiency testing) system have been encouraging. Most laboratories have been able to detect extended-spectrum β-lactamases in *K. pneumoniae*, methicillin resistance in *Staphylococcus aureus*, and vancomycin resistance in *Enterococcus faecium*. However, laboratories continue to have problems with detection of penicillin resistance in pneumococci. Almost half of the laboratories participating in the PT challenge failed to detect a zone diameter of <19 mm in a strain of pneumococci with low-level penicillin resistance. Thus, the need for both quality control and proficiency testing systems must be emphasized, coupled with educational programs to improve our surveillance for antimicrobial resistance.

In summary, surveillance for antimicrobial resistance usually requires that laboratories employ more than one method of susceptibility testing. These may include disk diffusion testing, broth microdilution testing, agar gradient dilution tests, and a variety of screening tests. Frequent examination of surveillance data is important to detect emerging resistance. Software programs, such as WHONET, can facilitate this task. Finally, quality control and proficiency testing programs help ensure the quality of the data generated by the laboratory.

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CONSIDERATIONS REGARDING THE NEED FOR BACTERIAL RESISTANCE NETWORKS

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In recent years the problem of antimicrobial resistance has been examined with growing interest in many countries (*1–3*). The world press has not been the only source of information on outbreaks of resistant germs such as enterococcus in the United States and vancomycin-tolerant staphylococcus in Hong Kong (*4*). In the scientific arena, international meetings sponsored by the World Health Organization (*5, 6*) have been held in collaboration with health agencies in a number of countries, and the topic is on the agenda of most congresses and conferences on clinical microbiology. Some countries have officially recognized that increased bacterial resistance to antibiotics is a threat to public health (*7*). As a result, associations such as the Alliance for the Prudent Use of Antibiotics (APUA) have been formed, and programs like WHONET (the World Health Organization network for monitoring bacterial resistance) have been established to combat the problem.

At the 1998 European Conference on Bacterial Resistance, Norman Simmons declared that physicians should own up and apologize for the fact that they had blundered; they had been given the marvelous gift of antimicrobial drugs, but were destroying it through inappropriate use (*8*).

The same issues have been recognized the world over in most of the meetings that have been held on this subject—namely, indiscriminate use of antibiotics for the treatment of disease in humans and increased use of these products in agriculture and the livestock industry (*9*). It has been suggested that the use of antimicrobial agents might be questionable in as much as 50% of human cases and 80% of the applications in agriculture (*10*). As studies have shown, the quality of microbiology laboratories is inadequate in many countries, and this has led to the inappropriate prescription of antimicrobial drugs (*11*). It has been proposed that basic education centers modify their infectious disease and microbiology curricula to give emphasis to this growing problem (*12*).

The pharmaceutical industry may take advantage of physicians' lack of up-to-date information about infectious diseases to try to sell new drugs by exaggerating their value (*13,*

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14). At the same time, the inadequacy of public health regulations in many countries and the unrestricted sale of antimicrobial drugs promote the use of these products *(15)*.

It has been pointed out that lack of knowledge about the epidemiology of local and regional bacterial resistance makes it impossible to issue guidelines for empirical treatment based on particular local situations. As a result, information from other places must be relied on without any certainty that it will be beneficial and without knowledge about the abuse of extended-spectrum antibiotics. In some cases, decisions are based on questionable sources, such as the publications referring to the resistance of *Pseudomonas aeruginosa* to vancomycin *(16)*.

International programs have been developed—for example, the Alexander, Sentry, and Resist Net projects, to mention the most serious ones—that help to address this situation because they concentrate on information that has not been produced for commercial purposes. (The Sentry and Resist Net projects are described elsewhere in this publication.)

One way to address this problem is to create networks to monitor bacterial resistance, or to adapt existing networks to ensure that they have all the necessary elements, including the degree of quality required to assure clinicians that they can have confidence in the data *(17, 18)*. There is no doubt that creating networks to monitor bacterial resistance in countries with only limited resources—that is, most of the Latin American countries—can yield benefits as long as certain basic points are kept in mind when they are created *(19)*.

First, quality control must be strict at the centers for monitoring bacterial resistance; otherwise their data cannot be used to generate an adequate regional consensus, nor can the data serve as a basis for recommendations on the empirical treatment of specific diseases or for the implementation of control measures *(20)*.

There should be a reference laboratory where problem clinical isolates can be tested, the quality control program (internal at first and later external) can be coordinated, shifting trends in bacterial resistance can be studied, changes in susceptibility can be verified, and recommendations for dealing with quality control problems can be formulated *(21)*.

The information, beginning with local data, should be analyzed by experts, with care taken to differentiate between pathogens in the community and those originating in hospitals. Later, data from other centers in the region can be included. It is essential to analyze trends in order to detect changes in the usual patterns of resistance and to recognize outbreaks. Regional data should be analyzed with caution, taking into consideration the number of isolates per center, the patient population at each participating site, and the use of antimicrobial drugs in each population group.

The results of the analysis should be passed on to physicians—most notably through periodic publications *(22)*—so that the empirical treatments in everyday use can be adapted and the utilization of antimicrobial drugs can be improved on a timely basis.

It would seem logical for the information to be handled using a statistical program that has been adapted to local needs, but the drawback to this approach is that the results would not be comparable with information gathered at other centers. WHONET, however, is an example of a statistical program for studying bacterial resistance that can analyze epidemiological data about the patient and at the same time information about the sensitivity of the germ under study *(23–26)*. This program makes it possible to conduct statistical studies by center or by region, since it can analyze jointly the information from several centers. It has the advantage of being compatible with several computer programs, thus facilitating the task of data collection. It is being used with increasing frequency in many countries, which will make it possible to conduct regional analyses for the entire world.

Monitoring bacterial resistance is fundamental to proposing measures to promote the rational use of antimicrobial drugs. Local information from centers where quality control is adequate should be used to create continu-

ing education programs for those who prescribe antimicrobial drugs and to draft policies for controlling infections. Regional information, in turn, can be used to prepare guidelines on empirical treatment, adjust the availability of drugs, and determine the true impact of bacterial resistance on morbidity and mortality.

In conclusion, networks for studying bacterial resistance are necessary in the developed countries, but they are even more useful in the developing nations because they promote the use of uniform methods for studying antimicrobial susceptibility and make it possible to fully and accurately appreciate the problem. However, it is essential to have quality control in the participating centers so that the results will be reliable and have a real impact in terms of reducing the toll of antibiotic consumption. In the future, they could also help to reduce the rates of bacterial resistance to antibiotics, as some hospital facilities *(27)* and other parts of the world *(28)* have already managed to do.

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DETECTION OF ANTIBIOTIC RESISTANCE USING THE BIOMÉRIEUX VITEK AND VITEK 2 SYSTEMS

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This review was undertaken to present the bioMérieux VITEK and VITEK 2 Systems in light of their ability to detect specific mechanisms of resistance found in clinically important bacteria. A description of both the systems is provided as well as a sampling of the literature from evaluations of these test methods. Two specific examples of detection of mechanisms of resistance utilizing the VITEK System are presented. These include detection of extended spectrum β-lactamases in Escherichia coli *and* Klebsiella pneumoniae *and vancomycin resistance in enterococci. Additionally, two examples utilizing the VITEK 2 are also presented: glycopeptide intermediate* Staphylococcus aureus *and penicillin resistance in pneumococci. The bioMérieux's Advanced Expert System (AES), a software tool employed to assist in the phenotypic interpretation of susceptibility results, is also described. From the review of the literature presented, it can be concluded that both the VITEK and the VITEK 2 provide a means to accurately and reproducibly detect resistance in the examples cited. Furthermore, the AES offers additional on-line validation and interpretation of susceptibility results and assists in better communication between the laboratory and other members of the health care team.*

INTRODUCTION

Antibiotic resistance is a serious problem that is both global in nature and expanding in scope. The American Society for Microbiology (ASM) states in a report by its Task Force on Antibiotic Resistance, that costs related to treatment of infections with antibiotic-resistant organisms are estimated by the National Centers for Disease Control and Prevention (CDC) to be over $4 billion annually. Concern continues to mount that resistant organisms may occur which cannot be effectively treated with available antimicrobial therapy *(1)*. Emergence of resistance in the Gram-positive arena including glycopeptide intermediate *Staphylococcus aureus* (GISA), vancomycin-resistant enterococci (VRE) and penicillin-resistant *Streptococcus pneumoniae* indicate an alarming trend toward that end *(2)*, as do Gram-negative organisms, especially strains of *Escherichia coli* and *Klebsiella pneumoniae*, which harbor extended spectrum beta-lactamases (ESBL). These enzymes confer

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resistance to all cephalosporins, penicillins, beta-lactam/beta-lactamase inhibitor combinations and monobactams. The selection of resistant pathogens in hospitalized patients with serious infections has generally been linked to the widespread use of antimicrobial agents *(3)*.

The ability to accurately and reliably detect resistance in pathogenic bacteria is a required component in the arsenal of tactics needed to reverse the trend of antimicrobial resistance. In 1991, C. Sanders proposed a somewhat revolutionary approach to susceptibility testing *(4)*. She suggested that rather than testing for susceptibility, specific tests for resistance should be developed and instituted in routine laboratory testing. Low and Scheld, in a 1998 editorial in the *Journal of the American Medical Association (5)*, stated the need for appropriate tools so that physicians may more accurately diagnose conditions for which an antibiotic is indicated, thereby decreasing the inappropriate use of antimicrobials. Additionally, the ASM Task Force on Antibiotic Resistance included as one of their recommendations to curb the excessive use of broad-spectrum antimicrobial agents, the requirement for reliable diagnostic tests. It was stated that when therapy is needed, appropriate diagnostic tests would lead to use of more targeted agents, again resulting in the reduction of the widespread administration of broad-spectrum antibiotics.

In this review, two systems from bioMérieux, Inc. will be discussed, the VITEK and VITEK 2. These systems will be examined in light of their ability to accurately detect resistance in four example situations: ESBL production in *K. pneumoniae* and *E. coli*, vancomycin resistance in enterococci, penicillin resistance in *S. pneumoniae* and glycopeptide intermediate *S. aureus*. Additionally, bioMérieux's Advanced Expert System (AES) will be presented. The AES is an adjunct software program associated with the VITEK 2 that validates and interprets susceptibility results, thus providing a means to identify the resistance mechanism present in an isolate and comment on its interpretation.

THE VITEK SYSTEM

The VITEK System is an automated instrument dedicated to identification of bacteria and yeasts and susceptibility testing of non-fastidious bacteria. The system consists of a reader/incubator, disposable test kits referred to as cards, a filler/sealer unit, and a workstation.

The reader/incubator is the analysis component of the system. It houses the optical system that monitors changes in transmitted light. The VITEK maintains a constant incubation temperature and automatically positions the cards for each reading. Readings are taken hourly. As the data are collected, they are passed to the workstation for analysis and presentation to the operator.

The filler/sealer prepares the cards for incubation. After the inocula are standardized by the microbiologist, the tubes are placed in a tray with an appropriate card associated with each tube. Contact between the inoculum and the card is provided by a small, thin transfer tube. The tray of tubes and cards are placed in the filling chamber. A vacuum is applied to the tray and the cards are inoculated and rehydrated concurrently. Following inoculation, the transfer tube is severed and sealed by the hot blade activity in the sealer portion of the unit. The cards are then ready to be inserted in the instrument and monitored for growth and metabolic activity.

The computer is a PowerPC, RISC based, UNIX workstation and serves as the central processing unit for the system. The software provided with the VITEK System includes analysis and data management programs. A bi-directional interface is provided so that results can automatically be transferred to the laboratory's information system and to the patient report. An Expert System is also available which provides on-line validation of results and interpretation of resistant phenotypes found during testing.

The card is a unique design that contains 30 or 45 miniaturized wells that house either biochemical substrates for identification prod-

ucts or various concentrations of antibiotics in susceptibility products. The appropriate substrate solutions are dispensed in each well and, after drying, the cards are overlaid with a thin, transparent film. The film seals the cards but allows appropriate transfer of gasses. The card is small (85mm by 55mm) and compact. Due to its sealed nature and compact design, it minimizes the risk of biohazard contamination and significantly decreases the amount of waste that must be decontaminated prior to disposal.

The VITEK allows identification testing of a variety of bacteria (Gram-negative rods, Gram-positive cocci and rods, fastidious and anaerobic bacteria) and yeasts and specific cards are designed to provide these identification results. Susceptibility testing can be performed on Gram-positive cocci (staphylococci, enterococci and *S. agalactiae*) and Gram-negative bacilli (*Enterobacteriaceae* and non-fermentative, Gram-negative bacilli). Fifty antibiotics are available for testing against Gram-positive cocci and 65 for Gram-negative bacilli. Additionally, specific tests to detect resistance are available (ESBL, beta-lactamase).

Since its introduction about 20 years ago, the VITEK has been extensively evaluated *(6, 7, 8, 9)*. In a more contemporary study, Doern and colleagues performed a multicenter evaluation of the VITEK antimicrobial susceptibility testing system with 11 antimicrobial agents versus members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Both recent clinical isolates and a challenge set of strains were included in the study. The total category error rate with the VITEK and the clinical isolates (11,902 organism-antimicrobial comparisons) was 4.5%, including 1.7% very major errors, 0.9% major errors and 1.9% minor errors. The authors stated that there was excellent overall interlaboratory reproducibility *(10)*. Two recent studies from the UK *(11, 12)* evaluated the VITEK in terms of its usefulness and cost effectiveness in a hospital laboratory. Shetty et al. found the VITEK to be valuable for the speed and accuracy with which results were available for the common pathogens.

Resistance detection on the VITEK

ESBL detection in K. pneumoniae *and* E. coli

In 1983, the first ESBL-producing strains were reported by Knothe et al. in *Klebsiella* and *Serratia*. The isolates contained plasmids which encoded a mutation of an older enzyme that made the bacteria resistant to cefotaxime *(13)*. The incidence of ESBLs has increased rapidly since that report. Numerous hospitals and extended care facilities have described epidemics, usually due to the spread of an endemic strain under the selective pressure of extensive use of broad-spectrum cephalosporins *(14, 15)*. The prevalence of ESBLs is likely to be greater than reported because of difficulties in their detection by clinical laboratories *(16)*. Evaluations to determine the ability to detect ESBLs in clinical strains have shown that standard or automated MIC methods were not sensitive enough to detect the presence of an ESBL that resulted in elevated MIC values that did not cross the resistant breakpoint *(17)*. The same can be said for disk diffusion testing against aztreonam, cefotaxime, ceftazidime, and cefoxitin *(18)*. The National Committee for Clinical Laboratory Standards (NCCLS) has provided guidelines for screening and confirmatory testing for detection of ESBL-producing strains that can be incorporated in clinical laboratory practices *(19)*. While the screening protocol can serve as an indicator *(20)*, the presumptive result must be confirmed by a test containing clavulanic acid.

The VITEK ESBL test was developed to provide a means for laboratories to routinely monitor the presence of ESBL-producing strains. The test is based on the double disk synergy test which was developed by French investigators *(21)*. The double disk test demonstrates the inhibitory effect of clavulanic acid on ESBLs. It utilizes two disks, one containing a third generation cephalosporin and the other clavulanic acid. The disks are placed 30 millimeters apart on a lawn of bacteria. An enhanced zone of inhibition between the disks indicates the presence of an ESBL-producing

strain. The VITEK ESBL test consists of four wells on a standard VITEK susceptibility panel. The four wells include one each of cefotaxime and ceftazidime with and without clavulanic acid. A significant reduction in growth in either well containing clavulanic acid compared to the growth in the well with the antibiotic alone indicates the presence of an ESBL-producing strain *(22)*.

In 1996, Sanders et al. reported on the ability of the VITEK ESBL test to detect these enzymes in *Enterobacteriaceae* when compared to the double disk method *(23)*. In all phases of the study, the VITEK ESBL test was comparable in performance (Table 1) yet much easier to perform than the double disk test. The authors concluded that the VITEK ESBL test is reliable for the detection of ESBLs in *E. coli* and *K. pneumoniae*. An additional evaluation of the VITEK ESBL test was presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy in 1998 by Spargo et al. *(24)*. In this study 32 characterized strains of *K. pneumoniae*, *K. oxytoca* and *E. coli* along with 11 strains negative for ESBL were tested with the VITEK ESBL test. Sensitivity was 100% (32/32) and specificity was 91% (10/11). Additionally, 108 random clinical isolates were tested at bioMérieux as part of this study. Eleven of these strains were positive and 10 confirmed by the double disk method. An additional 20 non-ESBL producing strains harboring TEM-1, TEM-2 and SHV-1 enzymes were negative on the VITEK ESBL test, allowing for 100% sensitivity and 99% specificity.

Vancomycin resistance in enterococci

The emergence of vancomycin-resistant enterococci (VRE) was first reported in the mid to late 1980s. Since that time, reports of VRE have continued. Among hospitals participating in the National Nosocomial Infection Surveillance System from 1989 to 1997, the percentage of enterococci reported as resistant to vancomycin increased from 0.4% to 23.3% in intensive-care settings *(25)*. In this report, Martone noted several factors as contributors to the spread of VRE in US hospitals which included suboptimal clinical laboratory recognition. Along with increased incidence, vancomycin resistance has a negative impact on the clinical outcome of patients infected with these strains of enterococci. In a recent study by Stosor et al. designed to determine the clinical impact of VRE, patients infected with these organisms were shown to have longer hospitalizations than those with vancomycin-susceptible enterococci (VSE) (35.8 versus 16.7 days, respectively) and hospitalization costs of more than $27,000 more per episode than

TABLE 1. Overall agreement of the Vitek ESBL test and the 2-disk test with characterizations of β-lactamases in strains evaluated in phase 1 and phase 2

	Result by indicated test compared with standard result	
Parameter	VITEK ESBL test	2-disk test
No. of strains with positive standard and test result	205	202
No. of strains with negative standard and test result	159	157
No. of strains with positive standard and negative test result	1	4
No. of strains with negative standard and positive test result	0	1
Agreement (%)	99.73	98.63
Sensitivity (%)	99.51	98.06
Specificity (%)	100	99.37

Source: Sanders et al. *J Clin Microbiol* 1996; 34:2997–3001.

did patients with VSE bloodstream infections ($83,897 versus $56,707) *(26)*.

As newly recognized genotypes of VRE emerged *(27)*, it was reported that various methods had difficulty detecting some strains of VRE, especially those of the VanB phenotype *(28, 29)*. Investigation into factors that influence the VITEK's ability to detect VRE of this phenotype *(30)* lead to a reformulation of this test for the VITEK System. This modified vancomycin-susceptibility test was compared to the agar screen method by Horvat and colleagues *(31)*. In that study, the VITEK showed 99% agreement with the standard method. In 1998, Endtz et al. from the Netherlands also studied the VITEK's ability to detect VRE *(32)*. They tested 145 VRE (50 VanA, 15 VanB and 50 VanC1 and 30 VanC2) and 50 vancomycin-susceptible strains The VITEK vancomycin test had no very major errors when compared to standard methods, thus demonstrating its ability to detect this resistance.

THE VITEK 2 SYSTEM

The VITEK 2 System is a fully automated microbiological testing system that integrates sample preparation, incubation, optical interrogation, and test kit handling in a single instrument. After the inoculum is prepared and standardized by the microbiologist, the instrument performs all the tasks necessary to complete identification and susceptibility testing. This includes inoculum dilution, test kit filling, sealing and transfer to the incubator, monitoring for metabolic changes, transfer of results to the workstation, and disposal of test kits *(33)*.

The optical system houses both multi-wavelength transmittance and fluorescence optics. Each well of the test kit is scanned 10–14 times every 15 minutes which allows precise, kinetic analysis. The cards are incubated in a temperature controlled carousel that carries the test kit to the read head for each interrogation.

The computer provided with the VITEK 2 is a PowerPC, RISC based, UNIX workstation. The software provided includes analysis and data management programs and is presented to the operator in a graphical user interface format. A bidirectional interface allows results to be automatically transferred to the laboratory's information system and to the patient report. bioMérieux's Advanced Expert System is included as an adjunct to susceptibility testing and will be presented in more detail.

The primary interface with the VITEK 2 is through a small, lap top computer-like device called the Smart Carrier Station. Here the user links the sample identification with the specific VITEK 2 card that will be tested. The information is provided via bar code or keyboard entry and is stored in a memory chip imbedded in the Smart Carrier cassette. The cassette is comparable to a test tube rack and it holds the tubes and cards as they are being processed at the bench and in the instrument.

bioMérieux has designed unique cards for the VITEK 2. They contain 64 wells, are opaque and packaged with a factory applied bar code and pre-inserted transfer tube. Identification products utilize fluorescent technology and currently include kits for Gram-negative bacilli, Gram-positive cocci and yeasts. Turbidity as a direct measurement of growth is utilized for susceptibility testing. Forty-six antibiotics are available for testing against *Enterobacteriaceae* and non-fermenting Gram-negative rods, 32 for staphylococci, enterococci and streptococci other than *Streptococcus pneumoniae* and 12 antibiotics are available for testing against *S. pneumoniae*. Additionally, a nitrocefin based beta-lactamase test for *Staphylococcus* and *Enterococcus* is provided.

Due to the rapid time to report for identification tests, automated processing and optimized workflow, the VITEK 2 has been shown to provide time savings when compared to other methodologies. In 1997, Auckenthaler discussed this concept at the European Congress on Clinical Microbiology and Infectious Diseases (presented at the 8th European Congress on Clinical Microbiology and Infectious Diseases, Lausanne, Switzerland, 1997). In a preliminary evaluation of the

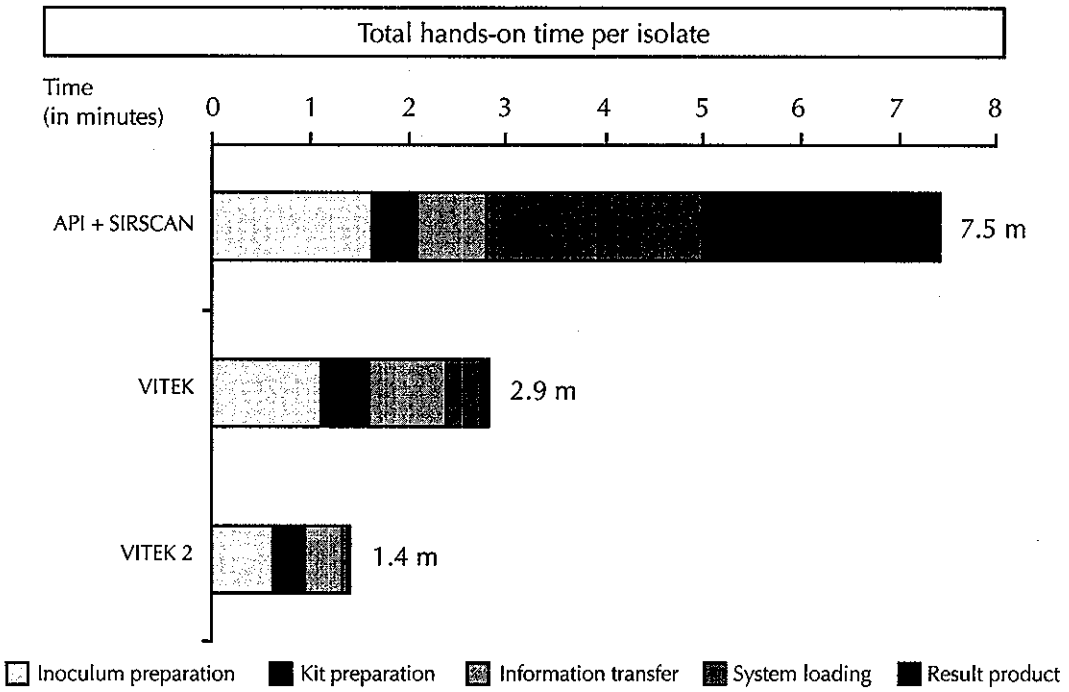
VITEK 2, he reported that it required 1.4 minutes hands-on time to obtain a result utilizing the VITEK 2 compared to 7.5 minutes per isolate using conventional methods (Figure 1). He concluded that the system should demonstrate a significant progress for automation of bacteriology. Funke et al. also evaluated the VITEK 2, particularly for its ability to identify medically important Gram-negative rods *(34)*. He found the system to be a promising, highly automated new tool for the rapid identification of Gram-negative bacilli. Evaluations of particular aspects of susceptibility testing on the VITEK 2 have been conducted by various groups *(35–38)* concluding that the VITEK 2 is reliable, reproducible and accurate (Table 2).

Resistance detection on the VITEK 2

Penicillin resistance and S. pneumoniae

Penicillin resistance in pneumococci is a serious threat to public health worldwide. The incidence of resistance has increased significantly over the past decade. In the United States, prevalence of pneumococci with decreased susceptibility to penicillin varies from 2% to 53% *(39)*. Some of these strains are also resistant to multiple antimicrobial agents, making them increasingly difficult to treat *(40)*. The NCCLS has focused its attention on clarifying its recommendations for MIC testing of pneumococcal isolates *(41)*. Those strains that are nonsusceptible by the oxacil-

FIGURE 1. Comparison of hands-on time for VITEK 2, VITEK, and manual methods



Source: Auckenthaler R. *Integrating automation in a microbiology lab.* ECCMID, Lausanne, 1997.

TABLE 2. Results of clinical isolates and reproducibility studies on the VITEK 2 with various antibiotics

Antibiotic	Clinical isolates essential agreement (%)	Reproducibility +/– 1 dilution card results only (%)
Amikacin	179/185 (96.8)	98.2
Amoxicillin-clavulanic acid	175/186 (94.1)	96.3
Ampicillin	188/196 (95.9)	98.8
Ampicillin-sulbactam	181/190 (95.3)	98.8
Aztreonam	181/197 (91.9)	97.0
Cefepime	190/199 (95.5)	92.8
Cefixime	186/198 (93.9)	95.2
Cefotetan	184/190 (96.8)	98.8
Gentamicin	183/185 (98.9)	98.8
Imipenem	188/198 (94.9)	93.4
Mezlocillin	173/197 (87.8)	91.6
Nalidixic acid	190/201 (94.5)	98.2
Netilmicin	180/185 (97.3)	96.4
Norfloxacin	190/201 (94.5)	96.4
Piperacillin	179/197 (90.9)	91.0
Tobramycin	183/185 (98.9)	98.8
Trimethoprim-sulfamethoxazole	191/196 (97.4)	98.2

Source: Modified from: Moland, Thomson, Sanders. ICAAC Toronto, 1997; D48.

lin screen method should have MIC determinations performed to penicillin, an extended-spectrum cephalosporin, chloramphenicol and vancomycin. Until recently, no automated, rapid MIC method has been available to provide this testing.

The VITEK 2 provides an automated, rapid method to determine MICs to relevant antimicrobial agents used in treating pneumococci. Ruesing et al. demonstrated the ability of the VITEK 2 to detect penicillin resistance in *S. pneumoniae (42)*. Two hundred and twenty-six strains were evaluated, covering a wide range of penicillin MIC values. This study showed 100% essential agreement and no major or very major errors were obtained. Additionally, a challenge set of 52 strains provided by the CDC were tested. Again, there were no major or very major errors with these strains and four minor errors. Finally, resistance to penicillin was reproducibly detected at a level of 99.3%. A multicenter evaluation performed by Jorgensen et al. examined susceptibility testing of *S. pneumoniae* on the VITEK 2 *(43)*. Of the 407 clinical isolates tested against penicillin, no major or very major errors were obtained. The CDC challenge set described in the study by Ruesing and colleagues was also tested by each of the three sites in this evaluation. Of the 162 tests, only one major and no very major errors were obtained. The authors concluded that the VITEK 2-determined MICs compared favorably with the reference MICs and that the tests were simple to perform with results available in an average of 8.4 hours.

Detection of glycopeptide intermediate *S. aureus*

In 1996, the first documented case of a strain of *S. aureus* with intermediate resistance to vancomycin was reported from Japan *(44)*. The following July, a GISA strain from a patient with peritonitis was reported in Michigan *(45)*, followed by one the next month from New Jersey *(46)*. These strains were characterized by Tenover et al. *(47)* and none of them were found to contain any known vancomycin-resistance genes. Moellering discussed this issue and reiterated the larger concern that vancomycin-resistance genes found in enterococci may be transferred to staphylococci or pneumococci *(48)*. The CDC presented guidelines

for the prevention and control of GISA *(49)*. The requirement for accurate laboratory testing was paramount in their recommendations.

Two *S. aureus* isolates provided by the CDC and known to demonstrate vancomycin MICs of 8 were tested on the VITEK 2 by Leahart et al. *(50)*. After multiple testing, the strains consistently gave MIC values of 8–16, intermediate by NCCLS breakpoints (Table 3). These results give an indication that GISA strains should be detected on the VITEK 2.

THE BIOMÉRIEUX ADVANCED EXPERT SYSTEM

The use of expert systems as an adjunct to susceptibility testing was discussed by Leclercq (presented at the 8th European Congress on Clinical Microbiology and Infectious Diseases, Lausanne, Switzerland, 1997). He noted that utilization of expert systems has become an accepted practice, especially due to their emphasis on detection of resistance mechanisms that may not be fully expressed or observed. Most expert systems are rule based which requires a careful choice of good marker antibiotics to test and the use of interpreted results, which can differ country to country.

The AES provides an expert system with a different approach. It utilizes a matrix of phenotype descriptions based on expected MIC ranges for antibiotics that can be associated with each phenotype. In this way, the AES offers a universally accepted basis of information (the MIC value) and uses a pattern-matching approach that compares the observed phenotype to the descriptions in the knowledge base. The AES knowledge base is built on reports from the scientific literature and supplemented with internal data. It contains 2,147 species-resistance mechanism patterns and over 17,000 MIC distributions *(51)*.

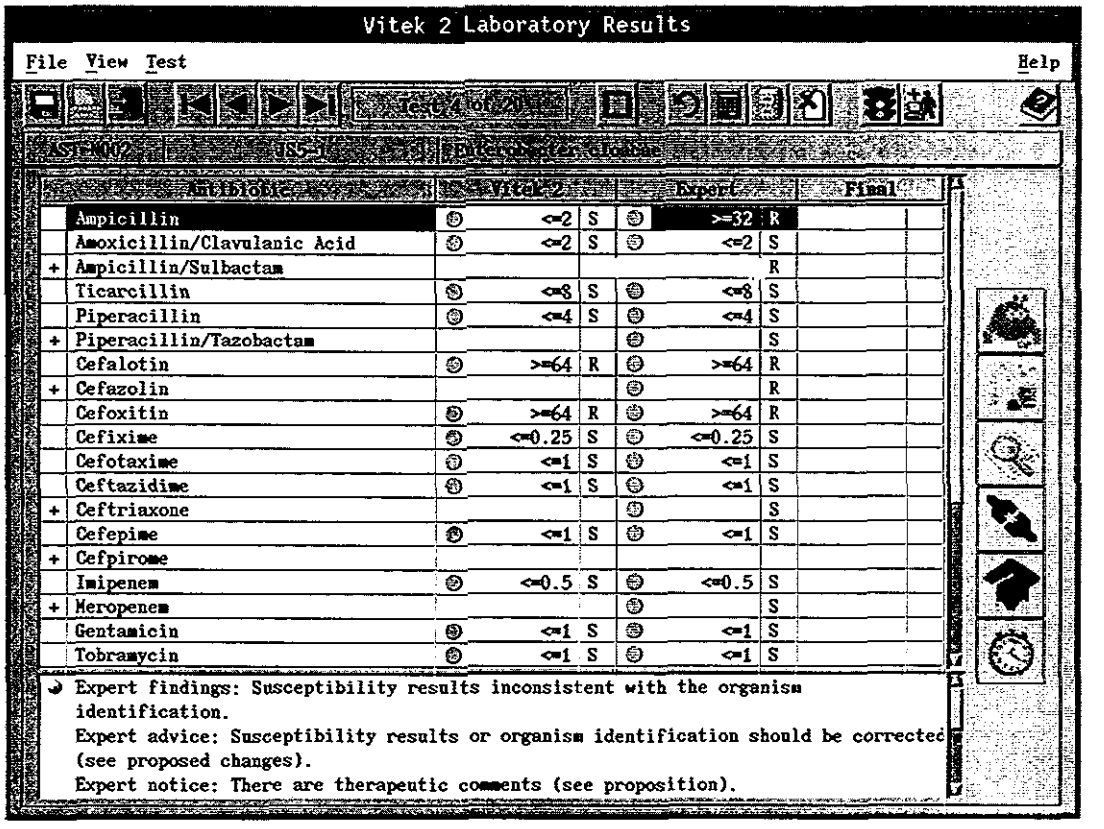
The AES follows a three step process: biological validation, therapeutic interpretation and commentary on the results. In the validation phase, the AES compares the observed MICs from the VITEK 2 susceptibility results and the identification of the isolate to the matrix of resistance mechanisms in the knowledge base. If AES determines a slight inconsistency between the observed and the best fit expected result, it will propose either an alternative identification or a modification to the susceptibility result (Figure 2). There may be instances when resistance is not fully expressed and therapy may be misdirected if the susceptibility results are not interpreted beyond the category breakpoints. This is the case, for instance, with oxacillin-resistant staphylococci. While other beta-lactam antibiotics may be susceptible as tested, they should be interpreted as resistant because these antibiotics would be an ineffective therapy choice. This is the second step in AES. Additionally, AES has the ability to deduce results for antibiotics that were not tested based on the MIC distributions and the resistance mechanisms it recognized during the biological validation phase. Finally, AES is able to attach comments to the susceptibility results that facilitate communication between the laboratory and the physician and staff. The AES can be customized to accommodate recommendations of local consensus committees (e.g. the NCCLS) and tailored to provide interpretations and comments that best suit each institution.

TABLE 3. VITEK 2 results of glycopeptide intermediate *Staphylococcus aureus* strains

Strain of *Staphylococcus aureus*	Vancomycin MIC (µg/ml)	Teicoplanin MIC (µg/ml)
CDC HIP-5827	8, 16, 8	4, 4, 4
CDC HIP-5836	16, 16, 8	8, 8, 16

Source: Leahart, Fitzsimmons, Peyret. ASM Atlanta, 1998.

FIGURE 2. VITEK 2 laboratory report showing bioMérieux Advanced Expert System recommendations



CONCLUSION

From the review of the literature presented, it can be concluded that both the VITEK and the VITEK 2 provide a means to accurately and reproducibly detect resistance in the examples cited. Furthermore, the AES offers additional on-line validation and interpretation of susceptibility results and assists in better communication between the laboratory and other members of the health care team.

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SENSITIVITY OF *SALMONELLA, SHIGELLA,* AND *VIBRIO CHOLERAE* TO ANTIMICROBIALS IN THE AMERICAS, 1948 TO 1998

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Antimicrobial resistance in general is currently a significant public health problem. In particular, the resistance of strains of Salmonella, Shigella, *and* Vibrio cholerae *affects not only human public health, but also the food industry. In order to better understand the antimicrobial resistance profiles of these microorganisms in the Region of the Americas, more than 80 articles on the subject published between 1961 and 2000 were reviewed. The results of that analysis reveal an increasing trend in the resistance of these infectious agents to the antimicrobial drugs included in this review: ampicillin, ciprofloxacin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. Even though the information obtained from the articles reviewed is partial and fragmented and does not allow trend assessment or the determination of patterns of resistance by country, much less by smaller geographic areas, its importance from the clinical, epidemiological, and socioeconomic standpoints cannot be dismissed. The studies reviewed show that, in most of the countries, more than 40 years after the appearance of resistance to antimicrobial agents there is still no monitoring or surveillance, or what there is refers to a limited number of samples, which affects its usefulness.*

INTRODUCTION

Acute infectious diarrhea is a common and significant cause of morbidity and mortality, particularly in developing countries. Due to the lack of laboratories, most of the time those infections are treated empirically, usually by administering antibiotics. In the last four decades, both the appearance of antimicrobial resistance and its increase have been observed, with a consequent rise in morbidity and mortality from infectious diseases. The resistance of microorganisms such as *Salmonella* and *Shigella* to antimicrobials is not a new phenomenon. Studies in the United States of America reported the appearance in the late 1940s of isolates of *Salmonella* and *Shigella* that were resistant to tetracycline and chloramphenicol, a phenomenon also seen in other countries of the Region.

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In 1991 there was a cholera epidemic in Peru which spread rapidly to other countries in South and Central America and which was followed by the detection, for the first time, of *Vibrio cholerae* strains resistant to antibiotics.

The objective of this literature review was to analyze for the first time the distribution of *Salmonella, Shigella,* and *Vibrio cholerae* resistance to antimicrobials over time and geographic location. These enteropathogens were selected for their importance to public health and their impact on the countries' economies, basically through their effects on the food industry and tourism.

MATERIALS AND METHODS

The articles about the resistance of *Salmonella, Shigella,* and *Vibrio cholerae* in countries of the Region of the Americas from the late 1940s to the present were identified through a search of the MEDLINE and LILACS databases and the use of the bibliographic references cited in these and other articles on the subject.

A total of 120 published articles were identified in the bibliographic search. The distribution by country was as follows: Argentina, 11; Bolivia, 1; Brazil, 22; Chile, 10; Colombia, 1; Ecuador, 2; Guatemala, 3; Honduras, 1; Jamaica, 3; Mexico, 16; Nicaragua, 1; Peru, 1; the United States, 35; and Venezuela, 3.

Some of the articles reviewed were excluded because they did not mention the study period or the laboratory technique used to determine how susceptible the organisms were to antibiotics. In most of the articles reviewed, sensitivity to antimicrobials was studied by means of the agar diffusion method; in a much smaller number, the dilution method was used. The antibiotics evaluated varied by microorganism, period, and study site. In order to simplify the presentation of data, this report focuses on the resistance of isolates of *Salmonella, Shigella,* and *V. cholerae* to ampicillin, ciprofloxacin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole, although occasionally other antibiotics are mentioned.

Tables 1 and 2 present information on the resistance of *Salmonella* and *Shigella* in study sample sizes of 30 or more isolates. However, in describing the results, reference is also made to works that contain data on fewer than 30 isolates. The number of isolates appearing in the tables represents the sum of all the isolates studied in all the articles reviewed for the specific period of study. The tables show the lowest and highest percentages published in the aforementioned articles for that period.

RESULTS

The results are presented by microorganism and by drug.

Salmonella

Salmonella resistance varied widely, depending on the antimicrobial drug, the geographic area, and the period of study (see Table 1).

Tetracycline

In the United States, the percentage of tetracycline-resistant *Salmonella* isolates increased steadily from 0% in 1948 to 9.7% in the 1959–1960 period *(1)*, and to 31% in 1975 *(2)*. From 1975 to 1997, the figures recorded varied widely (between 8% and 55%), but the general trend was upward *(2–8)*. In considering the *S. typhimurium* isolates separately from those of other species of the genus *Salmonella*, it was noted that resistance had been greatest among the former *(3, 5, 9–13)*. In Mexico, the percentage of tetracycline-resistant *Salmonella* isolates also increased, from 7.1% in the 1953–1965 period to 84.2% in 1979–1980 *(14)*, with a subsequent decline to 48% in the 1980s *(15)*. In Brazil, the tetracycline resistance in *Salmonella* isolates remained stable from 1978 to 1992, hovering around 60% *(16–17)*.

TABLE 1. Resistance (%) among isolates of *Salmonella*, by antibiotic, period, and country, 1948–1998

Country		Antibiotic											
		Ampicillin		Chloramphenicol		Tetracyclin		Trimethoprim-sulfamethoxazole		Ciprofloxacin		Trovafloxacin	
Period (*Bibliographic reference*)	*Microorganism*	Sample *size*	Resistant *percentage*	Sample *size*	Resistant *percentage*	Sample size	Resistant percentage	Sample size	Resistant percentage	Sample size	Resistant percentage	Sample size	Resistant percentage
Argentina													
1968–1971 (*24*)	*S. typhimurium*	...	...	68	100	...	...	68	24	...	...	...	...
1990–1991 (*23, 37*)	*Salmonella*	173	100	173	5.3	...	...	...	...	...	...	...	...
	S. typhimurium	64	92.1	64	88.9			128	24–100				
	Salmonella, other	...	...	...	...			109	45.4				
1995–1998 (*38–39*)	*Salmonella*	667	14–57	667	2–4	...	...	667	10–33	215	0–2	616	–
Brazil													
1978–1983 (*16*)	*Salmonella*	291	77	291	74.2	...	...	291	69.8	...	...	...	...
1987–1992 (*17*)	*Salmonella*	116	73.2	116	62.9	...	...	116	50	...	...	...	...
Chile													
1972–1974 (*32*)	*S. typhimurium*	34	97	34	67.6	34	97	...	...	...	...	...	...
1975–1983 (*30–31*)	*S. typhi*	2,594	0.03	2,594	0.23	2,594	0.23	...	...	...	...	...	...
1996–1996 (*34*)	*Salmonella*	471	0.2	471	0.6	...	...	471	0.85	...	...	...	...
Colombia													
1997– (*57*)	*Salmonella*	92	6–76	92	0–9	...	...	92	6–76	471	–	...	...
Jamaica													
1964–1965 (*35*)	*S. typhi*	44	4.5	44	...	44	2.3	44	–	...	...	...	...
	Salmonella, other	53	5.6	53	5.6	53	9.4	53	–	...	...	...	...
1969–1970 (*35*)	*S. typhi*	19	...	19	...	19	6.3	19	–	...	...	...	...
	Salmonella, other	92	9.7	92	3.2	92	4.3	92	–	...	...	...	...
1974–1975 (*35, 61*)	*S. typhi*	21	–	21	–	21	–	21	–	...	...	...	...
	Salmonella, other	338	41.1–52	338	35–52	338	14.7–16	338	7.8–13	...	...	...	...

Mexico													
1978–1980 (*14, 25*)	*Salmonella*	870	59 to 66	870	39–48	870	75–84	870	36.5–42	...	...	...	...
	S. typhimurium	129	71	121	45	105	92	129	37	...	...	...	...
	S. typhi	16	12	16	12	14	29	7	79	...	...	...	...
1982–1985 (*25*)	*Salmonella*	298	28 to 50	298	46–50	298	33–48	198	2.6	...	...	...	...
	S. typhi	305	5.1–15.4	305	4.2–5.1	...	...	305	8.7–23.4	...	...	...	...
United States													
1948 (*1*)	*S. typhimurium*	...	...	100	–	100	–	...	...	...	...	...	...
1956–1957 (*1*)	*S. typhimurium*	...	...	100	–	100	5	...	...	...	...	...	...
1959–1960 (*1*)	*S. typhimurium*	...	...	158	1.9	158	13.9	...	...	...	...	...	...
	Salmonella, other	...	...	150	–	150	5.3	...	...	...	...	...	...
1962–1966 (*18, 84–85*)	*S. typhimurium*	188	20	401	0–2	401	9.8–38	...	...	...	...	...	...
	Salmonella, other	28	–	144	–	144	0–11	...	...	...	...	...	...
1967–1969 (*9–10*)	*Salmonella*	692	8–13.5	692	–	692	2.6–12.5	...	...	...	...	...	...
	S. typhimurium	230	16.5–25.5	230	–	230	4.4–31.4	...	...	...	...	...	...
1970–1980 (*2–3, 5, 9–13*)	*Salmonella*	13,083	3.5–24	11,298	0–7	14,343	8.6–31	10,748	0–7	...	...	...	...
	S. typhimurium	1,441	4.3–42.8	1,441	0–2.9	1,441	21–46.2	263	0–1.2	...	...	...	...
1981–1990 (*2, 7, 20*)	*Salmonella*	11,332	9–24	11,332	2–6	11,332	8–24	11,332	0.6–3	200	–	...	...
1994–1995 (*21*)	*Salmonella*	...	...	...	...	...	...	...	...	4,008	0.02	...	...
1997 (*8*)	*Salmonella*	1,301	181.5	1,301	10.1	1,301	21.8	1,301	1.8	...	...	...	...

– = zero.
... = no data.

Chloramphenicol

In the United States, *Salmonella* resistance to chloramphenicol has generally been low. Resistance was not detected in the first studies in 1948; it was observed in 1959–1960 solely among isolates of *S. typhimurium* (1.9%) *(1)*. In the other species, the phenomenon was not noted until 1972, when resistance in California was detected in 1.5% of the *Salmonella* isolates and in 16.5% of those of *S. typhi (12)*. From 1973 to 1997, resistance of *Salmonella* isolates in the United States remained between 0% and 10% *(2–5, 7–8, 13, 18–21)*. In Argentina, the percentage of chloramphenicol-resistant *Salmonella* isolates has also been zero or low: 0% in the 1980s and 5.3% in the 1990s *(22–23)*. However, there are data indicating that *S. typhimurium* resistance to chloramphenicol has been higher, both in the 1960s and in the 1990s *(23–24)*. In Mexico, resistance by *Salmonella*, which was nil during the 1953–1965 period, reached 50% in the 1980s *(25–26)*. In 1972 Mexico City was hit by a major epidemic of typhoid fever, with more than 10,000 cases *(27)*. The principal characteristic of that outbreak was the high percentage of chloramphenicol-resistant isolates of *S. typhi* (91.7%), which up to that time had been a rare phenomenon *(27)*. In several studies conducted in the United States, the resistance of *Salmonella* strains was found to have a prevalence of 1 per 1,000 isolates, except for a study conducted in California between 1971 and 1972. In that study, 33 isolates out of a total of 2,246 were resistant to chloramphenicol and 19 of the resistant isolates corresponded to *S. typhi (12)*. One possibility considered is that the chloramphenicol-resistant strains originated in Mexico *(28)*. In Chile, where most of the information available pertained to *S. typhi*, this microorganism has remained susceptible. In 1951 and 1952 and 1958–1974, there were, respectively, 8, 2, and 0 resistant isolates (the referenced article does not include the numbers of isolates tested). Of the 2,594 isolates studied between 1975 and 1983, 6 were resistant (0.23%) *(29–31)*. In contrast, resistance by *S. typhimurium* has been higher, though there was a downward trend between 1972 and 1996: 67.6% in 1972–1974; 25% in 1983–1984; and 0.9% in 1996 *(32–34)*. In Jamaica, between 1964 and 1975 no resistance was detected among isolates of *S. typhi*, and resistance in isolates of the other species ranged between 5.6% in 1964–1965 and 35% in 1975 *(35)*. In Brazil, *Salmonella* resistance ranged from 37.5% to 74.2% in the period from 1973 to 1992 *(16, 17, 36)*.

Ampicillin

In the United States, resistance to ampicillin by *Salmonella* strains, particularly *S. typhimurium*, has increased since the late 1960s *(9–10)*. The highest resistance values were reached in 1975, approximately 43% for *S. typhimurium* and 17% for all species of *Salmonella (5)*. There were no changes in the period from 1979 to 1997 *(8, 20)*. In Brazil, 77% of the *Salmonella* isolates were found to be resistant to ampicillin between 1978 and 1983 *(16)* and 73% between 1987 and 1992 *(17)*. In Argentina, resistance of *Salmonella* spp. showed large variations—from 14% to 100% between 1985 and 1995 *(22, 23, 37)*. In Chile, 97% of the isolates of *S. typhimurium* were resistant to ampicillin during the 1972–1974 period *(32)*.

Trimethoprim-sulfamethoxazole

In the United States, the resistance of *Salmonella* to trimethoprim-sulfamethoxazole rose from 0% in 1973 to 7% in 1978 *(2–3)*, and then ranged between 0% and 5% through 1997 *(2, 7–8, 20)*. In Argentina, between 1969 and 1998 resistance by *Salmonella* spp. did not exceed 33% *(24, 38)*, a lower value than the figure reported for Brazil, 69.8% between 1978 and 1992 *(16–17)*, and for Mexico, which was 36% in 1979 *(25)*.

Quinolones

Fluoroquinolones were introduced into the U.S. market in the mid-1980s. A national sur-

vey conducted in 1994–1995 by the Centers for Disease Control and Prevention (CDC) in that country showed that, of 4,008 *Salmonella* isolates studied, only one (0.02%) was resistant to ciprofloxacin *(21)*, a frequency similar to that found in another survey conducted from 1989 to 1990 *(7)*. In Argentina, the resistance to ciprofloxacin and trovafloxacin in 1998 was 0% *(39)*, whereas between 1995 and 1996 it had been 2% *(38)*. In Chile, resistance to quinolones was 0% in 1996 *(34)* and in several other Latin American countries it was 0% to ciprofloxacin and 0.9% to trovafloxacin *(40)* in 1998.

Shigella

Strains of the genus *Shigella* currently show high percentages of resistance in the Region and, as in the case of *Salmonella*, a review of the published articles indicates wide variations by species, geographic area, antibiotic, and period of study (see Table 2).

Ampicillin

In the United States, *Shigella* resistance to ampicillin increased from 0% to 95% between 1967 and 1974, in both east- and west-coast states *(41–46)*. Two surveys conducted by the CDC, in 1979 and 1985–1986, revealed resistance in 35% and 32% of isolates, respectively *(47)*. In Venezuela, resistance to ampicillin increased from 7% in 1972 to 54% in 1992 *(48–50)*. In Rio de Janeiro, Brazil, resistance to the drug increased from 0% between 1960 and 1969 to 2.2% in the 1970–1978 period *(51)*; and in the Northeast, by more than 50% between 1988 and 1993 *(52)*. In Mexico, in the period from 1978 to 1985 the figures ranged from 30% to 67% *(14, 25–26)* and, in Argentina, from 42% to 83% in the 1980s *(22, 53)* and from 51% to 95% in the 1990s *(54)*. When considering the species *S. flexneri* and *S. sonnei* separately, it was found that resistance has occurred most frequently among the strains of *S. flexneri (38–39, 54)*. For example, in Chile it was around 50% in the 1980s *(33, 55)*, while in Bolivia, Colombia, Cuba, Ecuador, and Nicaragua it was around 95%, 76%, 80%, 90%, and 70%, respectively, in the early 1990s *(56–60)*. In Jamaica, by contrast, resistance was low both in the 1970s (7%) and in the 1980s (2.6%) *(61–62)*.

Trimethoprim-sulfamethoxazole

In Argentina, the resistance of *Shigella* isolates to trimethoprim-sulfamethoxazole has been high since 1985, with values ranging from 57% to 85% *(22, 38, 53–54)*. In Rio de Janeiro, Brazil, resistance was 12.4% in the 1960s, increasing to 20.9% and then 91.7% during the 1970s and 1980s, respectively *(36, 51)*. However, in the Northwest of Brazil, resistance of *Shigella* isolates to trimethoprim-sulfamethoxazole was nonexistent until the end of the 1970s, but increased to 75% at the beginning of the 1980s and to 100% in the 1990s *(52, 63–64)*. In Chile, resistance to that drug was 7.6% between 1983 and 1984 *(33)* but it increased to 45% in 1989–1990 *(55)*. Since then it has remained stable; in 1996, 43.7% of the isolates were resistant *(34)*. Resistance in Mexico in the 1980s ranged from 15% to 33% *(26)*, and in Bolivia, Nicaragua, and Venezuela in the 1990s it was approximately 90% (90%, 86%, and 93%, respectively) *(56, 60, 65)*. In this same period, the resistance of *Shigella* strains to trimethoprim-sulfamethoxazole in Colombia, Cuba, and Ecuador reached values of 93%, 92%, and 48%, respectively *(57–59)*. In Jamaica, resistance to that antibiotic, as well as to ampicillin, was low during the 1980s (2.2%) *(62)*. In the United States, in Houston, Texas, 100% of *Shigella* isolates were sensitive to trimethoprim-sulfamethoxazole in 1974 *(66)* and only 1% presented resistance in New York in 1973 *(13)* and in the national survey conducted by the CDC in 1979, but this had increased to 7% by 1985–1986 *(47)*. In another region, at a Navajo reservation, resistance increased from 3% in 1983 to 21% in 1985 *(67)*.

Chloramphenicol

The information analyzed shows that during 1967–1990 resistance of *Shigella* isolates to chloramphenicol in the United States was a

TABLE 2. Resistance (%) among isolates of *Shigella*, by antibiotic, period, and country, 1950–1998

Country		Antibiotic									
		Ampicillin		Chloramphenicol		Tetracyclin		Trimethoprim-sulfamethoxazole		Ciprofloxacin	
Period *(Bibliographic reference)*	Microorganism	Sample size	Resistant percentage	Sample size	Resistant percentage	Sample size	Resistant percentage	Sample size	Resistant percentage	Sample size	Resistant percentage
Argentina											
1985–1988 (*22, 53*)	*Shigella*	68	42.1–83	68	7.9–73	30	73	68	57–78.9	...	...
1990–1998 (*38–39, 54*)	*Shigella*	771	5–95	771	3–62	...	...	771	57–85	1,391	–
	S. flexneri	1,957	60–100	1,957	13–71	...	...	1,957	79–84	458	0–1
	S. Sonnei	579	36–54	503	1.6–5	...	...	567	55–82	...	...
Bolivia											
1995 (*56*)	*Shigella*	39	95	39	62	...	...	39	90	...	...
	S. flexneri	...	...	28	86	...	...	...	...	...	...
Brazil											
1950–1959 (*70*)	*Shigella*	...	...	51	–	51	1.9	...	...	...	...
1960–1969 (*68–70*)	*Shigella*	89	–	521	0–24.7	534	6.7–32.5	89	12.36	...	...
1970–1978 (*51*)	*Shigella*	86	2.23	86	17.4	86	41.9	86	20.93	...	...
1988–1993 (*52*)	*S. flexneri*	39	50–90	39	64	39	95	39	50–90	...	...
Chile											
1983–1990 (*35, 55*)	*Shigella*	130	7–42	94	–	...	...	235	19.5–45	...	...
1996–1996 (*34*)	*Shigella*	142	57.7	142	28.9	...	...	142	43.7	142	...
	S. flexneri	73	63	73	32.9	...	...	73	57.5	73	...
	S. sonnei	46	54.3	234	21.7	...	...	46	34.8	46	...
1997–1998 (*71*)	*S. flexneri*	218	87	215	52	59	73	216	76	...	...
	S. sonnei	241	91	234	81	55	75	242	21	...	...

Colombia											
1997–1997 (*57*)	*Shigella*	55	40–80	55	40–80	...	...	55	33–93	...	...
Cuba											
1990–1997 (*58*)	*Shigella*	2,250	80–98.2	2,253	70–83	2,253	65–76	2,250	66.5–91.9	...	...
Ecuador											
1994–1997 (*59*)	*Shigella*	380	79–90	380	68–78	64	90	380	30–48	380	–
Jamaica											
1975 (*61*)	*Shigella*	125	7	125	6	125	8	125	2	...	...
1983–1985 (*62*)	*S. flexneri*	217	0.5	217	0.5	...	...	217	1	...	...
	S. Sonnei	117	2.6	117	0.9	...	...	117	0.9	...	...
Mexico											
1978–1980 (*14, 25*)	*Shigella*	306	33.5–43	310	22–24	310	77–82.3	260	33	...	...
1982–1985 (*26*)	*Shigella*	187	30–37.7	187	17.4–30	...	...	187	15–33.3	...	...
United States											
1967–1970 (*41–43, 86*)	*Shigella*	1,358	0–84	1,276	0–3	...	...	1,358	1–40	...	...
1971–1978	*Shigella*	1,654	5–95	1,548	0–4	1,654	5–82.3	...	...	...	...
(*41, 43–46, 66, 87*)	*S. flexneri*	56	3	56	69	56	33	56	100	...	...
	S. sonnei	215	45–60	215	1–98	215	35	113	100	...	...
1983–1986 (*47, 67*)	*Shigella*	252	32	252	5	252	46	252	7	...	...
	S. flexneri	692	19–40	389	0–11	480	8–67	682	3–20	...	...
	S. sonnei	382	21–85	294	0–9	391	1–34	466	3–21	...	...

– = zero.
... = no data.

low 0% to 5% *(7, 42, 45, 47, 67)*. In Argentina, however, it was quite variable during the period from 1985 to 1997, ranging from 3% to 73%; resistance was higher among the isolates of *S. flexneri* than among those of *S. sonnei (22, 38, 54)*. On the other hand, resistance in Brazil was 0% between 1950 and 1959 but rose to between 14% and 24% in the 1960s and 1970s *(51, 68–70)*. In Chile, resistance was also variable, with an upward trend in the 1980s (0% to 23%) and 1990s (28.9% to 81%) *(33–34, 55, 71)*. In Mexico, resistance of *Shigella* isolates to chloramphenicol ranged from 22% to 24% between 1978 and 1980 *(14, 25)* and was approximately 24% in the period between 1982 and 1985 *(26)*. The data from Colombia for 1996 and from Cuba for the period between 1990 and 1997 indicate that resistance ranged from 40% to 80% in the former and from 70% to 83% in the latter *(57–58)*. In Jamaica, between 1983 and 1985 *Shigella* resistance to chloramphenicol was less than 1% *(62)*.

Quinolones

In the United States, resistance of *Shigella* isolates to ciprofloxacin was 0% from 1989 to 1990 *(7)*. In Argentina, from 1995 to 1996 resistance to ciprofloxacin and trovafloxacin was 0% *(38)* and in 1998, resistance to ciprofloxacin was 1% among *S. sonnei* isolates and 0% in *Shigella* spp. and *Shigella flexneri*. Also, resistance to trovafloxacin was 0% for *Shigella* spp., *S. sonnei*, and *S. flexneri (39)*. The data from Chile for 1996 and from Ecuador for 1990 to 1997 indicate that the resistance of *Shigella* isolates to quinolones was also 0% *(34, 57)*. During 1998, the resistance of this genus of bacteria to ciprofloxacin and trovafloxacin remained at 0% in several Latin American countries (Argentina, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Peru, Uruguay, and Venezuela) *(40)*.

Vibrio cholerae

From around 1900 to 1973 in North America and to 1991 in South America, there were no autochthonous cases of cholera reported. In January 1991, there was an outbreak of cholera in Peru caused by *V. cholerae* 01, El Tor, serotype Inaba *(72)*. Cases of cholera were subsequently reported in Bolivia, Brazil, Chile, Colombia, Ecuador, El Salvador, Guatemala, Mexico, Panama, the United States, and Venezuela *(73)*. Between 1991 and 1996, a total of 1,189,553 cases were reported, with more than 11,000 deaths *(74)*.

A review of published articles shows that in Latin America, multiple resistance in *V. cholerae* appeared almost simultaneously with the 1991 cholera outbreak in Peru (personal communication, Dr. Eduardo Gotuzzo). In 1991, 36% and 9%, respectively, of a series of 42 and 11 samples from Guayaquil, Ecuador, were multiresistant *(75)*. Between 1992 and 1993, 0.9% of some samples from northern Ecuador were resistant to tetracycline and trimethoprim-sulfamethoxazole *(76)*. In addition, in Chile multiresistance to tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole was reported in a *V. cholerae* strain in 1991 *(77)*. In Guatemala, 47% of the isolates studied in 1993 were resistant to furazolidone, sulfisoxazole, and streptomycin, although all the strains isolated in 1991 had been sensitive to all the antibiotics evaluated *(78)*. Similarly, 27% of the strains of *V. cholerae* evaluated in Honduras were multiresistant to ampicillin, cefalotin, chloramphenicol, doxycycline, gentamicin, kanamycin, tetracycline, and trimethoprim-sulfamethoxazole *(79)*. In Mexico, resistance to ampicillin, chloramphenicol, and tetracycline in 1991 was 6.9%, 0.1%, and 3.6%, respectively, declining in 1992 to 2.3%, 0%, and 0.1%, respectively *(80)*.

Since the reappearance of cholera in Argentina, there has been strict monitoring of the resistance of *V. cholerae* to the following drugs: ampicillin, tetracycline, nitrofurantoin, erythromycin, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, sulfonamides, and streptomycin. Of the 122 samples studied at the beginning of the cholera outbreak in 1992, none was resistant to the antimicrobials tried. However, 1.2% of the 681 isolates studied dur-

ing the outbreak of 1992–1993 presented different resistance profiles: one isolate was resistant to sulfonamides and ampicillin; three to tetracycline, chloramphenicol, sulfonamides, and trimethoprim-sulfamethoxazole; three others to tetracycline, erythromycin, chloramphenicol, sulfonamides, trimethoprim-sulfamethoxazole, ampicillin, and streptomycin; and one to sulfonamides, ampicillin, and streptomycin. Similarly, 3% of 511 isolates studied during the 1993–1994 outbreak showed variable resistance: one was resistant only to ampicillin; one to erythromycin, ampicillin, and streptomycin; one to sulfonamides and trimethoprim-sulfamethoxazole; one to tetracycline, sulfonamides, trimethoprim-sulfamethoxazole, ampicillin, and streptomycin; and a group of 12 isolates was resistant to streptomycin only. No resistance was reported to any of the antimicrobials tested during the fourth (n=133) and fifth outbreaks (n=277) of cholera. During the sixth epidemic period (1996–1997), of the 252 isolates studied, 20 (7%) presented multiple resistance; they were sensitive to ciprofloxacin and to erythromycin. Furthermore, in that same period four other isolates had different resistance profiles: one showed resistance to sulfonamides, trimethoprim-sulfamethoxazole, and nitrofurantoin; one to erythromycin, nitrofurantoin, and ampicillin; one to tetracycline, erythromycin, chloramphenicol, nitrofurantoin, ampicillin, and streptomycin; and one to nitrofurantoin and ampicillin. The 13 isolates recovered in the seventh outbreak (1997–1998) presented resistance only to sulfonamides and trimethoprim-sulfamethoxazole. During 1999 no cases of cholera were reported in Argentina (personal communication, Dr. Norma Binztein and Dr. Marcelo Galas, Antimicrobial Service, National Institute of Infectious Diseases "Dr. Carlos G. Malbran," ANLIS; unpublished data).

In Nicaragua, analysis of 120 isolates in 1993 indicated that all were sensitive to all the antibiotics tested, including ampicillin and trimethoprim-sulfamethoxazole. The first resistant strains, recovered from two patients who had come from Nicaragua, were isolated in 1995 in Costa Rica. The study of 143 isolates in Nicaragua in 1995 indicates that 11% were resistant to ampicillin, 27% to trimethoprim-sulfamethoxazole, and 29.3% to both antibiotics (*81*). In Colombia, the isolates recovered between 1991 and 1996 were all sensitive to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, norfloxacin, and ciprofloxacin. However, 21% of 176 isolates recovered in 1997 were resistant to chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole (*57*).

DISCUSSION

Antibiotics can be administered, depending on the type of syndrome manifested by the patient, for the treatment of enteric infections caused by any of the etiologic agents mentioned in this review. Usually, the infection is more serious in children under 5 years of age, the elderly, and individuals with immunodeficiency. In general, salmonellosis or shigellosis in transitory carriers, and mild enterocolitis do not require treatment. When the predominant symptom is diarrhea with or without vomiting and dehydration occurs, the therapy of choice is rehydration. However, in cases presenting bacteremia from *Salmonella*, enteric fever or among chronic carriers, administration of antibiotics is indispensable; ampicillin or trimethoprim-sulfamethoxazole are the drugs of choice. With regard to shigellosis, in cases in which the infection is not self-limiting, administration of ampicillin or tetracycline reduces the period of bacillary excretion and limits the course of the disease. Obviously, the treatment of choice will vary with the pattern of resistance of the microorganisms to the antibiotic being considered.

In the case of *Vibrio cholerae*, treatment of dehydration is usually sufficient. However, the period of bacillary dissemination and, therefore, of environmental pollution is decreased with antibiotic treatment. In addition, shortening the period of diarrhea shortens the period of dehydration in the patient.

The administration of antibiotics in any of the aforementioned circumstances has the potential for developing resistance through selection of the resistant bacterial population or the transfer of genetic material through plasmids or transposons, either between the same species or between different enterobacteria. Thus, for example, the increased frequency of *Salmonella* resistance to some antibiotics is related to their therapeutic use in humans (*5*). The information available shows that 40% of the cases were treated with antibiotics and that almost 9% had received antimicrobials a month prior to the onset of salmonellosis, which strengthens the presumption that part of the resistance is related to the use of antibiotics in the treatment of the human salmonellosis (*6*). The resistance in *Salmonella* was also linked with the introduction of antibiotics in livestock or poultry to promote growth or to prevent or treat infections, thus giving rise to the selection and persistence of resistant bacteria in animals destined for human consumption and, potentially, cases of human disease due to multiresistant bacteria (82).

Although this literature review included more than 80 articles published in the Region between 1961 and 2000 on the sensitivity of *Salmonella, Shigella,* and *V. cholerae* to different antibiotics, in several studies the number of isolates whose sensitivity was studied was small. In addition, information on the antimicrobial resistance of isolates of *Salmonella* and *Shigella* was obtained from only 10 of the 35 countries of the Region. With regard to the antimicrobial resistance of *V. cholerae,* information was obtained on seven of the 20 countries that reported having had outbreaks of cholera between 1991 and 1993 (*73*). The information reviewed made it possible to determine the resistance to certain antibiotics and, to a lesser extent, the trends in some countries and during specific periods. In only part of the published articles, mostly those originating in the United States, was it possible to relate the data on resistance to antibiotics with the temporal, geographic, and demographic context. Among the various countries there were differences as to the year in which the first studies on *Salmonella* and *Shigella* resistance to antimicrobials were conducted and published. Thus, for example, the information reviewed on the resistance of *Salmonella* in United States was published in 1961, based on data from studies conducted in 1948 (*1*), while for Colombia, the information was published in 1999 (*57*) but the studies had been done between 1991 and 1997. For Brazil, the information reviewed on the resistance of *Shigella* to antimicrobials was from studies conducted between 1950 and 1959 and published in 1970 (*70*).

In all the countries in which it was possible to obtain data for successive periods, resistance tended to increase over time. However, there were some differences among countries. Thus, for example, the frequency of resistance to chloramphenicol among isolates of *Salmonella* in the United States during the period 1948 to 1997 (0% to 10%) was lower than that detected in Mexico (30% to 50%) in the period 1978 to 1985. Susceptibility to that antibiotic was also marked (>90%) in Argentina, even between 1995 and 1998, and in Chile in 1996. In addition, Jamaica was the only country in which the resistance of *Shigella* to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole was equal to or lower than 8%. In the other countries, except for the first articles published in Brazil in 1950, in Chile in 1983, and in the United States from 1970 to 1986, resistance rates were higher.

The data presented in this review should be interpreted carefully, since the patterns of resistance in each country can be influenced by local variations in the circulation of the microorganisms. Furthermore, the differences found may be due simply to an artifact, since, with few exceptions, the rates of resistance presented in the articles reviewed are based on potentially biased samples of microorganisms, because the isolates analyzed might have been sent to the laboratory for diagnostic purposes or because of failure of the empirical therapy instituted, or because they are from

patients who came from different types of health care facilities. Furthermore, the differences among various periods in a single country or different countries may be the product of sample size, publication bias *(83)*, or the use of different laboratory techniques or cut-off points for the evaluation of sensitivity, or even of quality control.

Even though the information obtained from the published articles is too partial and fragmented to be used in assessing trends and determining resistance patterns by country and even less by smaller geographic areas, its importance from the clinical, epidemiological, and socioeconomic standpoints cannot be dismissed. The findings point out that, in most countries, over 40 years after the appearance of resistance to antimicrobials, monitoring or surveillance is not carried out, or has been restricted to a limited number of samples, which negatively affects its usefulness.

Inasmuch as antibiotic therapy will continue to be utilized, surveillance of resistance should be routine in all the countries of the Region, not only as a part of activities to promote the rational use of antibiotics, but because of its importance in relation to patient treatment and to the introduction of changes in medication in a timely fashion.

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ANTIBIOTIC RESISTANCE IN LATIN AMERICA: IMPORTANCE OF THE ARTEMIS AND RESIST NET PROGRAMS

Resist Net Collaborative Group

The Artemis project was designed to eliminate a series of intrinsic problems associated with international surveillance studies, such as the lack of standardized testing methodologies and the limited number of isolates and sites for the studies and for validation of the information. This study applied a standardized methodology for sensitivity testing, together with standardized reagents and materials. Of the isolates studied, 10% were validated in a central laboratory, where they were stored for future reference and research. The Artemis project, which began in January 1997 and ended in June 1998, was conceived as a global study for in vitro monitoring of the patterns of resistance to various antimicrobials in many countries. In Latin America, 10 countries and 30 hospitals participated. Each center collected 190 isolates of Gram-positive or Gram-negative aerobic bacteria or facultative anaerobes, and a total of 100 isolates of the following bacteria: Staphylococcus aureus, Streptococcus pyogenes, *and difficult-to-grow bacteria, such as* Haemophilus influenzae, Streptococcus pneumoniae, *and* Moraxella catarrhalis. *A total of 7,300 isolates of various types of infections were collected in the period in question. This is the information analyzed here.*

Artemis served as the inspiration for the Resist Net project, launched in April 1998, which is also described here. Resist Net is a multicenter international project involving 11 countries. As of May 1999 a total of 131 centers were participating in the project, whose design is more long-term. Some changes were introduced, such as the use of the disk diffusion method (Kirby-Bauer-Sherry). Like Artemis, Resist Net was created as a program for continuous monitoring of the sensitivity patterns of the most common pathogens in community and hospital environments.

As of 1 November 1998 a total of 26,999 isolates had been obtained from all the participating countries. By May 1999 a total of 84,044 isolates had already been collected. The information below is incomplete and represents only a general analysis of the microorganisms of current epidemiological and clinical importance most commonly isolated in the period April to September 1998.

INTRODUCTION

Resistance to antibiotics and antimicrobials has been a highly important topic of common interest in scientific fora over the past 10 years. Although this phenomenon was first recorded in the 1940s in the description of a strain of β-lactamase-producing *Staphylococcus aureus,*

the scientific community has witnessed the advent of new antibiotics of different classes. Strains of infectious agents that are initially susceptible to these drugs soon become resistant to them, a phenomenon that has been reported in every country in the world. There are currently strains of *Staphylococcus aureus* susceptible only to vancomycin. If such strains acquire genes that confer resistance to that antibiotic, for example from *Enterococcus*, we will soon find ourselves in a situation similar to that of the era prior to the discovery of antibiotics.

In some countries and regions such as the United States of America, Canada, and Europe, resistance to various classes of antibiotics or to specific antimicrobials is more or less well known. Nevertheless, specialists in this field have pointed out that much remains to be known and described. In other regions, such as Africa, Southeast Asia, and Latin America, little is known about antimicrobial resistance and there is no real understanding of the magnitude of the resistance of the vast majority of bacteria, whether community- or hospital-acquired. Attempts have been made to describe what is happening in the countries of Latin America. However, given the scarcity of data and regular publications with up-to-date information, it is difficult to draw any conclusions *(1–3)*.

National and international antimicrobial resistance surveillance studies have contributed to an understanding of this problem *(4–7)*. These surveillance systems have reported on the susceptibility patterns of a variety of pathogens and have investigated demographic and geographical differences in the in vitro efficacy of numerous antimicrobials. The data generated by these studies should be interpreted with caution, since local differences can be masked in the national information *(8)*. Similarly, information generated in one country cannot be applied to others, since conditions may be different. The Artemis project was designed to eliminate a series of intrinsic problems associated with international surveillance studies—for example, the lack of standardized test methods *(9)* and the limited number of specimens and sites *(10)* for studies and validation of local information. The Artemis study utilized a standardized method (E-test®) for conducting susceptibility tests, in addition to standardized reagents and materials. Ten percent of the specimens studied were confirmed in a central laboratory,[1] where they were conserved for future reference and research.

Drawing its inspiration from this project, the Resist Net project, described below, was launched in April 1998. Capitalizing on the experience of Artemis made it possible to design a new, longer-term study. Some changes were introduced, like the use of a more practical and accessible methodology, such as disk diffusion (Kirby-Bauer-Sherry).

THE ARTEMIS PROJECT[2]

The Artemis project was conceived as a global study for the in vitro monitoring of patterns of resistance to different antimicrobials in many countries *(11)*. In Latin America, 10 countries and 30 hospitals participated.

The project was launched in January 1997 and concluded in June 1998. Participating countries included: Argentina (6 centers), Brazil (5 centers), Chile (1 center), Colombia (4 centers), Ecuador (1 center), Guatemala (1 center), Mexico (5 centers), Peru (1 center), Uruguay (1 center), and Venezuela (5 centers). Table 1 presents the number and percentage distribution of the isolates, by country.

Materials and Methods

All the laboratories participating in the project were requested to collect a minimum of 290 recently isolated clinical specimens of

[1] International Health Management Associates, Mount Pleasant, IL, USA.

[2] The bulk of the information cited here was published in *Revista Panamericana de Infectología* Volume 1 Number 2, pages 68–75, 1999.

TABLE 1. Number of clinical isolates from the respiratory tract in Latin America and percentage distribution per country between January 1997 and June 1998. Artemis Study

	Number of isolates (%)										
Pathogen	Argentina	Brazil	Chile	Colombia	Ecuador	Guatemala	Mexico	Peru	Uruguay	Venezuela	Total
Haemophilus influenzae	125 (18.6)	114 (17.0)	38 (5.7)	108 (16.1)	63 (9.4)	3 (0.5)	79 (11.7)	26 (3.9)	27 (4.0)	89 (13.2)	672
Klebsiella pneumoniae	116 (25.6)	70 (15.5)	17 (3.8)	55 (12.1)	19 (4.2)	8 (1.8)	68 (15.0)	–	9 (2.0)	91 (20.1)	453
Moraxella catarrhalis	99 (32.1)	45 (14.6)	10 (3.2)	17 (5.5)	12 (3.9)	–	28 (9.1)	31 (10.1)	32 (10.4)	34 (11.0)	308
Pseudomonas aeruginosa	138 (25.3)	96 (17.6)	19 (3.5)	58 (10.6)	20 (3.7)	24 (4.4)	60 (11)	10 (1.8)	21 (3.8)	100 (18.3)	546
Staphylococcus aureus, methicillin-sensitive	135 (17.2)	125 (16.0)	25 (3.2)	133 (17.0)	21 (2.7)	30 (3.8)	104 (13.3)	25 (3.2)	19 (2.4)	166 (21.2)	783
Staphylococcus aureus, methicillin-resistant	23 (27.3)	26 (30.9)	–	9 (10.7)	5 (5.9)	–	8 (9.5)	–	3 (3.6)	10 (11.9)	84
Streptococcus pneumoniae	210 (24.4)	123 (14.3)	40 (4.6)	119 (13.8)	20 (2.3)	4 (0.5)	131 (15.2)	23 (2.7)	38 (4.4)	153 (17.8)	861

community or hospital origin. Duplicate specimens were not accepted. Each isolate was identified down to its species and was subjected to antimicrobial susceptibility tests at the collection center. Also recorded were demographic data, including the size of the hospital, the patient's location, and the source of the infection. Every center collected 190 isolates of facultative Gram-positive or Gram-negative aerobic or anaerobic bacteria and a total of 100 isolates of the following: *Staphylococcus aureus*, *Streptococcus pyogenes*, and fastidious species, such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*. The antimicrobial resistance of all isolates was evaluated with the E-test® (AB BIODISK, Solna, Switzerland), following the manufacturer's recommendations. A panel of antimicrobials was used that included: trovafloxacin, ciprofloxacin, amoxicillin-clavulanate, ampicillin-sulbactam, cefoperazone-sulbactam, cefaclor, ceftazidime, ceftriaxone, and cefuroxime. In addition, all isolates of *S. aureus* were tested for resistance to methicillin; all *S. pneumoniae* isolates for resistance to penicillin; and all *H. influenzae* and *M. catarrhalis* isolates, for production of β-lactamases. A central laboratory (International Health Management Associates, Mount Pleasant, Illinois, USA) was in charge of providing all E-test® strips and the culture media. The isolates were sent to this laboratory and frozen for future research. All results are recorded in a centralized project database.

Validation of the results took place at the central laboratory, where 10% of the isolates were reidentified and the minimum inhibitory concentrations (MIC) evaluated by the broth microdilution reference method, following the procedures recommended by the U.S. National Committee for Clinical Laboratory Standards (NCCLS) *(12)*. When discrepancies were higher than 10%, laboratory tests were repeated. Each local laboratory was also asked to conduct quality control tests at intervals of at least one week, using the standard ATCC control strains for *S. pneumoniae* # 49619, *H. influenzae* # 49247, *Escherichia coli* #25922, *S. aureus* #29213, *Pseudomonas aeruginosa* #27853, and *Enterococcus faecalis* #29212. The cutoff point for determining the susceptibility of pathogens to antimicrobials was the one indicated in the guidelines approved by the NCCLS *(12)* and the U.S. Food and Drug Administration (FDA).

Results

A total of 7,300 diverse specimens were collected from infections in the period in question. The initial analysis of this information was conducted exclusively with the isolates from infections of the respiratory tract—3,707 in all, both community- and hospital-acquired. This information is shown here. There was a clear predominance of specimens from community-acquired infections.

The distribution of the isolates, which appears in detail in Table 1, was as follows: 861 isolates of *S. pneumoniae* (23%), 867 of *S. aureus* (23%), 672 of *H. influenzae* (18%), 546 of *P. aeruginosa* (15%), 453 of *Klebsiella pneumoniae* (12%), and 308 of *M. catarrhalis* (8%). The majority of *H. influenzae* and *M. catarrhalis* isolates were sensitive to tested antibiotics (>97%). Just under 3% of these isolates were resistant to trovafloxacin, ciprofloxacin, ampicillin-sulbactam, amoxicillin-sulbactam, cefoperazone-sulbactam, ceftazidime, and ceftriaxone.[3] Only 9% of *H. influenzae* isolates were resistant to cefaclor, and 10% of *M. catarrhalis* isolates were resistant to cefuroxime.

Of the 861 isolates of *Streptococcus pneumoniae*, only 643 were tested for sensitivity to penicillin. Of these, 524 (81.5%) were sensitive and 119 (18.5%), resistant. Of the resistant strains, 66 had intermediate resistance (55.4%), and 53 (44.6%) were highly resistant. Of the total *S. pneumoniae* isolates tested for resistance to penicillin, 66 (10.2%) had intermediate sensitivity to penicillin and 53 (8.2%) were highly resistant (Table 2).

[3] This same phenomenon has been observed with third-generation cephalosporins (cefotaxime and ceftriaxone) by various investigators in the Resist Net study (see further on). This information was discussed within this group at their last meeting (May 1999) and must be confirmed.

TABLE 2. Comparative in vitro activity against penicillin-sensitive and penicillin-resistant strains of *Streptococcus pneumoniae*. Artemis Study

	Sensitive (MIC ≤ 0.06 mg/L) *n*=524 (81.5%)		Intermediate Resistance (MIC= 1 mg/L) *n*=66 (10.3%)		High Resistance (MIC ≥ 2 mg/L) *n*=53 (8.2%)	
Antimicrobial	MIC_{90}	Susceptibility (%)	MIC_{90}	Susceptibility (%)	MIC_{90}	Susceptibility (%)
Trovafloxacin	0.25	99.6	0.5	100	0.38	100
Ciprofloxacin	2	N/D	2	N/D	2	N/D
Cefaclor	1	N/D	24	N/D	>256	N/D
Ceftazidime	1.5	N/D	16	N/D	64	N/D
Ceftriaxone	0.094	97.7	1	87.7	3	24.5
Cefuroxime	0.25	95.6	2	68.2	12	11.3
Amoxicillin-clavulanate	0.047	97.5	1	84.4	2	32.1
Ampicillin-sulbactam	0.094	N/D	1.5	N/D	8	N/D
Cefoperazone-sulbactam	0.38	N/D	2	N/D	4	N/D

N/D = Not determined.

More than 99% of the isolates of *S. pneumoniae* were susceptible to trovafloxacin, regardless of their sensitivity to penicillin (MIC_{90} 0.25 mg/L). Ciprofloxacin was the least active drug against *S. pneumoniae*, with an MIC_{90} of 3.0 mg/L. Second-generation (cefaclor and cefuroxime) and third-generation cephalosporins (ceftazidime, ceftriaxone, and cefoperazone-sulbactam) were powerful against the majority of *S. pneumoniae* isolates. The MIC_{90} were 32, 4, 24, 1, and 2, respectively (Table 3). The prevalence of penicillin-resistant *S. pneumoniae* was 27% in Argentina, 21% in Mexico, 15% in Colombia, 11% in Venezuela, and 3% in Brazil. Given the limited number of specimens collected in the other countries, it is not possible to draw conclusions about the prevalence of penicillin-resistant *S. pneumoniae* (Table 4).

The prevalence of β-lactamase-positive *Haemophilus influenzae* throughout the study was 16%, but varied from country to country: it was 22% or more in Argentina, Mexico, and Uruguay; between 15% and 19% in Brazil, Chile, and Venezuela; and less than 10% in Colombia, Ecuador, and Peru (see Table 4). Overall frequency of β-lactamase-positive *M. catarrhalis* was 76%; the lowest figures were for Peru and Venezuela, with 9% and 15%, respectively. The rest of the countries had frequencies that ranged from 79% to 100%.

In total, 867 isolates of *Staphylococcus aureus* were collected in the countries participating in the study. Of these, 84 (9.7%) were methicillin-resistant. All the antimicrobial agents tested exhibited a comparable degree of activity (>90%) against methicillin-resistant *Staphylococcus aureus* strains (see Table 3), with the exception of ceftazidime and ceftriaxone, which yielded sensitivities of 24% and 76%, respectively.

With regard to *Klebsiella pneumoniae*, 407 (90%) of the 453 isolates were sensitive to the two quinolones tested and 84% to cefoperazone-sulbactam. Some 40% to 60% of the strains exhibited sensitivity to the rest of the antibiotics (see Table 3). The sensitivity of *Pseudomonas aeruginosa* to antibiotics with activity against *Pseudomonas* (ciprofloxacin, ceftazidime, cefoperazone-sulbactam, and trovafloxacin) was similar, ranging from 63% to 68%. Resistance to ciprofloxacin, ceftazidime, cefoperazone-sulbactam, and trovafloxacin varied from country to country, ranging from 66.7% to 48.6% in Mexico and Argentina, 70.7% to 59.6% in Colombia, and 82.3% to 70.8% in Brazil and Venezuela (Table 5).

RESIST NET PROGRAM

Like Artemis, Resist Net was created as a program for continuous surveillance of sensitivity patterns among the most common pathogens in community and hospital environments. It was also a response to the need for an extensive, highly reliable database. The

TABLE 3. In vitro susceptibility of respiratory pathogens in Latin America, by infectious agent. Artemis Study

Antimicrobial	*Haemophilus influenzae* (*n*=672)	*Klebsiella pneumoniae* (*n*=453)	*Moraxella catarrhalis* (*n*=308)	*Pseudomonas aeruginosa* (*n*=546)	*Staphylococcus aureus* methicillin-sensitive (*n*=783)	*Staphylococcus aureus* methicillin-resistant (*n*=84)	*Streptococcus pneumoniae* (*n*=861)
Trovafloxacin							
Susceptibility (%)	99.9	91.8	99.3	62.5	99.2	90.5	99.5
MIC_{90}	0.064	1.5	0.125	32	0.064	2	0.38
Ciprofloxacin							
Susceptibility (%)	98.1	90.9	96.7	65.7	95.2	41.5	N/D*
MIC_{90}	0.047	1.0	0.25	32	0.5	32	3
Cefaclor							
Susceptibility (%)	90.9	50.9	99.0	0.8	94.6	26.8	N/D*
MIC_{90}	8	256	2	256	4	256	32
Ceftazidime							
Susceptibility (%)	97.2	62.1	97	66.2	23.6	1.2	N/D*
MIC_{90}	0.75	256	2	256	32	256	24
Ceftriaxone							
Susceptibility (%)	99.6	61.5	99	3.1	76.3	14.6	87.5
MIC_{90}	0.032	32	1	32	32	32	1
Cefuroxime							
Susceptibility (%)	97.8	46.4	90.1	1	95.2	28.6	78.9
MIC_{90}	2	256	4	256	3	256	4
Amoxicillin-clavulanate							
Susceptibility (%)	99.1	46.3	100	2.1	95.5	32.9	86.8
MIC_{90}	1.5	256	0.75	256	3	64	1
Ampicillin-sulbactam							
Susceptibility (%)	97	41.4	100	3.6	96.4	41.7	80.8
MIC_{90}	1.5	256	0.75	256	4	48	1.5
Cefoperazone-sulbactam							
Susceptibility (%)	N/D*	83.8†	100	67.6†	97.9†	43.4†	N/D*
MIC_{90}	0.5	32	1	128	4	256	2

* NCCLS has not determined cutoff points.
†Adapted on the basis of cutoff points for cefoperazone, according to the NCCLS.

TABLE 4. Percentage distribution of penicillin-resistant isolates of *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus*, β-lactamase-producing *Haemophilus influenzae* and β-lactamase-producing *Moraxella catarrhalis*, by country. Artemis Study

	S. pneumoniae			*S. aureus*			*H. influenzae*			*M. catarrhalis*		
	Resistance to penicillin			Resistance to methicillin			β-lactamase			β-lactamase		
Country	Total	No.	%	Total	No.	%	Total	No.	%	Total	No.	%
Argentina	165	45	27	158	23	15	123	29	24	92	86	93
Brazil	98	3	3	151	26	17	93	18	19	39	39	85
Chile	33	14	42	25	–	–	38	6	16	10	10	100
Colombia	86	13	15	142	9	6	102	6	6	17	17	94
Ecuador	16	2	13	26	5	19	62	4	6	12	12	83
Guatemala	4	21	–	30	–	–	3	–	–	–	–	–
Mexico	100	–	21	112	8	7	72	16	22	24	24	79
Peru	6	1	17	25	–	–	13	1	8	22	22	9
Uruguay	34	9	26	22	3	13	27	7	26	32	32	97
Venezuela	103	11	11	176	10	6	72	11	15	27	27	15
Total	645	119	18	867	84	10	605	98	16	275	275	76

– = zero.

TABLE 5. Pattern of *Pseudomonas aeruginosa* susceptibility in Latin America (MIC mg/L and percentage of susceptibility) to antimicrobial, by country. Artemis Study

Antimicrobial	Argentina (*n*=138)	Brazil (*n*=96)	Chile (*n*=19)	Colombia (*n*=58)	Ecuador (*n*=20)	Guatemala (*n*=24)	Mexico (*n*=60)	Peru (*n*=10)	Uruguay (*n*=21)	Venezuela (*n*=100)
Trovafloxacin										
Susceptibility (%)	48.6	71.9	52.6	63.8	60	72	66.7	40	57.1	82
MIC_{90}	32	32	32	32	8	6	32	32	32	32
Ciprofloxacin										
Susceptibility (%)	49.3	70.8	42.1	65.5	85	91.3	65	20	65	82
MIC_{90}	32	32	32	32	2	1	32	32	32	32
Ceftazidime										
Susceptibility (%)	64	72.9	73.7	70.7	40	66.7	53.3	60	52.4	76
MIC_{90}	256	96	256	64	256	64	256	48	256	96
Cefoperazone-sulbactam										
Susceptibility (%)	59.1	82.3	89.5	59.6	40	75	59.3	60	52.4	78
MIC_{90}	96	64	32	128	256	48	256	256	128	64

initial proposal was to include as many Latin American countries and centers as possible and, in the course of one year, to collect from 60,000 to 80,000 isolates. These were tested for susceptibility by the disk diffusion method. All the centers used this method, according to the standards set by the NCCLS *(13)*. Specimen collection began in April 1998, and the first phase concluded in April 1999.

External quality control for the study was the responsibility of the microbiological research laboratory at Brigham and Women's Hospital in Boston, Massachussets (Dr. Thomas O'Brien coordinated this part of the study). Test strains were sent three times a year to each country coordinator for distribution to the centers, which identified the genus and species; the strains were tested for sensitivity to certain antibiotics and their inhibition zones measured. Reports were sent to the country coordinator, who, in turn, informed the external quality control coordinator. The country coordinator or the person whom he or she designated conducted periodic audits to verify internal quality control. The Pan American Sanitary Bureau, Regional Office for the Americas of the World Health Organization, collaborated in these audits.

In order to conduct the study, a manual of procedures and quality control was prepared based on the NCCLS standards for sensitivity testing; the manual also includes the necessary steps to ensure adequate internal quality control. The manual was reproduced and distributed to all participating centers. Among the many topics addressed in the manual is the medium selection, control strains, storage of disks, preparation and standardization of the inoculum, length and conditions of the incubation, and reading and interpretation of the results. The manual was prepared following the guidelines published by the NCCLS, the American College of Pathologists and the American Society for Microbiology.

The following countries participated in the Resist Net project; the number of centers in each country appears in parenthesis: Argentina (31), Brazil (10), Chile (5), Colombia (6), Costa Rica (1), Ecuador (3), Guatemala (1), Mexico (13), Peru (3), Uruguay (7), and Venezuela (7). A total of 87 centers had participated by November 1998. In May 1999, there was a total of 131 centers participating in the project. More centers will be added in the second phase of the project.

Method

Organization: The program has a general coordinator and is sponsored by Pfizer Labo-

ratories. Each country has a group of researchers in various centers, as well as a country coordinator, who is in charge of determining which laboratories meet the requirements for participation in the program; selecting bacteria not specified in the protocol that should be tested with different antimicrobials; selecting antimicrobials not listed as compulsory in the protocol; preparing local reports; verifying internal quality control at the participating laboratories; holding meetings with other local researchers; analyzing the information generated; cleaning up databases, and other functions. Participating laboratories had to meet quality standards and keep up-to-date with sensitivity testing using the disk diffusion method. They also had to be able to receive bacterial specimens of clinical isolates obtained within communities or hospitals. These laboratories, moreover, acted as representatives of their respective region or country. The standards issued by the American Society for Microbiology, the American College of Pathologists, and the NCCLS were accepted as international standards for laboratory procedures.

The WHONET program was utilized to collect the data generated during the project. This software is free and has already been tested and validated *(14, 15)*. Pfizer distributed the software to all country coordinators and participating centers. WHONET is used to record the following information: patient identification, age, and sex; data on the specimen; service from which the isolate was recovered; whether it was from an ambulatory or hospitalized patient; type of microorganism involved (genus and species); and cutoff points and the diameter of the inhibition zone, in millimeters, for each antibiotic tested.

The participating laboratories agreed to follow the method and guidelines proposed by the NCCLS for sensitivity tests using disk diffusion (Kirby-Bauer-Sherry) as published in 1997 and any subsequent updates *(13)*. The measurements were always reported in millimeters and the diameter of the inhibition zones were measured by ruler, manual or semiautomatic calipers, or BIOMIC reading. In addition, there was a continuous update of the aforementioned method. Sensitivity readings classified as sensitive, intermediate, or resistant were not acceptable.

The control strains utilized were: *E. coli* ATCC 25922; *P. aeruginosa* ATCC 27853; *S. aureus* ATCC 25923; *E. faecalis* ATCC 29212; *E. coli* ATCC 35218; *N. gonorrhoeae* ATCC 49226; *H. influenzae* ATCC 49247; *H. influenzae* ATCC 49766, and *S. pneumoniae* ATCC 49619. β-lactamase-producing *E. coli* ATCC 35218 was recommended only for quality control of disks that contained the combination "β-lactam – β-lactamase inhibitor", which include clavulanic acid, sulbactam, and tazobactam.

All bacteria isolated in the laboratory were identified down to the genus and species. All participating centers conducted sensitivity tests of the following bacteria isolated in the community: *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes,* other β-hemolytic streptococci, *Staphylococcus aureus, Salmonella* spp., *Shigella* spp, diarrheogenic *Escherichia coli,* and other bacterial enteropathogens such as *Yersinia enterocolitica,* and *Vibrio cholerae.* Other types of pathogenic bacteria, such as *Neisseria gonorrhoeae* and *N. meningitidis,* were also included. As in the Artemis project, collection of duplicate isolates was not permitted. Sequential, but unrepeated, isolates from hemocultures and sterile fluids were allowed.

Isolates of enterobacteria, *Staphylococcus* spp., *Pseudomonas* spp., and other nonfermenting bacteria (e.g., *Acinetobacter* and *Stenotrophomonas)* were also recovered from the different services of participating hospitals. For *Pseudomonas* and other nonfermenting bacteria, specifically, sensitivity tests were done using the microdilution method.

The most common sites of specimen recovery were: respiratory tract, abdomen, genitourinary tract, and surgical and nonsurgical wounds. Every bacterium isolated from abscesses or from fluids and tissues considered sterile (blood, cerebrospinal fluid, pleural

fluid) was identified and tested for sensitivity. Each participating center had to obtain a minimum of 75 specimens per month.

Each country analyzed the epidemiological and resistance patterns of antimicrobials selected for routine testing. It was determined that each bacterium isolated would be tested with at least seven obligatory antimicrobials, and that the agents proposed in the NCCLS standards *(13)* would be included among them. Trovafloxacin was included in various panels as a courtesy to Pfizer. Table 6 lists the bacteria and compulsory antibiotics. Another five antibiotics could be tested with each bacterium, and the decision on which antimicrobials to test rested with each country coordinator, provided that there was a rational basis for their selection. A maximum of 12 antimicrobials were tested for each strain.

During the study, other tests, such as the nitrocefin test, were conducted to detect β-lactamases in *H. influenzae, M. catarrhalis, N. gonorrhoeae, S. aureus,* and nonurinary *Enterococcus* spp. In addition, tests were done to detect broad-spectrum β-lactamases using aztreonam, ceftazidime, or cefotaxime, as indicated by the NCCLS.

As of 1 November 1998 a total of 26,999 isolates had been collected in all the participating countries. Part of this information was presented during the Pan American Conference on Antimicrobial Resistance in the Americas, held in Caraballeda, Venezuela from 2 to 4 November 1998. By May 1999, a total of 84,044 isolates had already been collected. The information below is the first to be released. It is not complete and represents only a general analysis of the microorganisms of current epidemiological and clinical importance most commonly isolated from April to September 1998. The final analysis of the full database of all the microorganisms isolated, by country and as a whole, will be published at a later date. Most of the information analyzed in this paper was presented at the 37th annual meeting of the Infectious Disease Society of America, held in Philadelphia, Pennsylvania, from 18 to 22 November.

SENSITIVITY OF *ESCHERICHIA COLI, KLEBSIELLA PNEUMONIAE, SALMONELLA* SPP. AND *SHIGELLA* SPP. TO VARIOUS ANTIMICROBIALS

The sensitivity of 10,687 isolates of *E. coli,* 2,347 isolates of *K. pneumoniae,* 403 isolates of *Salmonella* spp., and 819 isolates of *Shigella* spp. taken from different clinical specimens was evaluated. Studies were conducted on the sensitivity patterns of these isolates with respect to the following antimicrobials:

- *Salmonella* spp. and *Shigella* spp.: ampicillin, ceftriaxone, ciprofloxacin, chloramphenicol, trimethoprim-sulfamethoxazole, and trovafloxacin.
- *Escherichia coli* and *Klebsiella pneumoniae*: ampicillin, ampicillin-sulbactam, cefalotin, ceftriaxone, ciprofloxacin, gentamicin, imipenem, trimethoprim-sulfamethoxazole, and trovafloxacin.

For *E. coli,* the antimicrobials with the greatest activity were imipenem, gentamicin, trovafloxacin, and ceftriaxone, which exhibited resistance rates of 0%, 8.9%, 10.5%, and 2.9%, respectively. For *K. pneumoniae,* the drugs with the greatest activity were imipenem and trovafloxacin (0.3% and 6.7% resistance). For *Salmonella* spp. and *Shigella* spp. the most active drugs were ciprofloxacin and trovafloxacin (resistance rates ranging from 0 to 0.9%). All isolates of *Shigella* spp. were sensitive to ceftriaxone. Only in Argentina were ceftriaxone-resistant isolates of *Salmonella* spp. detected; these were isolated during an outbreak of salmonellosis.

SENSITIVITY OF *STAPHYLOCOCCUS AUREUS,* COAGULASE-NEGATIVE *STAPHYLOCOCCUS,* AND *ENTEROCOCCUS* SPP. TO VARIOUS ANTIMICROBIALS

As mentioned earlier, 11 countries participated in the network. For analytical purposes, the information on *Staphylococcus aureus,*

TABLE 6. Compulsory antibiotics to be tested in the Resist Net Study, by type of bacterium

Bacterium	Compulsory antibiotics						
Haemophilus spp. *	Ampicillin	Tmp/Smx †	Ceftriaxone	Trovafloxacin	Azithromycin	Chloramphenicol	Cefaclor
Streptococcus pneumoniae	Oxacillin	Erythromycin	Vancomycin	Tetracycline	Trovafloxacin	Tmp/Smx	Chloramphenicol
Enterococcus spp.	Ampicillin	Vancomycin	Gentamicin‡	Streptomycin‡	Trovafloxacin	Ampicillin-sulbactam§	Nitrofurantoin
Staphylococcus spp.¶	Oxacillin	Penicillin	Vancomycin	Clindamycin	Azithromycin	Tmp-Smx	Trovafloxacin
Streptococcus spp., except for *S. pneumoniae*	Ampicillin	Erythromycin	Vancomycin	Chloramphenicol	Clindamycin	Trovafloxacin	Ofloxacin
Neisseria gonorrhoeae	Penicillin	Ciprofloxacin	Trovafloxacin	Ceftriaxone	Spectinomycin	Azithromycin ‖	Tetracycline
Enterobacteria	Ampicillin	Cefalotin	Gentamicin	Trovafloxacin	Tmp-Smx	Ceftriaxone	Ampicillin-sulbactam
Salmonella spp. and *Shigella* spp.	Ampicillin	Chloramphenicol	Tmp/Smx	Ciprofloxacin	Ceftriaxone	Trovafloxacin	Aztreonam
Pseudomonas aeruginosa	Piperacillin	Gentamicin	Ceftazidime	Cefoperazone	Imipenem	Ciprofloxacin	Ampicillin-sulbactam
Acinetobacter spp.	Gentamicin	Ceftazidime	Aztreonam	Imipenem	Amikacin	Ciprofloxacin	

* Susceptibility tests of *Haemophilus spp.* are conducted in HTM medium.

† Tmp-Smx = trimethoprim-sulfamethoxazole.

‡ Gentamicin: High level (120 µg); Streptomycin (300 µg).

§ Optional, based on the researcher's criterion.

¶ It was felt that, in the case of *Staphylococcus*, gentamicin should be added to seven antibiotics mentioned.

‖ Optional. There was no cutoff value at the start of the study.

coagulase-negative *Staphylococcus*, and *Enterococcus* spp. was broken down into three groups of countries:

Group 1: Argentina, Chile, Peru, and Uruguay; Group 2: Brazil; and Group 3: Colombia, Costa Rica, Ecuador, Guatemala, Mexico, and Venezuela.

Isolates of methicillin-resistant *Staphylococcus aureus* were more common in Groups 1 and 2 and were more frequently associated with resistance to gentamicin (Group 1, 91%; Group 2, 99%; and Group 3, 41%). The isolates of coagulase-negative *Staphylococcus* were highly resistant to virtually all antimicrobials analyzed. Among *Enterococcus* isolates (n=1,832), high resistance to streptomycin was more frequent than to gentamicin, especially in Chile and Mexico. The percentages of *Enterococcus* resistance to trovafloxacin and ciprofloxacin were 27% and 63%, 12% and 51%, and 24% and 74% for Groups 1, 2, and 3, respectively. Trovafloxacin was more active than ciprofloxacin against all the species analyzed, exhibiting marked activity against methicillin-resistant *S. aureus*. Vancomycin-resistant isolates of *Staphylococcus aureus* were not detected. In this phase of the study, only a few vancomycin-resistant isolates of *Enterococcus* were detected (Group 1, 2%; Group 2, 2%, and Group 3, 11%) (Table 7).

SENSITIVITY OF *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* TO VARIOUS ANTIMICROBIALS

A total of 6,891 isolates of *Pseudomonas aeruginosa* and 1,322 isolates of *Acinetobacter baumannii* were analyzed to determine their patterns of resistance to the various antimicrobials used in their management. Only one isolate per patient was analyzed. Studies were conducted on the sensitivity patterns of these isolates with respect to the following antimicrobials:

- *Pseudomonas aeruginosa*: amikacin, aztreonam, ceftazidime, ciprofloxacin, gentamicin, imipenem, and piperacillin.
- *Acinetobacter baumannii*: amikacin, ceftazidime, ciprofloxacin, gentamicin, and imipenem. This analysis did not include information on ampicillin-sulbactam.

The rate of resistance of *P. aeruginosa* was over 20% in Argentina, Brazil, Mexico, Guatemala, and Uruguay; the lowest resistance was found in Costa Rica. Ecuador exhibited the highest resistance to gentamicin (57%), with figures of over 35% for Brazil, Colombia, Guatemala, Peru, Argentina, and Mexico;

TABLE 7. Percentage of *Staphylococcus aureus*, coagulase-negative *Staphylococcus* and *Enterococcus* spp. resistance to various antimicrobials, by group of countries. Resist Net Study

			Percentage of resistance												
	Group*	Number	O	G	Z	D	X	A	S	GH	SH	N	C	T	V
SA	1	3,275	39	38	38	31	22	–	–	–	–	–	29	5	–
	2	383	37	39	40	37	38	–	–	–	–	–	–	2	–
	3	1,529	26	16	30	24	14	–	–	–	–	–	26	4	–
CNS	1	1,603	64	42	44	32	37	–	–	–	–	–	25	14	–
	2	121	50	21	26	5	33	–	–	–	–	–	–	10	–
	3	1,386	69	48	56	44	50	–	–	–	–	–	36	23	–
E	1	827	–	–	–	–	–	8	7	24	31	8	63	27	2
	2	102	–	–	–	–	–	1	1	17	29	–	51	12	2
	3	903	–	–	–	–	–	12	11	17	43	6	74	24	11

– = zero.

* Country groupings: Group 1: Argentina, Chile, Peru and Uruguay; Group 2: Brazil; Group 3: Colombia, Costa Rica, Ecuador, Guatemala, Mexico, and Venezuela.

SA = *Staphylococcus aureus*; CNS = coagulase-negative *Staphylococcus*; E = *Enterococcus*.

O = oxacillin; G = gentamicin; Z = azithromycin; D = clindamycin; X = trimethoprim-sulfamethoxazole; A = ampicillin; S = ampicillin-sulbactam; GH = gentamicin 120 µg; SH = streptomycin 300 µg; N = nitrofurantoin; C = ciprofloxacin; T = trovafloxacin; V = vancomycin.

again, the lowest rate was found in Costa Rica. Resistance to ceftazidime was highest in Venezuela (29%), Mexico (25%), and Brazil (21%) and lowest in Costa Rica (4%) and Peru (5%). For piperacillin, the following values were obtained: Ecuador, 46%; Venezuela, 36%; Brazil, 34%; Mexico, 36%, Costa Rica, 11%. Resistance to aztreonam was high in Colombia (20%), Ecuador (18%), and Mexico (9%) and low in Peru and Uruguay, where it was 6%.

Imipenem resistance was highest in Brazil (19%), Ecuador (15%), Argentina (14%), Mexico, and Uruguay (12% each) and lowest in Colombia, Guatemala, and Peru. With regard to ciprofloxacin, resistance rates of 52%, 39%, 35%, and 33% were found in Ecuador, Peru, Colombia, and Brazil, respectively, with lower figures for Costa Rica (9%).

A. baumannii exhibited a high rate of resistance to amikacin and gentamicin: Argentina, 65%, and 72%; Venezuela, 56% for both drugs; Mexico, 55% and 42%, and Brazil, 73% and 52%. Uruguay had the lowest rate: 21% for both antimicrobials. Resistance to ceftazidime was high in Argentina and Brazil (63% each), Mexico (37%), and Uruguay (48%), with the lowest rate in Venezuela (9%). Resistance to aztreonam was very high throughout the Region: Uruguay, 92%; Argentina, 78%; Brazil, 72%, and Mexico, 68%. Resistance to imipenem was high in Mexico (19%) and Venezuela (33%), but insignificant in Brazil (2%). High rates of resistance to ciprofloxacin were found in Argentina (78%), Brazil (76%), and Uruguay (62%), while in Mexico the rate of resistance was 26%.

SENSITIVITY OF RESPIRATORY ISOLATES OF *STREPTOCOCCUS PNEUMONIAE, HAEMOPHILUS INFLUENZAE,* AND *MORAXELLA CATARRHALIS* TO VARIOUS ANTIMICROBIALS

During the period April to September 1998, a total of 1,085 isolates of *Streptococcus pneumoniae*, 541 of *Haemophilus influenzae* (26% type b), and 304 of *Moraxella catarrhalis* were analyzed. Penicillin resistance in *Streptococcus pneumoniae* was determined by disk diffusion with a 1 μg oxacillin disk.

Overall *Streptococcus pneumoniae* resistance to penicillin was 38.2%. Resistance to erythromycin, trimethoprim-sulfamethoxazole (tmp-smx), chloramphenicol, tetracycline, and trovafloxacin was 12.1%, 45.2%, 6.7%, 19.4% and 0.4%, respectively.

Of all the isolates of *Haemophilus influenzae*, 20.4% were resistant to ampicillin. Resistance to chloramphenicol, tmp-smx, and trovafloxacin was 8.5%, 22.4%, and 0.2%, respectively. Of all the isolates of *Haemophilus influenzae* type b, 13.5% produced a halo of inhibition of approximately ± 26 mm for the 30 μg ceftriaxone disk. Other tests to characterize this type of potential resistance were not conducted in this initial stage of the study.

Thirty-six percent of *Moraxella catarrhalis* isolates were sensitive to ampicillin. Trovafloxacin-resistant strains were not identified among these isolates.

REMARKS

Artemis is the first study in Latin America to collect a large number of isolates of respiratory origin in a brief period of time. However, as observed in Tables 1 and 4, in some countries the number of isolates of some bacteria were very low, making it impossible to draw any conclusions about local sensitivity patterns. Most collection sites did not obtain a significant number of isolates of *M. catarrhalis*, with the exception of Argentina, where almost 100 were collected. In contrast, at least five countries sent a significant number of *S. aureus* isolates. The level of resistance to methicillin detected was approximately 6% in Colombia, Mexico, and Venezuela, and 15% in Argentina and Brazil. In Chile, Guatemala, and Peru no isolates of methicillin-resistant *S. aureus* were obtained during this sampling.

Resist Net is a valuable epidemiological surveillance system for identifying resistance

patterns in Latin America. Research into potential new resistance mechanisms can emerge from the information generated by epidemiological surveillance programs such as the one described here.

This study confirms that bacterial resistance continues to present a significant problem in Latin America and underscores the need to maintain epidemiological surveillance programs without interruption.

One of the objectives of the Resist Net study is to collect a sufficient number of isolates of the principal pathogens in each center, country, or region to obtain a better understanding of the phenomenon of resistance in Latin America. The first phase of Resist Net should be completed by the end of 1999.

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ANTIMICROBIAL RESISTANCE AMONG PATHOGENS CAUSING NOSOCOMIAL AND COMMUNITY INFECTIONS IN LATIN AMERICA: A COMPREHENSIVE REVIEW OF 1997 STATISTICS

Helio S. Sader[1] and Ronald N. Jones[2]

The SENTRY Antimicrobial Surveillance Program began in January 1997 and was designed to monitor nosocomial and community-acquired infections via a worldwide surveillance network of sentinel laboratories. This report will present the results obtained with the 10 sites (six countries) that participated in the SENTRY Program in 1997. Rank order of occurrence and antimicrobial susceptibility of pathogenic species causing bloodstream infections, pneumonia, wound or skin and soft tissue infections, and urinary tract infections in hospitalized patients were determined by collecting consecutive isolates over a specified period of time. Selected species causing community-acquired respiratory infections were also evaluated. All isolates were tested by reference broth microdilution, and molecular studies were performed on selected isolates.

A total of 3,468 bacterial strains were obtained. The largest number of isolates were collected from patients with bloodstream infections (1,642 cases), followed by pneumonia (557 cases), wound or skin and soft tissue infections (470 cases), urinary tract infections (469 cases), and community-acquired respiratory tract infections (330 cases). Escherichia coli *was the most frequently isolated pathogen in general (19.8%).* Staphylococcus aureus *was the most common species isolated from bloodstream infections and wound or skin and soft tissue infections, and* Pseudomonas aeruginosa *was the most frequent species isolated from patients with nosocomial pneumonia (26.9%). The main resistance problems found in Latin American medical centers were: imipenem resistance among* P. aeruginosa *(72.4%–84.2% susceptibility) and* Acinetobacter *(88.5–92.0% susceptibility); extended-spectrum β-lactamases (ESBL) production among* Klebsiella pneumoniae *(35.0%–44.4%) and* E. coli *(4.9–30.3%); fluoroquinolone resistance among* E. coli *(as high as 40% of strain), resistance to third-generation cephalosporins among* Enterobacter; *and oxacillin resistance*

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among staphylococci. Vancomycin resistance rates remain low among enterococci in Latin America.

Molecular typing showed a great genomic variability among extended-spectrum β-lactamase-producing strains with some cases of clonal dissemination within and between medical centers. Only 47.7% of pneumococci *(197 isolates evaluated) were susceptible to penicillin and approximately 10% of the isolates showed high-level resistance to penicillin and resistance to third-generation cephalosporins. Trimethoprim-sulfamethoxazole showed low activity against pathogens causing community-acquired respiratory tract infection (50.6%–63.5% susceptibility) and some newer quinolones were active against 100% of these pathogens. The high rates of resistance observed in this study underscore the need for ongoing global surveillance and local corrective action.*

INTRODUCTION

Resistance among many nosocomial and community-acquired pathogens to standard antimicrobial agents has become a major problem throughout the world, with no evidence of potential deceleration. This has been associated with changing patterns of pathogen occurrence e.g., the emergence of Gram-positive organisms as the dominant species causing bloodstream infections (BSI) in patients with neutropenia (*1*). The most recent discussions of this negative event have focused on the misuse of antimicrobial agents in the outpatient setting and elsewhere (*2*). Actions necessary to limit the selection of resistance have been summarized in the comprehensive report by the American Society for Microbiology Task Force on Antimicrobial Resistance in 1995 (*1*). That report emphasized the need for: 1) surveillance networks to recognize emerging resistance and direct interventions; 2) education of health care professionals and the public as to effective prescribing habits or therapeutic expectations; and 3) basic research for developing new modes of therapy or infection prevention.

In general, resistance to commonly used antimicrobial agents in predominant pathogens is of concern and is a result of selective pressure due to frequent use of these agents (*3, 4*), regardless of the clinical setting. Pathogens of primary concern throughout most countries include oxacillin-resistant staphylococci, penicillin-resistant pneumococci, vancomycin-resistant enterococci, and the *Enterobacteriaceae* resistant to "third-generation" cephalosporins or extended-spectrum penicillins due to production of extended-spectrum β-lactamases (ESBL) or chromosomally-mediated inducible cephalosporinases (Bush group 1 or AmpC). The *Enterobacteriaceae* strains resistant to the fluoroquinolones and nonfermentative Gram-negative bacilli resistant to the carbapenems are also troublesome.

Resistance patterns among pathogens may vary from country to country; thus, global surveillance programs using standardized susceptibility testing methods are needed to provide reliable information about emerging resistance problems throughout the world and for individual countries. Information from surveillance programs can guide appropriate selection of empiric therapy as well as focus implementation of preventive measures. In Brazil, two recent multicenter studies provided antimicrobial susceptibility data on local pathogens (*5, 6*). Due to the change in resistance patterns that are occurring in Latin America and worldwide, ongoing surveillance of predominant pathogens and antimicrobial susceptibilities is needed in order to optimize patient care. The SENTRY Antimicrobial Surveillance Program, started in January 1997, was designed to monitor nosocomial and selected community-acquired infections via a worldwide surveillance network of sentinel hospitals distributed by geographic location and hospital size. The goal of this lon-

gitudinal surveillance program is to track commonly occurring pathogens and their antimicrobial resistance trends in Latin American countries, and internationally (United States, Canada, Europe, Asia, the Middle East, South Africa, and Australia) over a 3- to 5-year period. Antimicrobial susceptibilities of pathogens causing BSI and fungemia, pneumonia, wound or skin and soft tissue infections (WSSTI), and urinary tract infections (UTI) in hospitalized patients, as well as fastidious organisms (*Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*) causing community-acquired respiratory tract infections (CARTI) are routinely monitored. Some of the SENTRY data collected in 1997 from Latin American countries has been published or is in press (7–12). This report will focus on Latin America and will include the antimicrobial susceptibility of predominant pathogens causing blood stream infections, pneumonia in hospitalized patients, wound and soft tissue infections, and urinary tract infections, plus antimicrobial susceptibility of *S. pneumoniae, H. influenzae,* and *M. catarrhalis.* The results were analyzed according to the site of infection and bacterial species. In general, the results were similar among the various countries evaluated and the discrepancies found among countries will be cited and discussed.

MATERIALS AND METHODS

Ten Latin American laboratories participated in the study, 8 hospital-based and 2 independent laboratories serving several medical centers. Of the 10 laboratories, 7 serve tertiary hospitals; 2 serve secondary hospitals, and 1, several primary hospitals. The laboratories were distributed throughout eight cities in six countries: Sao Paulo, Rio de Janeiro and Florianopolis, Brazil; Buenos Aires and San Isidro, Argentina; Santiago (two centers), Chile; Montevideo, Uruguay; Medellin, Colombia; and Mexico City, Mexico.

Bacterial isolates were collected according to the site of infection:

- Blood stream infections—each participating center sent 20 consecutively collected isolates per month. Thus, each center contributed approximately 240 BSI isolates per year.
- Community acquired respiratory infections—each participating center contributed over a 6-month period (1 July through 31 December 1997) consecutive clinically significant outpatient isolates (one isolate per patient) of *S. pneumoniae* (n=40), *H. influenzae* (n=40), and *M. catarrhalis* (n=20) that were considered pathogens. Isolates were collected from both adults and children.
- For pneumonia in hospitalized patient—each participant center collected approximately 100 isolates in the second semester of 1997.
- For wound and soft tissue infections—each participating center contributed approximately 50 isolates collected between 1 April and 30 June 1997.
- For urinary tract infections—each participating center collected approximately 50 isolates from 1 February through 1 May 1997.

Data collected for each isolate from each infection site included species identification, local antimicrobial susceptibility profile, date of isolation, specimen type, and various risk factors (BSI only). Isolates were placed on specified transport media or agar slants and sent to the coordinating/monitoring laboratory at the University of Iowa College of Medicine (Iowa City, Iowa, USA).

Organism identification. All pathogens were identified at the participating center using routine methods for that laboratory and were confirmed at the coordinating laboratory using automated (Vitek and API, bioMerieux, St. Louis, MO, USA) or conventional methods. Isolates were stored at –70 °C at the coordinating laboratory.

Susceptibility testing. Antimicrobial susceptibility testing was performed at the coordinating laboratory using broth microdilution methods as described by the National Com-

mittee for Clinical Laboratory Standards (NCCLS) (*13*). Antimicrobial agents were obtained from respective manufacturers as laboratory-grade powder and included macrolides (erythromycin, azithromycin, clarithromycin), the streptogramin quinupristin-dalfopristin, glycopeptides (vancomycin, teicoplanin), fluoroquinolones (gatifloxacin, ciprofloxacin, ofloxacin, sparfloxacin, levofloxacin, trovafloxacin), aminoglycosides (amikacin, gentamicin, tobramycin), carbapenems (imipenem, meropenem), a monobactam (aztreonam), cephalosporins (cefepime, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefazolin, cefoxitin, cefaclor, cefixime), penicillins (ampicillin, penicillin, amoxicillin, oxacillin, ticarcillin, piperacillin), β-lactamase inhibitor combinations (amoxicillin-clavulanate, ticarcillin-clavulanate, piperacillin-tazobactam), and other drugs including clindamycin, chloramphenicol, tetracycline, rifampin, and trimethoprim-sulfamethoxazole. Quality control measures were utilized by testing *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853. Breakpoint interpretive criteria used were those established by the NCCLS (*14*).

Molecular methods. Selected isolates were genotyped by ribotyping using the RiboPrinter™ Microbial Characterization System (i.e. duPont de Nemours, Wilmington, Delaware, United States of America) according to the manufacturer's instructions (*15*). The patterns were electronically imaged, stored, and compared. Pattern comparison was based on both band position and signal intensity (*15*).

The strains with identical ribogroups were also molecular typed by pulsed-field gel electrophoresis (PFGE). The PFGE was performed using restriction endonuclease *SpeI*, as previously described (*16*). Analysis of PFGE patterns was performed by visual inspection of photographs of ethidium bromide-stained gels. All bands had to match exactly for isolates to be classified as "identical." Banding patterns differing by one or two bands were termed "similar" and were grouped under the same DNA type. Isolates with a difference of at least three bands were considered "different" DNA types.

The studies of the ESBL-producing *Klebsiella pneumoniae* and *E. coli* with isoelectric focusing (IEF) were performed utilizing crude β-lactamase extracts prepared by freeze-thaw lysis (*17*). IEF tests utilized a Multiphore II electrophoresis system in ampholine-polyacrylamide gels, pI 3.5–9.5 (Pharmacia Biotech Inc., Piscataway, NJ, USA). Gels were run for 90 minutes at a potential difference of 1,500 V, 30 mA and 30 W and were stained with 0.5 mM Cefinase 2 (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) (*18*). Preparations of TEM-1, TEM-4, SHV-1, SHV-3 and SHV-5 were included as standards in each gel (*19*).

RESULTS AND DISCUSSION

A total of 3,468 bacterial strains were obtained from 10 laboratories participating in SENTRY in Latin America in 1997, of which 330 were fastidious organisms from community-acquired respiratory infections, and the remainder were cultured from hospitalized patients. Based on the study design, the largest number of isolates were obtained from patients with BSI (1,642 cases), followed by pneumonia (557 cases), WSSTI (470 cases), and UTI (469 cases). The 15 most frequently occurring pathogens isolated from all nosocomial infection sites are listed in Table 1. The top seven pathogens accounted for more than 80% of all isolates. *E. coli* was the most frequently isolated pathogen in general (19.8% of the total isolates). However, *S. aureus* was the most common species isolated from BSI and WSSTI, and *P. aeruginosa* was the most frequent species isolated from patients hospitalized with pneumonia (26.9%). Gram-negative bacteria were responsible for approximately two-thirds of pathogens isolated (Table 1).

Bloodstream infections. The predominant bacterial pathogens causing bloodstream infections in Latin America were *S. aureus*

TABLE 1. Occurrence (%) of the 15 major pathogen isolates (3,138 strains) from 10 medical centers in Latin America. Each strain tested by reference antimicrobial susceptibility methods (1997)

	% occurrence in (no. tested):*				All sites
Organism in rank order†	BSI (1,642)	LRTI (557)	SSTI (470)	UTI (469)	No. (%)
1. *Escherichia coli*	17.1	5.9	13.6	52.5	620 (19.8)
2. *Staphylococcus aureus*	20.5	23.0	28.2	0.6	600 (19.1)
3. *Pseudomonas aeruginosa*	5.6	26.9	12.3	7.5	335 (10.7)
4. *Klebsiella* spp.	10.3	10.2	8.3	12.8	325 (10.4)
5. CoNS*	15.2	0.7	4.7	1.3	282 (9.0)
6. *Acinetobacter* spp.	5.3	11.7	5.5	3.2	193 (6.2)
7. *Enterobacter* spp.	5.8	7.0	7.9	4.3	192 (6.1)
8. *Enterococcus* spp.	2.9	2.0	7.9	4.3	116 (3.7)
9. *Serratia* spp.	2.2	3.6	2.1	2.1	76 (2.4)
10. *Proteus mirabilis*	1.0	0.5	2.8	4.9	55 (1.8)
11. *Salmonella* spp.	3.0	0.0	0.6	0.2	53 (1.7)
12. *Streptococcus pneumoniae*	1.9	1.4	0.0	0.0	40 (1.3)
13. *Citrobacter* spp.	0.6	0.9	2.1	2.6	37 (1.2)
14. Indole-positive *Proteus*	0.5	0.7	1.6	2.3	33 (1.1)
15. β-haemolytic streptococci	1.6	0.2	0.8	0.2	32 (1.0)
16. *Stenotrophomonas maltophilia*	0.9	2.3	0.8	0.0	32 (1.0)

*BSI = blood stream infections; LRTI = lower respiratory tract infections; SSTI = skin and soft tissue or wound infections; UTI urinary tract infection; and CoNS = coagulase-negative staphylococci. A total of 330 fastidious organisms from community-acquired respiratory tract and other infections are listed in Table 5.

†Top-ranked pathogens listed by all-site occurrence. The top 10 pathogens accounted for nearly 90% of all isolates.

(20.5%), *E. coli* (17.1%), coagulase-negative staphylococci (CoNS) (15.2%), *Klebsiella* spp. (10.3%), *Enterobacter* spp. (5.8%), *P. aeruginosa* (5.6%), and *Acinetobacter* spp. (5.3%). The rank order of occurrence differed only slightly from country to country, but greater differences were found between Latin America and other regions of the world (*10*). *S. aureus*, *E. coli* and CoNS were the three most frequent pathogens in most Latin American countries (except for Uruguay where *Klebsiella* spp. was more frequent than CoNS) as well as in the United States and Canada.

When comparing the data from Latin America to that of the United States and Canada we found that *Acinetobacter* spp., *Enterobacter* spp., and *Salmonella* spp. were more common in the former, while *Enterococcus* spp., and streptococci (*S. pneumoniae*, β-haemolytic and viridans group) were more frequently isolated from BSI in the latter two countries (*10*).

The most prevalent Gram-positive organisms causing BSI (Table 2) were *S. aureus* (336 isolates), CoNS (250 isolates), and *Enterococcus* spp. (48 isolates). The antimicrobial activity of 11 drugs against these pathogens is shown in Table 2. Among the *S. aureus* isolates, 29.2% were resistant to oxacillin. Oxacillin resistance was much higher among the CoNS (68.4%). For *S. aureus*, activity of the other β-lactam agents tested paralleled that of oxacillin (*13*, *14*). Trovafloxacin and gatifloxacin were more active than ciprofloxacin against *S. aureus* and CoNS, and gatifloxacin was markedly more active than other fluoroquinolones against CoNS. No resistance to vancomycin was observed among *S. aureus* or CoNS. Only one isolate of *Enterococcus* spp. resistant to vancomycin was detected. High-level resistance to gentamicin was documented in 41.7% of the enterococcal isolates.

The most prevalent Gram-negative pathogens causing BSI and their susceptibility to selected agents are listed in Table 3. Cefepime was the most active cephalosporin tested against the five predominant Gram-negative pathogens, although none of the cephalospor-

TABLE 2. Antimicrobial susceptibility of the three most frequent Gram-positive pathogens from bloodstream infections in Latin America. Sentry Antimicrobial Surveillance Program, 1997

	Pathogen (prevalence rank/number tested)		
	S. aureus (1/336)	CoNS (3/250)*	*Enterococcus* spp. (9/48)
Antimicrobial agent	% susc.†	% susc.†	% susc.†
Oxacillin	70.8	31.6	0.0
Penicillin	6.3	10.0	81.3
Clindamycin	74.4	59.2	0.0
Erythromycin	38.9	41.6	–
Ciprofloxacin	72.3	60.0	33.3
Gatifloxacin	82.1	91.6	54.2
Trovafloxacin	85.4	72.8	45.8
Gentamicin	69.9	47.6	58.3
Tetracycline	74.7	74.8	37.5
Trimethoprim-sulfamethoxazole (1:19)	78.2	49.6	–
Vancomycin	100.0	100.0	97.9

*CoNS = coagulase negative staphylococci (10 species).

†Categories of susceptibility interpreted by NCCLS [1998] criteria. For gatifloxacin, a fluoroquinolone under investigation, susceptible was defined as ≤2 μg/mL.

ins demonstrated significant activity against *Acinetobacter* spp. (0.0%–41.4% susceptible). Imipenem and tetracycline were the only agents that exhibited reasonable in vitro activity against *Acinetobacter* spp. (92.0% and 70.1% susceptible, respectively). However, there is no significant data in the literature regarding the clinical efficacy of the tetracyclines for the treatment of *Acinetobacter* infections. Against the *Enterobacteriaceae*, cefepime was the most active cephalosporin; however, activity of all the β-lactams (except imipenem) against *Klebsiella* spp. was reduced. This trend of lower β-lactam spectrum against *Klebsiella* spp. was also seen in most Latin American countries and was secondary to a high percentage of ESBL-producing strains *(7, 20)*.

Utilizing the screening concentrations recommended by the NCCLS *(14)* to predict isolates of *Klebsiella* spp. and *E. coli* suspected of harboring an ESBL (MIC ≥2 μg/mL to ceftazidime or aztreonam or ceftriaxone), 8.5%–8.9% of *E. coli* and 43.2–44.4% of *Klebsiella* spp. isolates met these criteria. When analyzing the data by country, the percentage of ESBL-producing strains causing BSI varied from 4.5% (Uruguay) to 12.0% (Chile and Mexico) among *E. coli*, and from 31.0% (Mexico) to 56.6% (Brazil) among *K. pneumoniae*. In a recent Brazilian study approximately 40% of *K. pneumoniae* isolates collected in a Sao Paulo University hospital (Hospital Sao Paulo, Federal University of Sao Paulo) were ESBL producers. The majority of isolates from that study corresponded to BSI (21). In another study, which evaluated isolates collected between December 1995 and March 1996 in Brazilian hospitals, only 87% of *Klebsiella* isolates were susceptible to cefoxitin, suggesting that resistance to β-lactams may also be due to mechanisms other than ESBL production (plasmid-mediated AmpC) (5). In a more recent surveillance study of over 30 Brazilian hospitals, we discovered that approximately 50% of *K. pneumoniae* and 15% of *E. coli* isolates were potential ESBL producers. The surveillance study included different types of hospitals, from small private clinics to large tertiary university medical centers (22).

Imipenem and the fluoroquinolones were the most active agents against *Klebsiella* spp., and cefepime and imipenem were the most active agents against *Enterobacter* spp. (96.4–98.2% susceptible). Resistance of *Enterobacteriaceae* to the fluoroquinolones appears to be rapidly increasing in Latin America; a susceptibility rate of 87% among *E. coli* to fluoroquinolones was significantly lower than that

TABLE 3. Antimicrobial susceptibility of the five most prevalent Gram-negative pathogens causing bloodstream infections in Latin America. SENTRY Antimicrobial Surveillance Program, 1997

	Pathogen (prevalence rank/number tested)				
	E. coli (2/281)	*Klebsiella* spp. (4/169)	*Enterobacter* spp. (5/96)	*P. aeruginosa* (6/92)	*Acinetobacter* spp. (7/87)
Antimicrobial class/agent	% susc.*	% susc.*	% susc.*	% susc.*	% susc.*
Cephalosporins					
Cefazolin	77.6	46.2	4.2	0.0	5.7
Cefuroxime	84.3	52.7	33.3	1.1	9.2
Ceftriaxone	93.2 (8.5)†	60.4 (43.2)†	76.0	7.6	17.2
Ceftazidime	95.0 (8.9)†	66.3 (44.4)†	72.6	71.7	39.1
Cefepime	97.2	79.9	94.8	72.8	41.4
Other β-lactams					
Ampicillin	40.9	24	2.1	0.0	11.5
Aztreonam	93.6 (8.5)†	61.5 (42.6)†	72.9 (32.3)	65.2	13.8
Imipenem	99.6	99.4	99.0	84.8	92.0
Piperacillin-tazobactam (4 μg/mL)	87.5	57.4	70.8	85.9	31.0
Aminoglycosides					
Amikacin	97.2	75.7	82.3	83.7	40.2
Gentamicin	90.7	59.8	75.0	71.7	40.2
Tobramycin	90.7	54.4	69.8	78.3	42.5
Fluoroquinolones					
Nalidixic Acid	83.3	74.6	79.2	3.3	35.6
Ciprofloxacin	86.9	84.0	91.7	73.9	35.6
Gatifloxacin	87.5	89.9	91.7	71.7	39.1
Levofloxacin	87.2	86.4	90.6	73.9	37.9
Trovafloxacin	87.2	84.0	88.5	68.5	42.5
Others					
Nitrofurantoin	87.1	33.8	26.9	0.0	8.1
Tetracycline	52.7	63.3	71.9	0.0	70.1
Trimethoprim-sulfamethoxazole (1:19)	51.6	60.4	76.0	0.0	34.5

*Categories of susceptibility interpreted by NCCLS [1998] criteria. For the investigational fluoroquinolone gatifloxacin, susceptible was defined as ≤2 μg/ml.

†Percentages of *E. coli* and *Klebsiella* spp. isolates meeting the screening criteria for possible ESBL production [NCCLS, 1998].

observed in Canada and the United States (97%) (7).

P. aeruginosa causing BSI (92 isolates evaluated) showed high rates of resistance to most antimicrobial agents tested. Imipenem and piperacillin-tazobactam were the most active drugs (approximately 85% susceptible) followed by amikacin (83.7% susceptible), tobramycin (78.3%), ciprofloxacin-levofloxacin (73.9%), and cefepime (72.8% susceptible). *P. aeruginosa* resistance to imipenem varied from around 7% in Argentina and Colombia, to as high as 24% in Uruguay and Mexico. The increasing resistance of *P. aeruginosa* to imipenem and fluoroquinolones in Latin America is of great concern.

Acinetobacter spp. was the seventh most frequent pathogen isolated from BSI. *A. baumannii* is a pathogen of considerable importance in this geographic area because of the high mortality related to hospital-acquired pneumonia, and the fact that said condition is usually resistant to multiple antimicrobial agents (23, 24). In our study, 92.0% of *Acinetobacter* spp. isolates were susceptible to imipenem (MIC 90, 2 μg/mL). Tetracyclines were also active in vitro (70.1% susceptible). The next most active compound was tobra-

mycin, which inhibited only 42.5% of the isolates tested (MIC 50, 16 μg/mL). Thus, only carbapenems, and possibly some tetracyclines, represent viable options for treating BSI due to *Acinetobacter* spp. in the hospitals evaluated. These findings are of great concern. The high prevalence of multiresistant, nonfermentative Gram-negative bacilli as a cause of BSI emphazise their importance as a true cause of other infections, such as pneumonia. In addition, these findings may stimulate the use of carbapenems, and possibly a greater carbapenem resistance in Latin American medical centers.

Salmonella spp. isolates were analyzed separately in Table 4. Some degree of resistance to penicillins was detected (8.2% resistance to ampicillin, ticarcillin, and piperacillin). Susceptibility was partially restored by β-lactamase inhibitors such as clavulanic acid. Resistance to trimethoprim-sulfamethoxazole was relatively high, with only 83.7% of isolates being susceptible (MIC ≤ 0.5/9.5 μg/mL). These findings are of concern because ampicillin (or amoxicillin) and trimethoprim-sulfamethoxazole are very frequently used to treat salmonella infections in Latin America.

Community-acquired respiratory tract infection caused by isolates of H. influenzae, M. catarrhalis, and S. pneumoniae. During approximately 6 months, 81 strains of *H. influenzae*, 52 strains of *M. catarrhalis*, and 197 strains of *S. pneumoniae* were obtained from patients with CARTI. It should be noted that, due to the study design, the number of isolates for each organism does not represent the frequency of occurrence. During 1997, low compliance by participating centers in the collection of fastidious organisms and the viability of the organisms when they reached the coordinating laboratory resulted in a low sample size.

The antimicrobial activity of 17 parenteral and orally administered drugs against these community-acquired pathogens is shown in Table 5. Most of the antimicrobial agents tested showed good activity against *H. influenzae*, with notable exceptions of trimethoprim-sulfamethoxazole (50.6% susceptible), and clarithromycin (61.7% susceptible). A comparison of the susceptibility of *H. influenzae* to amoxicillin and amoxicillin-clavulanate suggests that 16.0% of the strains were β-lactamase producers. As expected, a larger percentage (~90%) of the *M. catarrhalis* strains were producers of β-lactamase. The β-lactamase stable compounds, including cefepime, cefotaxime, and cefixime were active against all of the *H. influenzae* and *M. catarrhalis* strains (100.0% susceptible) (see Table 5).

TABLE 4. Antimicrobial susceptibility of 50 *Salmonella* spp. bloodstream isolates tested against 15 potential therapeutic compounds. SENTRY Antimicrobial Surveillance Program, 1997

Antimicrobial agent	MIC 90 (μg/ml)	% Susceptible	% Resistant
Ampicillin	2	91.8	8.2
Amoxicillin-clavulanic acid (2:1)	1	93.9	2.0
Piperacillin	4	91.8	8.2
Piperacillin-tazobactam (4 μg/ml)	4	98.0	2.0
Ticarcillin	4	91.8	8.2
Ticarcillin-clavulanic acid (2 μg/ml)	4	91.8	6.1
Aztreonam	≤0.12	100.0	0.0
Ceftriaxone	≤0.25	100.0	0.0
Ceftazidime	0.25	100.0	0.0
Cefepime	≤0.12	100.0	0.0
Imipenem	0.5	100.0	0.0
Ciprofloxacin	0.06	100.0	0.0
Ofloxacin	0.5	100.0	0.0
Gatifloxacin	0.12	100.0	0.0
Trimethoprim-sulfamethoxazole (1:19)	>1	83.7	16.3

Among the 197 outpatient isolates of *S. pneumoniae* obtained, only 47.7% were susceptible to penicillin (MIC ≤0.06 μg/mL), and approximately 10% had high-level resistance (MIC, ≥2 μg/mL) to penicillin. Most β-lactams, including cefepime, cefotaxime, amoxicillin-clavulanate, and amoxicillin (except for cefaclor and cefixime where the susceptibility is determined by the penicillin test results *[14]*) were active against more than 90% of strains of *S. pneumoniae*. Macrolides and chloramphenicol were active against 90.9%–93.4% of isolates, while only 52% of isolates were susceptible to trimethoprim-sulfamethoxazole. Compared to a previous report of pneumococcal resistance in clinical isolates from Brazil (*25*), it appears that resistance is increasing. In an evaluation of 50 strains of *S. pneumoniae* obtained from patients with pneumonia or meningitis, intermediate resistance to penicillin among these strains was 24%; high-level resistance was not detected. Resistance to trimethoprim-sulfamethoxazole was 32%, and all but one isolate was susceptible to erythromycin (*25*).

A large Latin American study conduced by the Pan American Health Organization (PAHO) analyzed 1,635 pneumococci isolates, and found that 75.1% were susceptible to penicillin (MIC ≤0.06 μg/mL). Among nonsusceptible strains (408 isolates), 274 (16.7%) had an intermediate resistance (MIC 0.06 – 1.0 μg/mL), and 134 (8.2%) presented high-level resistance (MIC ≤2 μg/mL). However, there was a great variation among countries. The highest rate of nonsusceptible strains was found in Mexico (47.3%) and the lowest in Colombia (12.1%). Mexico also presented the highest rate of high-level resistance (21.3%), while the lowest rate was observed in Brazil (1.4%) (*26*).

Pneumonia in hospitalized patients. The most prevalent pathogens causing pneumonia in hospitalized patients were *P. aeruginosa* (26.9%), *S. aureus* (23.0%), *Acinetobacter* spp. (11.7%), *Klebsiella* spp. (10.4%), and *Enterobacter* spp. (7.0%). Gram-negative bacilli were responsible for almost 75% of monitored cases. Table 6 shows the antimicrobial activity of 15 drugs against the five most prevalent Gram-

TABLE 5. Antimicrobial activity and spectrum for 17 selected parenteral and orally administered drugs tested against *S. pneumoniae* (197 strains), *M. catarrhalis* (52 strains), and *Haemophilus influenzae* (81 strains) in Latin America. SENTRY Antimicrobial Surveillance Program, 1997

	H. influenzae		*M. catarrhalis*		*S. pneumoniae*	
Antimicrobial class/agent	MIC 90	% susc.*	MIC 90	% susc.*	MIC 90	% susc.*
Penicillin	>16	–	8	7.7	2	47.7
Amoxicillin	>8	84.0	8	7.7	2	94.9
Amoxicillin-clavulanate	1	100.0	0.25	98.1	2	94.9
Cefaclor	8	93.8	1	100.0	>32	47.7†
Cefuroxime	2	98.8	2	100.0	4	77.3
Cefixime	0.12	100.0	0.5	100.0	>4	47.7†
Ceftriaxone¶	0.06	100.0	0.5	100.0	0.5	92.4
Cefepime	0.25	97.5	4	100.0	1	85.9
Azithromycin	4	97.5	≤0.12	100.0	0.25	90.9
Clarithromycin	16	61.7	≤0.25	100.0	≤0.25	90.9
Ciprofloxacin	0.03	100.0	0.06	100.0	2	–
Gatifloxacin	≤0.03	100.0	0.06	100.0	0.5	100.0
Levofloxacin	≤0.5	100.0	≤0.5	100.0	2	100.0
Chloramphenicol	≤2	97.5	≤2	100.0	≤2	93.4
Tetracycline	≤2	97.5	≤2	100.0	>16	82.8
Trimethoprim-sulfamethoxazole (1:19)	8	50.6	2	63.5	4	52.0
Vancomycin	>16	0.0	>16	0.0	0.5	100.0

*Interpreted by NCCLS [1998] criteria, where available. Criteria for susceptibility of gatifloxacin was defined as ≤2 μg/ml.

†Percentage susceptible determined by the penicillin test results [NCCLS, 1998].

¶Also indicates spectrum of cefotaxime.

TABLE 6. Antimicrobial activity and spectrum for 20 drugs tested against the five most prevalent Gram-negative pathogens causing pneumonia infections in Latin America. SENTRY Antimicrobial Surveillance Program, 1997

	Pathogen (prevalence rank/number tested)				
	P. aeruginosa (1/150)	*Acinetobacter* spp. (3/65)	*Klebsiella* spp. (4/57)	*Enterobacter* spp. (5/39)	*E. coli* (6/33)
Antimicrobial class/agent	% susc.*	% susc.*	% susc.*	% susc.*	% susc.*
Cephalosporins					
Cefazolin	0.0	1.5	56.1	5.1	42.4
Cefuroxime	0.0	0.0	50.9	35.9	57.6
Ceftriaxone	14.7	9.2	64.9 (38.6)†	53.8	69.7 (30.3)†
Ceftazidime	65.3	20.0	73.7 (38.6)†	53.8	84.8 (30.3)†
Cefepime	69.3	30.8	73.7	89.7	72.7
Other β-lactams					
Ampicillin	0.0	0.0	1.8	5.1	27.3
Aztreonam	54.7	0.0	66.7 (38.6)†	53.8	69.7 (30.3)†
Imipenem	74.0	89.2	98.2	100.0	100.0
Piperacillin-tazobactam (4 μg/ml)	77.3	18.5	64.9	56.4	57.6
Aminoglycosides					
Amikacin	75.3	24.6	84.2	74.4	81.8
Gentamicin	64.0	29.2	70.2	69.2	69.7
Fluoroquinolones					
Ciprofloxacin	68.0	16.9	82.5	79.5	93.9
Levofloxacin	66.0	18.5	80.7	84.6	93.9
Gatifloxacin	61.3	23.0	86.0	82.1	93.9
Other					
Trimethoprim-sulfamethoxazole (1:19)	2.7	24.6	63.2	61.5	48.5

*Categories of susceptibility interpreted by NCCLS [1998] criteria. For the investigational fluoroquinolone gatifloxacin, susceptible was defined as ≤2 μg/ml.

†Indicates the percentage of isolates having MICs consistent with possible ESBL production [NCCLS, 1998].

negative pathogens causing bacterial pneumonia. The strains of *P. aeruginosa* isolated from patients with pneumonia showed high rates of resistance to most drugs tested. Rates of resistance found in Brazil were generally higher than those seen in Latin American as a whole (*20*). Piperacillin-tazobactam was the most active drug (77.3% susceptible), followed by amikacin (75.3%), imipenem (74.0%), and cefepime (69.3%).

Resistance rates were even higher for *Acinetobacter* spp., against which only imipenem showed reasonable activity (88.5% susceptibility). Piperacillin-tazobactam was active against only 18.5% of the isolates. All other compounds inhibited less than 40% of the isolates evaluated. Cefepime was the most active cephalosporin (30.8% susceptibility) and none of the quinolones exhibited reasonable activity against this pathogen. The increasing frequency of *Acinetobacter* spp. causing pneumonia in hospitalized patients in Latin America has been reported previously (*20, 27*), and *Acinetobacter* spp. was recently reported to be the third leading pathogen in hospitalized patients with pneumonia in Latin American countries participating in SENTRY (*11*). The high rate of mortality associated with this pathogen in patients with hospital-acquired pneumonia (*28*) and its resistance to multiple antimicrobial agents makes it a particularly acute therapeutic problem. The prevalence of *P. aeruginosa* and *Acinetobacter* spp. in hospitalized patients with pneumonia, and the high

resistance rates of both pathogens seen in this study suggest that these organisms were cultured from patients with prolonged hospitalization and/or previous multiple courses of antimicrobial agents, who may not be representative of the general population of hospitalized patients with pneumonia. This is also supported by the low occurrence in this study of *S. pneumoniae* and *H. influenzae* as pneumonia-causing pathogens. However, with the increasing trend to provide more outpatient treatment rather than hospital-based therapy, we may have observed a shift in the pathogens causing pneumonia towards those that are indicative of nosocomially-acquired infections.

Cefepime and imipenem were the most active agents against *Enterobacter* spp. (89.7% and 100.0% susceptibility, respectively), whereas susceptibility rates to the other β-lactams and cephalosporins were less than 60.0% (5.1% to 56.4% susceptible). The reduced susceptibility of *Enterobacter* spp. to ceftazidime, ceftriaxone, aztreonam (53.8% each), and to piperacillin-tazobactam (56.4% susceptible) suggests the occurrence of chromosomally inducible (Class 1 or AmpC) β-lactamase producing strains. Based on the screening concentrations recommended by the NCCLS *(14)*, approximately 30% of *E. coli* and 40% of *Klebsiella* spp. were possibly ESBL producers. According to the NCCLS, this suggests that 84.8% of *E. coli* isolates were susceptible in vitro to ceftazidime, but 30.3% may be clinically refractory to therapy by virtue of ESBL production. Amikacin was the most active aminoglycoside. The four fluoroquinolones tested were uniformly active against the *Enterobacteriaceae*, with slightly less activity against *Enterobacter* spp. (79.5–84.6% susceptible) and *Klebsiella* spp. (80.7%–86.0% susceptible) than *E. coli* (93.9% susceptible). As was noted for the blood stream isolates, the fluoroquinolones showed greater activity against Brazilian isolates of *Klebsiella* (92.6% susceptible—data not shown) than against those from other Latin American SENTRY sites (80%–82% susceptible).

S. aureus was the primary Gram-positive pathogen causing pneumonia; very few other isolates (enterococci [11 isolates] and CoNS [four isolates]) were processed. As was demonstrated with the Gram-negative pathogens causing pneumonia, resistance rates of *S. aureus* were high. Of the 128 strains isolated, 50.0% were resistant to oxacillin (29.2% for BSI). Similar rates of resistance were seen with cephalosporins, other β-lactams, clindamycin, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole. The newer fluoroquinolones, gatifloxacin and trovafloxacin, had greater activity (77.3% and 78.1% susceptible, respectively). All isolates were susceptible to vancomycin. Similar susceptibility patterns, including ~50% resistance rate to oxacillin, were seen in isolates of *S. aureus* from each country when the data were analyzed separately (data not shown).

Wound or skin and soft tissue infections in hospitalized patients. The frequency of occurrence of pathogens causing WSSTI was, in rank order, *S. aureus* (28.2%), *E. coli* (13.6%), *P. aeruginosa* (12.3%), *Klebsiella* spp. (8.3%), *Enterobacter* spp. (7.9%), and *Enterococcus* spp. (7.9%) As expected, *S. aureus* was the predominant pathogen, but as observed with other sites of infections, Gram-negative organisms played a significant role. Table 7 summarizes the antimicrobial susceptibility results for the five most prevalent Gram-negative pathogens listed in the order of occurrence. As seen with the pseudomonal isolates causing pneumonia, the antimicrobial susceptibility rates were also low among the 58 isolates of *P. aeruginosa* causing WSSTI. Piperacillin-tazobactam was the most active compound with 84.5% susceptibility followed by aminoglycosides (72.4–77.6% susceptible), ciprofloxacin and imipenem (both 72.4% susceptible).

Against *Enterobacter* spp., cefepime was the most active cephalosporin (86.5% susceptible) and was significantly superior to ceftazidime (59.5% susceptible, $P < 0.05$). Imipenem inhibited all strains at concentrations less than or equal to the susceptibility breakpoint while the

TABLE 7. Antimicrobial susceptibility of the five most prevalent Gram-negative pathogens causing skin and soft tissue infections in Latin America. SENTRY Antimicrobial Surveillance Program, 1997

	Pathogen (prevalence rank/number tested)				
	E. coli (1/60)	*P. aeruginosa* (3/58)	*Klebsiella* spp. (4/39)	*Enterobacter* spp. (5/37)	*Acinetobacter* spp. (6/26)
Antimicrobial class/agent	% susc.*	% susc.*	% susc.*	% susc.*	% susc.*
Cephalosporins					
Cefazolin	58.3	0.0	46.2	5.4	0.0
Cefuroxime	73.3	0.0	48.7	16.2	0.0
Ceftriaxone	90.0 (11.7)†	6.9	64.1 (43.6)†	70.3	19.2
Ceftazidime	88.3 (15.0)†	69.0	69.2 (43.6)†	59.5	26.9
Cefepime	93.3	64.9	76.9	86.5	26.9
Other β-lactams					
Ampicillin	28.3	0.0	2.6	8.1	0.0
Aztreonam	90.0 (15.0)†	58.6	66.7 (43.6)†	56.8	15.4
Imipenem	100.0	72.4	100.0	100.0	88.5
Piperacillin-tazobactam (4 μg/ml)	78.3	84.5	61.5	56.8	61.5
Aminoglycosides					
Amikacin	91.7	77.6	84.6	91.9	38.5
Gentamicin	70.0	72.4	61.5	83.8	30.8
Tobramycin	71.7	74.1	56.4	78.4	38.5
Fluoroquinolones					
Ciprofloxacin	60.0	72.4	87.2	83.8	30.8
Levofloxacin	60.0	62.1	89.7	83.8	30.8
Gatifloxacin	60.0	58.6	92.3	83.8	30.8
Others					
Tetracycline	40.0	0.0	66.7	67.6	42.3
Trimethoprim-sulfamethoxazole (1:19)	41.7	1.7	61.5	81.1	23.1

*Categories of susceptibility interpreted by NCCLS [1998] criteria. For the investigational fluoroquinolone gatifloxacin, susceptible was defined as ≤2 μg/ml.

†Proportion of isolates having NCCLS [1998] screening criteria consistent with ESBL production.

fluoroquinolones were active against 83.8% of the *Enterobacter* spp. isolates. Interestingly, trimethoprim-sulfamethoxazole showed reasonable in vitro activity against *Enterobacter* spp. and inhibited 81.1% of the isolates. Piperacillin-tazobactam and aztreonam exhibited marginal activity against *Enterobacter* spp. (56.8% susceptible).

A notable finding in the susceptibility of the 60 *E. coli* isolates was the high rate of resistance to the fluoroquinolones (40.0%), which was similar to that exhibited to cefazolin. The other β-lactams, except ampicillin, and aminoglycosides remained active against most *E. coli* isolates. Fifteen percent of the isolates were considered ESBL-producers, based on increased MICs to ceftazidime and/or aztreonam (NCCLS criteria). Resistance rates to gentamicin and tobramycin were also high.

In contrast, all *Klebsiella* spp. isolates were susceptible to the fluoroquinolones, but susceptibility to β-lactams and cephalosporins was variable. Based on NCCLS (1998) criteria for production of ESBL, 43.6% of the *Klebsiella* spp. were possible producers of these enzymes. Imipenem was active against all *Klebsiella* spp. strains, and amikacin was markedly more active than gentamicin and tobramycin (84.6%, 61.5% and 56.4%, respectively) (see Table 7).

TABLE 8. Antimicrobial susceptibility of the three most common Gram-positive pathogens from skin and soft tissue infections in Latin America. SENTRY Antimicrobial Surveillance Program, 1997

	Pathogen (prevalence rank/number tested)		
	S. aureus (1/133)	*Enterococcus* spp. (5/37)[a]	CoNS (7/22)[b]
Antimicrobial class/agent	% susc.[1]	% susc.[1]	% susc.[1]
Penicillin	3.8	94.6	4.5
Oxacillin	68.4	0.0	9.1
Clindamycin	72.9	0.0	54.5
Erythromycin	27.1	10.8	36.4
Ciprofloxacin	67.7	27.0	45.5
Gatifloxacin	84.2	64.9	77.3
Trovafloxacin	79.7	56.8	54.5
Gentamicin	69.2	83.8	31.8
Tetracycline	75.9	37.8	54.5
Trimethoprim-sulfamethoxazole (1:19)	82.0	70.3	40.9
Vancomycin	100.0	97.3	100.0

[a]Includes *E. faecalis* (31 strains), *E. faecium* (three strains), *E. avium* (two strains), and one unspecified *Enterococcus* spp.

[b]CoNS = coagulase-negative staphylococci that includes 11 unspeciated *Staphylococcus* spp., *S. epidermidis* (six strains), *S. haemolyticus* (four strains), and *S. equorum* (one strain).

[1]Categories of susceptibility interpreted by NCCLS [1998] criteria. For the investigational fluoroquinolone gatifloxacin, susceptible was defined at ≤2 μg/ml.

S. aureus was the most common Gram-positive organism isolated from hospitalized patients with WSSTI, and the antimicrobial susceptibility of the 133 isolates is shown in Table 8. Resistance to oxacillin was detected in 31.6% of the isolates, with similar cross-resistance to other β-lactams and cephalosporins. However, piperacillin-tazobactam was slightly less active (35.3% resistant). Gatifloxacin, a fluoroquinolone still under research, was the most active fluoroquinolone (84.2% susceptible) and was the second most active compound for systemic infection after vancomycin (100% susceptible). This susceptibility profile was similar to that observed with BSI strains, whereas pneumonia isolates were markedly more resistant. Coagulase-negative staphylococci showed higher resistance rates in general. Only 9.1% of the isolates were susceptible to oxacillin. The newer fluoroquinolone gatifloxacin was markedly more active than ciprofloxacin (77.3% vs. 45.5% respectively). In contrast, *Enterococcus* sp. isolates remained very susceptible to penicillin (94.6%) and vancomycin (97.3%).

Urinary tract infections (UTI) in hospitalized patients. The rates of occurrence (Table 1) of the most prevalent pathogens causing UTI in Latin America were *E. coli* (52.5%), *Klebsiella* spp. (12.3%), *P. aeruginosa* (7.5%), *Proteus mirabilis* (4.9%), and *Enterobacter* spp. (4.3%). These results varied slightly from country to country. Table 9 lists the susceptibility testing results of these pathogens in the order of occurrence. The cephalosporins and other β-lactams (except ampicillin and cefazolin) were active against most of the *E. coli* isolates. Cefepime was the most active of the cephalosporins (98.4% susceptible) and imipenem was the most active of the other β-lactams. Of the *E. coli* strains, 8.1% had an ESBL phenotype by NCCLS criteria *(14)*. The fluoroquinolones were less active than many of the other agents, and cross-resistance was apparent among these drugs. Trimethoprim-sulfamethoxazole, a commonly used agent for urinary tract infections, was not active against the majority of *E. coli* isolates (43.1% susceptible). Nitrofurantoin, an older agent with exclusive UTI use, was active only against *E. coli* (84.1% susceptible).

Against *Klebsiella* spp. and *Enterobacter* spp., cefepime was the most active cephalosporin. More than 35% of the *Klebsiella* strains had

TABLE 9. Antimicrobial susceptibility of the five most prevalent Gram-negative pathogens causing urinary tract infections in Latin America. SENTRY Antimicrobial Surveillance Program, 1997

	Pathogen (prevalence rank/number tested)				
	E. coli (1/246)	*Klebsiella* spp. (2/60)	*P. aeruginosa* (3/35)	*P. mirabilis* (4/23)	*Enterobacter* spp. (5/20)
Antimicrobial class/agent	% susc.*	% susc.*	% susc.*	% susc.*	% susc.*
Cephalosporins					
Cefazolin	74.4	53.3	0.0	65.2	0.0
Cefuroxime	90.7	60.0	0.0	78.3	15.0
Ceftriaxone	96.7 (4.9)†	75.0 (35.0)†	0.0	78.3	65.0
Ceftazidime	97.2 (8.1)†	71.7 (37.7)†	54.3	87.0	60.0
Cefepime	98.4	81.7	37.1	91.3	90.0
Other β-lactams					
Ampicillin	40.7	3.3	0.0	56.5	0.0
Aztreonam	96.3 (7.3)†	70.0 (37.7)†	28.6	82.6	60.0
Imipenem	99.2	98.3	77.1	91.3	100.0
Piperacillin-tazobactam (4 μg/ml)	87.8	61.7	62.9	87.0	65.0
Aminoglycosides					
Amikacin	98.4	83.3	71.4	82.6	80.0
Gentamicin	89.4	71.7	25.7	73.9	75.0
Tobramycin	90.7	68.3	40.0	73.9	60.0
Fluoroquinolones					
Nalidixic acid	75.6	58.3	0.0	60.9	40.0
Ciprofloxacin	82.5	75.0	37.1	65.2	65.0
Gatifloxacin	83.7	85.0	34.3	65.2	65.0
Levofloxacin	81.3	81.7	28.6	65.2	65.0
Trovafloxacin	82.5	68.3	20.0	65.2	55.0
Others					
Nitrofurantoin	84.1	20.0	0.0	0.0	20.0
Tetracycline	50.4	58.3	0.0	4.3	40.0
Trimethoprim-sulfamethoxazole (1:19)	43.1	53.3	0.0	56.5	30.0

*Categories of susceptibility interpreted by NCCLS [1998] criteria. For the investigational fluoroquinolones gatifloxacin and trovafloxacin, susceptible was defined as MIC ≤2 and ≤1 μg/mL, respectively.

†Indicates the number of strains (%) having positive screening criteria for ESBL production.

MICs for aztreonam, ceftriaxone or ceftazidime of ≥ 2 μg/mL, suggesting an ESBL phenotype (*14*). Patient-to-patient transmission was observed in several centers where ESBL-producing isolates were detected (Table 10). Less than 70% of isolates of *Klebsiella* spp. and *Enterobacter* spp. were susceptible to piperacillin-tazobactam. The fluoroquinolones had good activity against *Klebsiella* spp. but 65% or less of *Enterobacter* spp. were susceptible to those agents. Amikacin was the most active of the aminoglycosides against both *Klebsiella* and *Enterobacter* (83.3% and 80.0% susceptible, respectively), suggesting that a plasmid-mediated aminoglycoside inactivating enzyme may be prevalent in the monitored Brazilian centers. Trimethoprim-sulfamethoxazole, tetracycline, and ampicillin (WHO essential drugs) had limited activity against the most prevalent pathogens causing nosocomial urinary tract infections in Brazil.

The most outstanding susceptibility testing results were those for *P. aeruginosa*: the number of susceptible isolates to antipseudomonal agents was extremely limited. The most active agents were imipenem (77.1% susceptible), amikacin (71.4% susceptible), and piperacillin-tazobactam (62.9% susceptible); less than 60.0% of the isolates were susceptible to the rest of the agents. In particular, the susceptibility to fluoroquinolones ranged from 0 (nalidixic acid)

TABLE 10. Comprehensive evaluation of ESBL-producing strains* of *Klebsiella* spp. and *Escherichia coli* isolates from bloodstream infections having potential epidemiological clustering characteristics, as obtained in the SENTRY Antimicrobial Surveillance Program from six Latin American countries† during 1997

Medical center/ strain number	Typing results‡		MIC (µg/ml)§			Coresistance‖	pI#
	Ribotype	PFGE	Meropenem	Cefepim	Cefoxitin		
Klebsiella pneumoniae							
41/K22	204-1	G	≤0.06	4	4	AG,S	5.4,7.6,8.2
41/K23	204-1	G	≤0.06	2	8	AG,S	5.4,7.6,8.2
41/K24	204-1	G	0.12	16	8	AG,S	5.4,7.6,8.2
41/K25	204-1	G	≤0.06	2	8	AG,S	7.6,8.2
41/K26	204-1	G	≤0.06	1	4	AG,S	5.4,7.6
42/K30	204-1	H	≤0.06	8	8	AG,F,T,S	5.4,6.8,7.6,8.2
42/K31	204-1	H	≤0.06	16	8	AG,F,T,S	5.4,7.6
43/K35	204-1	H	0.12	>16	16	AG,F,T,S	5.4,5.6,6.8,7.6,8.2
40/K15	614-1	F	≤0.06	8	16	AG,T,S	5.4,7.6
40/K16	614-1	F	≤0.06	>16	>32	AG,F,T,S	5.4,7.6
42/K29	614-4	I	≤0.06	16	4	AG,F,T,S	5.4,7.6
42/K32	614-4	I	≤0.06	8	8	AG,F,T,S	5.4,7.0,7.6
42/K33	614-4	I	≤0.06	16	4	AG,T,S	5.4,7.0,7.6
42/K34	614-4	I	≤0.06	8	4	AG,F,T,S	5.4,7.0,7.6
39/K12	621-1	E	≤0.06	>16	8	AG	5.4,6.5,6.8,7.6,8.0
39/K13	621-1	E	0.25	>16	8	AG,F,T,S	5.4,6.5,6.8,7.6,8.0
Escherichia coli							
48/E8	182-2	G	≤0.06	2	2	AG,S	5.4,7.0,7.6
48/E9	182-2	G	≤0.06	1	2	AG,S	5.4,7.0,8.2
48/E10	182-2	G	≤0.06	4	2	AG,S	5.4,7.0,8.2
40/E4	241-4	J	≤0.06	>16	32	AG,F,T,S	5.4,8.0
42/E5	241-4	K	≤0.06	>16	8	AG,F,T,S	5.4,7.0
43/E6	241-4	L	≤0.06	8	2	AG,S	5.4,7.6
46/E7	241-4	M	≤0.06	8	16	AG,S	5.4,7.0,7.6

*ESBL production was confirmed by the E-test method with a fixed concentration of clavulanic acid (2 µg/ml). A >2 $\log_2$ dilution step reduction in the MIC with clavulanic acid compared to cephalosporin alone was defined as a positive test.

†Medical center locations: Brazil (three), Argentina (two), Chile (two), Colombia (one), Mexico (one), and Uruguay (one).

‡Molecular typing with RiboPrinter and PFGE = pulsed field gel electrophoresis.

‖Coresistance defined as a MIC in the intermediate or resistant range by NCCLS criteria [1998]. F = fluoroquinolone (using ciprofloxacin as an index drug), AG = aminoglycoside (using tobramycin as an index drug), T = tetracycline, and S = trimethoprim-sulfamethoxazole.

#Isoelectric focus analysis.

to 37.1% (ciprofloxacin). The predominance of *P. aeruginosa* and the resistance profile of the isolates again suggest that these isolates were cultured from patients with prolonged hospital stays and/or multiple courses of antimicrobial therapy for chronic urologic disorders. Nevertheless, the occurrence of the multiresistant strains is worrysome.

Most enterococci isolated from patients with urinary tract infections were identified as *E. faecalis* (90.0%); no *E. faecium* was detected. All isolates were susceptible to penicillin and one non-*faecalis* isolate presented intermediate resistance to vancomycin. High-level resistance to gentamicin was detected in 40% of the enterococci.

Molecular Investigations of Antimicrobial-Resistant Strains

During 1997, ESBL phenotype isolates from blood cultures were subjected to further studies (*7, 20*). Those isolates that produced an inhabitable (2 µg/mL clavulanic acid) enzyme by the E-test method (*29*) were then studied using ribotyping, PFGE and IEF (*15, 16, 19*).

Molecular epidemiology techniques are useful as tools to assess resistance mechanisms dissemination within a hospital, community, country or even a continent. The selective pressure created by the use of antimicrobial agents is the main factor for the appearance of numerous mechanisms of resistance. However, the dissemination of the mechanism may be the result of clonal spread of resistant strains (patient-to-patient transmission), the spread of resistance genome (DNA from strain-to-strain), or independent selection of resistant mutants. When isolates with a specific mechanism of resistance present little genetic variability (identical or similar typing patterns), clonal dissemination (patient-to-patient) of resistant strains is highly suspected. Oxacillin resistance among staphylococci is probably the best example of this kind of resistance dissemination (*30*). Other resistance mechanisms that may disseminate in this manner include penicillin resistance among *S. pneumoniae*, vancomycin resistance among enterococci, and imipenem resistance among *Acinetobacter* spp. (*31*).

On the other hand, great genomic variability among isolates demonstrating a certain resistance mechanism indicates that selection of resistant mutants is occurring in different strains, probably due to the selective pressure produced by antimicrobial use. The best example of this type of resistance dissemination in Latin America is β-lactam resistance due to the production of ESBL. Data from the SENTRY, as well as from other local studies, has documented an enormous genomic variability among ESBL-producing strains. Selective pressure also appears to be the main factor for the dissemination of resistance to "third-generation" cephalosporins due to the production of chromosomally-mediated inducible class 1 β-lactamases. Although imipenem resistance among *P. aeruginosa* may disseminate from patient-to-patient, ample genomic variability among some of these multiresistant strains in our hospitals indicate that selective antibiotic pressure may play an important role in the dissemination of this resistance.

A total of 7 *E. coli* and 16 *K. pneumoniae* were characterized and the results are listed in Table 10. Possible patient-to-patient transmission was observed (*E. coli* ribotype 182-2 and PFGE pattern G; *K. pneumoniae* ribotype 204-1 and PFGE pattern G) in both institutions. Wide genetic diversity was found among the remaining ESBL isolates. Examination of other phenotypic and molecular data (pI) among the five potential epidemic strains of *K. pneumoniae* in medical center 041 notes only two isolates (K24, K26) with identical molecular results, but these strains were markedly different in their phenotypic expression of β-lactam activity; transmission appears doubtful. Similarly, for medical center 048, among the three potential epidemic *E. coli* (E8–10) only two had identical molecular and phenotypic results, indicating only one probable nosocomial transmission episode.

An extreme variation in pI results was documented and coresistances were found most commonly for aminoglycosides (84.6%), tetracycline (30.8%), and sulfonamides (69.2%). Fluoroquinolone resistance was rarer (7.7%). The carbapenems (imipenem, meropenem) remained active, and intermediate or susceptible results for cefoxitin were observed with 25 of 26 isolates (low probability of AmpC). Cefepime MICs were ≤ 16 μg/mL (intermediate) and ≤ 1 μg/mL (NCCLS ESBL screen concentration) for 96.2% and 25.0% of strains, respectively.

CONCLUSIONS

Gram-negative bacilli and *S. aureus* were the predominant pathogens causing serious infections in Latin American medical centers participating in the SENTRY Antimicrobial Surveillance Program. The predominance of *P. aeruginosa* and *Acinetobacter* spp. and the significant levels of resistance to most agents are of major concern in Brazil and all of Latin America. With only imipenem and tetracycline exhibiting in vitro activity against *Acinetobacter* spp., frequent use of the carba-

penems could lead to even higher levels of resistance. Currently, imipenem-resistant *P. aeruginosa* is increasing in Brazil. Of additional concern is the epidemic rate of ESBL-producing strains of *Klebsiella* spp. and *E. coli* in many Latin American medical centers, which is much higher than rates found in other areas of the world. Resistance among *P. aeruginosa* and the *Enterobacteriaceae* to fluoroquinolones, oxacillin-resistant *S. aureus*, and penicillin- and trimethoprim-sulfamethoxazole-resistant pneumococci were other significant resistance issues identified in this ongoing surveillance study. Vancomycin resistance among the enterococci (42 invasive isolates), *S. aureus*, and *S. pneumoniae* was not identified in 1997.

Reasons for the high rates of resistance to other agents observed in this study are unclear, but this appears to be a common problem throughout Latin America. These resistance patterns may be related to compromised infection control practices, wide antimicrobial availability and high usage, climate conditions, or other undetermined medical practices that vary geographically. This benchmark study (1997) will serve as comparison for future surveillance studies, including the ongoing SENTRY program, to monitor emerging resistance issues in Latin American nations. The high rates of resistance observed in this study underscore the need for surveillance and local agressive corrective action.

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NEISSERIA GONORRHOEAE IN CANADA

Lai-King Ng[1]

This is a review of the occurrence of antibiotic-resistant Neisseria gonorrhoeae *in Canada. Isolates characterized at the National Laboratory for Sexually Transmitted Diseases (NLSTD), Winnipeg, Canada, were collected from public health and reference laboratories.*

The isolates were screened by the participating laboratories for plasmid-mediated resistance to penicillin or tetracycline and chromosomal-mediated resistance. The resistant isolates were sent to the NLSTD for further characterization. In addition, all the isolates from children in Canada were tested by NLSTD for antimicrobial susceptibility and typing. The typing methods included auxotype, serotype, and plasmid profile analysis. The data were analyzed in regard to the infection rate of gonorrhea, the prevalence of antimicrobial resistance and the distribution of different types of resistance in the country.

Gonorrhea in Canada is decreasing, but antibiotic resistance remains a national concern. Although the trends and low incidence may lead us to believe that the national goal to eliminate endemic gonorrhea in Canada by 2010 is realistic, success will partly depend on the effectiveness of antimicrobial therapies. Therefore, it is important to continue national surveillance of antibiotic resistance in the gonococcus.

INTRODUCTION

Infection caused by *Neisseria gonorrhoeae* is one of the most common sexually transmitted diseases (STD) of bacterial origin in the world, and it is the second most often reported STD in Canada. In 1996, the highest rates of gonococcal infection occurred in those regions with the highest concentration of First Nation communities, including the Northwest Territories (187.8 per 100,000 population), Yukon (31.8 per 100,000) and Manitoba (48.4 per 100,000); the lowest rates were found in the Maritimes (0.4 to 10.3 per 100,000) *(1)*.

There are multiple strategies used to control STDs in Canada, including the use of antimicrobial therapies, sensitive and accurate detection and diagnostic methods, and partner notification. Also, educational programs are in place to increase public awareness of STDs. With the implementation of these strategies, the number of cases of *N. gonorrhoeae* reported by Canadian hospitals has fallen from 56,330 in 1981 to 5,023 in 1996 (Figure 1). This represents a decrease in the rate of infection from 226.2 per 100,000 population to 16.8 per 100,000 population in those same years, respectively.

Despite a tenfold reduction in the reported incidence of gonorrhea in Canada, the disease

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is still an important public health concern, causing between 20% and 40% of cases of pelvic inflammatory disease (PID) and 14% of all tubal infertility. During the period from 1985 to 1996, the highest incidence rates of gonorrhea among females were found in the 15–19 year age group, indicating a shift in the disease toward younger women (Figure 2) *(1)*. The estimated annual cost for the Canadian health care system of female gonococcal infections and the associated impact of the disease is approximately CAN$ 43 million.

Although effective antimicrobial treatment is an important intervention for controlling gonorrhea, antimicrobial resistant *N. gonorrhoeae* is a major concern, given that inappropriate therapy may result in treatment failure and cause an increase in the risk of transmission. This report reviews the trends, the distribution, and the current status of resistant *N. gonorrhoeae* in Canada.

NATIONAL SURVEILLANCE OF ANTIBIOTIC RESISTANT *NEISSERIA GONORRHOEAE*

The National Laboratory for Sexually Transmitted Diseases (NLSTD) of the Laboratory Centre for Disease Control, Health Canada, has been monitoring gonococcal susceptibility to antibiotics since the 1970s to ensure that treatment recommendations are effective. In 1976, the first plasmid-mediated penicillinase-producing *N. gonorrhoeae* (PPNG) was identified and characterized at the NLSTD; in 1986, two cases of plasmid-mediated high level tetracycline-resistant *N. gonorrhoeae* (TRNG) were identified. Besides the PPNG and TRNG, chromosomal resistance is also a concern, since it has contributed to increased frequencies of treatment failure *(2)*.

The monitoring program has evolved as a result of the increased capability of public

FIGURE 1. Reported cases of *Neisseria gonorrhoeae* in Canada, from 1940 to 1996, by year

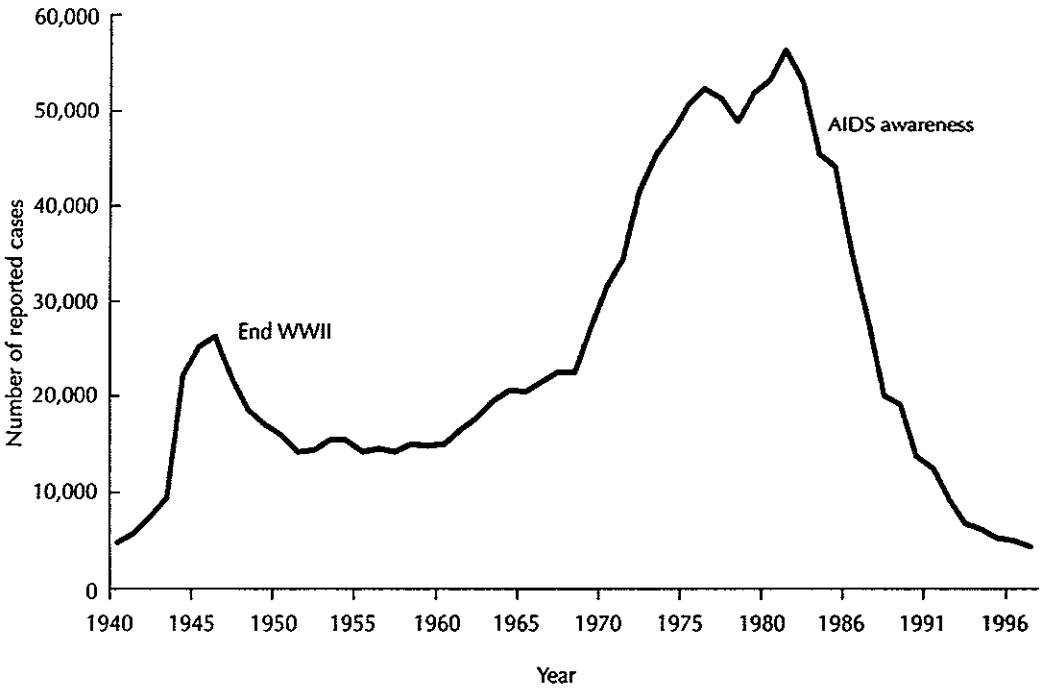
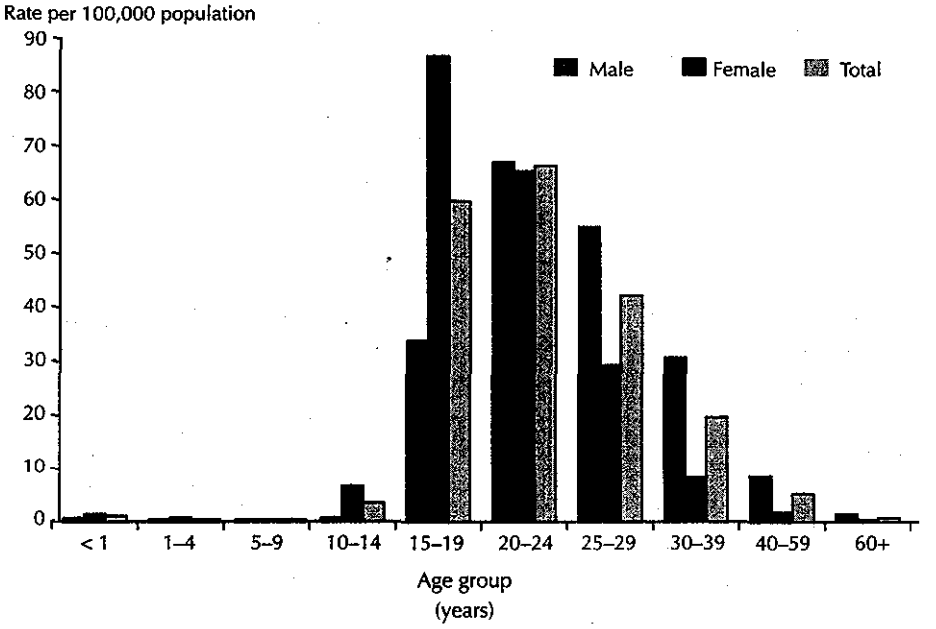


FIGURE 2. Distribution of cases of gonorrhea, by age and sex, in Canada, 1996



health laboratories throughout the country, and due to the use of different antibiotic therapies. At present, isolates from clinics and hospital laboratories are sent to the corresponding provincial public health laboratory or the local reference laboratory for susceptibility testing. Penicillin- or tetracycline-resistant and chromosomal-mediated resistant isolates are forwarded, on a voluntary basis, to the NLSTD for further characterization. In addition, all isolates from children less than 14 years of age are also forwarded to the NLSTD. To ensure that antimicrobial data are comparable across laboratories, a national proficiency program was implemented in 1992. As not all laboratories monitor the same panel of antibiotics, the NLSTD performed susceptibility tests for antibiotics not tested in other laboratories.

The NLSTD monitored resistance to penicillin, ceftriaxone, cefixime, tetracycline, spectinomycin, ciprofloxacin, erythromycin and azithromycin. Isolates were differentiated using auxotype, serotype and plasmid profile analysis. To confirm β-lactamase production in PPNG isolates, hydrolysis of nitrocefin was used. The mechanism of high level plasmid-mediated tetracycline resistance was confirmed by DNA hybridization using a DNA probe containing the *tet(M)* sequence, or by polymerase chain reaction (PCR). The latter method has been used since 1997, using primers described by Xia et al. which could distinguish plasmids of Dutch or American origin *(3)*.

GEOGRAPHICAL DISTRIBUTION OF RESISTANT *N. GONORRHOEAE* IN CANADA

In 1996, 69% of the 1,256 isolates received at NLSTD were from Ontario. Among the resistant isolates from Ontario, 71% were TRNG; 6.4%, PPNG; 8.5%, PP/TRNG; and 7.5%, chromosomal multiple-resistant *N. gonorrhoeae* (CMRNG). Tetracycline resistant *N. gonorrhoeae* in Ontario accounted for 97%

of all Canadian TRNG isolates received by the NLSTD during 1996. In contrast, resistant strains from Quebec showed a higher proportion of PPNG (34%) than TRNG (9.7%) and PP/TRNG (9.7%). Most of the resistant *N. gonorrhoeae* strains had chromosomal resistance (44%), but there was no CMRNG (multiple resistance to penicillin, tetracycline and erythromycin) reported from Quebec in 1996. The different types of resistance in Alberta and British Columbia were much lower and more evenly distributed than in Ontario and Quebec.

CHARACTERISTICS OF RESISTANT *N. GONORRHOEAE* ISOLATES IN CANADA

The characteristics of gonococcal resistance in Canada in 1995 may be summarized as follows. Most (81%) of the resistant gonococci are wild-type (auxotypes not requiring proline, NR), followed by proline-requiring auxotype (P^-)(8%) (Figure 3). The most common serotypes of resistant gonococcal isolates were 1B-1 and 1B-2, and correspond to the NR auxotype (Figure 4). The plasmid profiles of the most common auxotype/serotypes (A/S types), that is NR/1B-1 and NR/1B-2, were mainly comprised of strains containing both the cryptic plasmid (2.6 Mda) and the tetracycline-resistant (25.2 Mda) plasmids (Figure 5).

In contrast, the distribution of plasmid profiles (Figure 6) of P^- strains was different from the NR strains (Figure 5). The plasmid profile containing the 2.6 Mda and 25.2 Mda plasmids of TRNG was less dominant in P^- strains than in NR/1B-1 and NR/1B2 strains. In fact the most common plasmid profile (46%) contained only the cryptic 2.6 Mda plasmid (Figure 6) collected in 1995. Other plasmid profiles observed in P^-/1B-1, 1B-2 and 1B-3 strains included those that were associated with PPNG and PP/TRNG. The 4.5 Mda β-lactamase plasmid was most common amongst PPNG isolates, followed by the 3.2 Mda plasmid (Figure 6). Only the 4.5 Mda plasmid was found associated with the 25.2 Mda plasmid in PP/TRNG isolates (Figure 6).

FIGURE 3. Distribution of auxotypes of resistant *Neisseria gonorrhoeae* in Canada, 1995

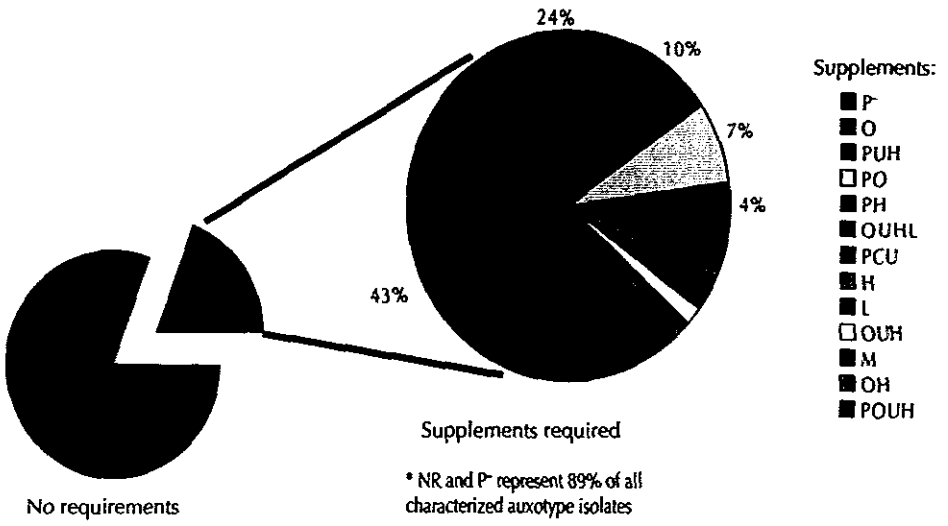
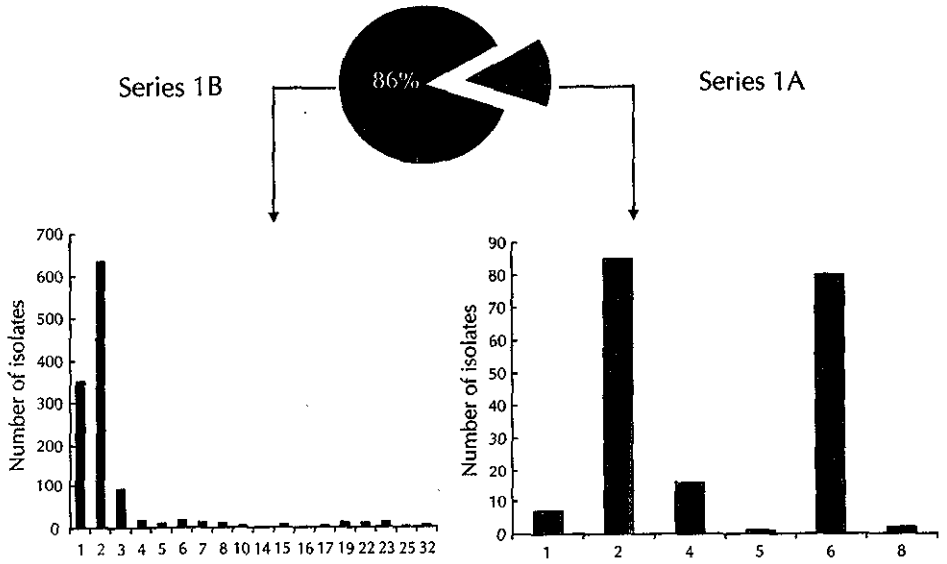


FIGURE 4. Distribution of serotypes of resistant *Neisseria gonorrhoeae* in Canada, 1995



PENICILLINASE-PRODUCING *N. GONORRHOEAE*

Following the first isolation of PPNG in 1976, the number of reported PPNG strains increased during the 1980s and in 1990, and represented approximately 7% of all reported cases. Concerns regarding treatment failure due to the high prevalence of penicillin resistance has resulted in the revision of case management guidelines in 1992 *(4)*. Penicillin has been replaced by third generation cephalosporins, such as ceftriaxone; penicillin was only recommended for use in areas where the prevalence of PPNG is less than 3% *(4)*. Recently, the reported rate of PPNG has decreased to 2.2% and 2.4% in 1995 and 1996, respectively. The decrease in prevalence of PPNG strains may be due, in part, to the change in the recommended therapy used in the country.

Plasmid profiles of the PPNG in Canada from 1991 to 1996 are shown in Table 1. The 3.2 Mda plasmid is the most prevalent β-lactamase-encoding plasmid found in Canadian isolates; and it may be found with (11% to 24.1% of isolates) or without (31.3% to 48.0% of isolates) the 24.5 Mda plasmid in gonococcal isolates (Table 1). The 3.05 Mda plasmid, which usually coexists with the 24.5 Mda plasmid, was less common during 1991 and 1992 among Canadian isolates. However, its prevalence increased during 1993 to 1996 (see Table 1). The importance of the 4.5 Mda plasmid seems to have fluctuated over time (see Table 1).

Phenotypic characteristics of the PPNG isolates are shown in Table 2. In recent years, about half of PPNG are NR. These NR isolates often corresponded to serotype 1B-1 or 1B-2. Proline-requiring auxotype was the next most common auxotype of PPNG. Although 1B-1 and 1B-2 were also found among P^- isolates, these serotypes were less prevalent than in the NR isolates.

FIGURE 5. The plasmid contents of resistant *Neisseria gonorrhoeae* isolates with NR/1B-1 o NR/1B-2 phenotypes

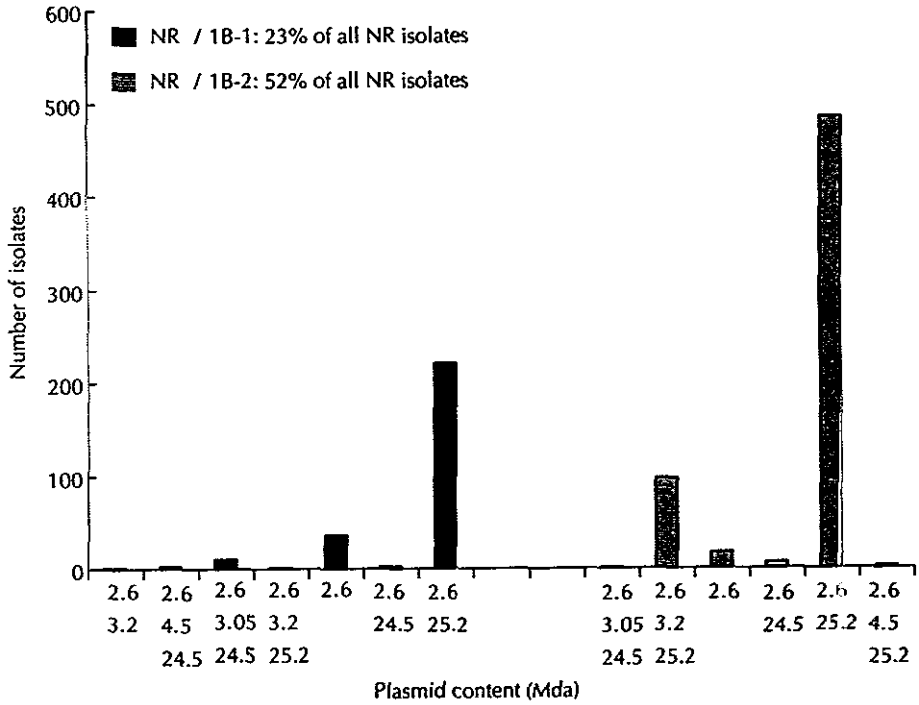


FIGURE 6. The plasmid contents of resistant *Neisseria gonorrhoeae* isolates with P⁻/1B-1, P⁻/1B-2 or P⁻/1B-3

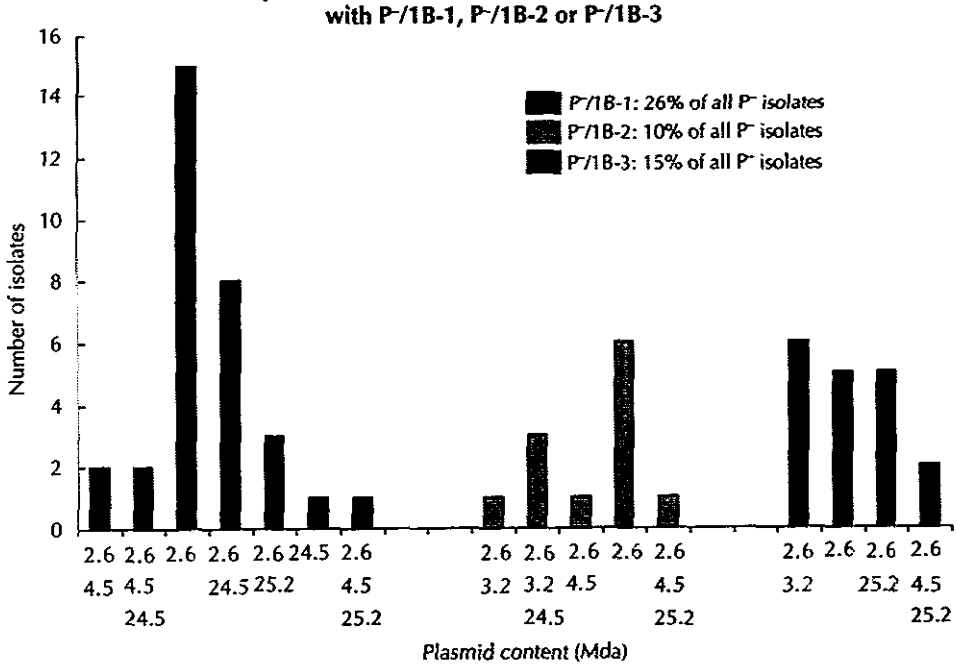


TABLE 1. The distribution of plasmid profiles of penicillase-producing *Neisseria gonorrhoeae* (PPNG) isolated in Canada, by year, between 1991 and 1996

Plasmid profile (size in Mda)	Percentage of PPNG isolates (by year)					
	1991 *n*=665	1992 *n*=572	1993 *n*=147	1994 *n*=163	1995 *n*=125	1996 *n*=121
2.6, 3.05, 24.5	3.8	3.0	17.0	15.0	19.0	12.4
2.6, 3.2	43.2	31.3	35.4	48.0	44.0	28.9
2.6, 3.2, 24.5	24.1	11.0	15.0	17.0	19.2	24.0
2.6, 4.5	20.5	10.5	15.0	10.0	11.2	9.9
2.6, 4.5, 24.5	8.6	44.2	17.3	10.0	6.4	24.8

TETRACYCLINE-RESISTANT *N. GONORRHOEAE*

High level tetracycline resistance was caused by the presence of *tet(M)* on the 25.2 Mda plasmid. Since the first isolation in 1986, there has been a steady increase in the isolation rates of TRNG, which reached about 2.6% in 1991. During the period 1992 to 1996, there was a sharp increase in the isolation rates of TRNG, peaking at 16% in 1995. In 1996, the rate was about 13%.

In the last five years, most of the TRNG have been a nonrequiring auxotype, often with serotype 1B-1 and 1B-2 (Table 2).

PENICILLINASE-PRODUCING AND TETRACYCLINE-RESISTANT *N. GONORRHOEAE*

Most PP/TRNG isolates (73% to 99%, 1991 to 1996) carry the 2.6, 3.2 and 25.2 Mda plasmid. The remainder have the 4.5 Mda plasmid instead of the 3.2 Mda plasmid. The tetracycline resistant 25.2 Mda plasmid was not found in other types of β-lactamase producing plasmids.

It is interesting to note that the auxotype/serotype classes of PP/TRNG were quite different in 1995 and 1996. There appears to have been a shift in the prevalence of strain types

TABLE 2. The five most common auxotypes and serotypes of penicillase-producing *Neisseria gonorrhoeae* (PPNG), tetracycline-resistant *Neisseria gonorrhoeae* (TRNG), and PP/TRNG isolates, Canada, 1994–1996

Resistance group	Rank	1994		1995		1996	
		Serotype	No.	Serotype	No.	Serotype	No.
PPNG	1	NR/IB-01	9	NR/IB-01	14	P⁻/IB-05	24
	2	NR/IB-02	8	NR/IB-03	8	NR/IB-01	20
	3	NR/IB-22	6	P⁻/IB-03	6	NR/IB-02	17
	4	P⁻/IA-06	6	NR/IA-06	6	P⁻/IB-02	13
	5	P⁻/IB-01	5	NR/IB-22	6	NR/IB-22	11
	6	Others	36	Others	47	Others	36
TRNG	1	NR/IB-02	600	NR/IB-02	485	NR/IB-01	328
	2	NR/IB-03	116	NR/IB-01	221	NR/IB-02	185
	3	NR/IB-01	116	NR/IA-02	54	NR/IA-02	57
	4	NR/IB-06	46	NR/IB-03	43	NR/IB-03	10
	5	NR/IA-02	31	NR/IA-06	12	PO/IB-11	6
	6	Others	85	Others	86	Others	48
PP/TRNG	1	NR/IB-02	51	NR/IB-02	99	NR/IA-03	18
	2	NR/IB-01	28	NR/IA-06	40	P⁻/IB-02	6
	3	NR/IA-06	14	NR/IB-03	7	NR/IB-03	6
	4	NR/IA-02	10	NR/IA-02	4	P⁻/IA-08	5
	5	NR/IB-08	8	P⁻/IA-06	4	O/IA-02	5
	6	Others	45	Others	35	Others	61

in Canada in 1996, with a smaller proportion of NR/1B-2 isolates than in 1994 and 1995.

CHROMOSOMAL RESISTANCE (CRNG AND CMRNG)

Chromosomal resistance in *N. gonorrhoeae* (CRNG) continues to cause concern. The emergence of ciprofloxacin resistance in different parts of the world alerted us to the cautionary use of this antibiotic. In 1989, six norfloxacin-resistant gonococci were isolated in Canada. These strains also had reduced susceptibility to lomefloxacin and ciprofloxacin, and all originated outside Canada. Five of these six CRNGs were from Asian countries. A study in Quebec conducted during 1994–1998 showed an emergence of decreased susceptibility to ciprofloxacin *(5)*. It was estimated that one out of every 100 isolates is resistant to ciprofloxacin, with the majority being of Asian origin *(5)*. To ensure that the prevalence of ciprofloxacin-resistant gonococcus remains low in Canada (Table 3), recommendations were made not to prescribe ciprofloxacin to patients who had traveled to Asian countries, where the incidences of quinolone resistance to gonococcus are high *(6)*. If either ciprofloxacin or ofloxacin were to be used in such cases, a test-by-cure is recommended *(6)*.

High levels of chromosomal resistance to penicillin, tetracycline and erythromycin (MIC ≥2 mg/L of each antibiotic, CMRNG) were also found in Canada. In 1996, 25% of the isolates tested at the NLSTD presented chromosomal resistance; amongst them, 5.5% were CMRNG. The highest incidence of CMRNG was observed between 1991 and 1994. The prevalence trend of CMRNG is similar to that of PPNG, a fact which may be due to reduced selective pressure of penicillin in the population, which, in turn, is probably due to changes in the recommended therapy for gonococcal infections. In Manitoba, resistant isolates were mainly PPNG; this province contributed about 21% of the Canadian PPNG isolates.

It should be noted that resistance to ceftriaxone or cefixime in Canadian isolates was not observed.

DISCUSSION AND CONCLUSIONS

The incidence of *N. gonorrhoeae* has been decreasing in the last decade. Incidence rates of infection were less than 17 per 100,000 population in the last few years. As a result, the consensus during the 1996 National Sexually Transmitted Diseases Forum was that the goal of eliminating endemic *N. gonorrhoeae* by 2010 is within reach *(7)*. One of the factors that

TABLE 3. Number of CMRNG* and *N. gonorrhoeae* isolates with reduced susceptibility to ciprofloxacin

		Number of isolates with RS or R† ciprofloxacin		CMRNG
Year	Reported cases	RS, MIC 0.125 to 0.5 (mg/L)	R, MIC ≥1.0 (mg/L)	No. of isolates
1989	19,110	2	0	60
1990	13,822	8	0	86
1991	12,457	0	0	184
1992	9,253	13	1	345
1993	6,832	30	2	186
1994	6,167	59	5	179
1995	5,715	36	15	147
1996	5,023	29	17	70

*CMRNG = gonococcus with MIC for penicillin, tetracycline and erythromycin ≥2 mg/L.
†RS = reduced susceptibility; R = resistance. The breakpoints are based on NCCLS 1998 criteria. *(8)*

could prevent the national goal from being achieved is antimicrobial resistance. Therefore, ongoing surveillance of antimicrobial resistance is vital to ensure that the effectiveness of current antimicrobial therapies remains. We have been fortunate in that the current recommended single dose therapy, i.e., ceftriaxone or third-generation cephalosporins, has not resulted in the emergence of pathogens resistant to these antibiotics, and in that there is no endemic ciprofloxacin resistant *N. gonorrhoeae*. Almost all of the isolates with reduced susceptibility or resistance to this drug were of Asian origin.

Another challenge of monitoring *N. gonorrhoeae* resistance in the next few years, is that some laboratories may use nonculture methods, e.g., PCR, to detect these organisms. Nonculture methods are much more sensitive for *Chlamydia* and the use of a dual test for both gonococcal and *Chlamydia* infections may be more practical. In view of the potential shift in techniques used for the diagnosis of gonorrhea, a plan to develop a nucleic acid-based method to monitor *N. gonorrhoeae* in the Canadian population is in progress.

Serotyping, together with auxotyping, has been useful for the differentiation of *N. gonorrhoeae* isolates. Unfortunately, commercial monoclonal antibodies are no longer available for serotyping. This is another reason for developing molecular methods for laboratory surveillance of this organism.

The antimicrobial resistance information collected at the NLSTD has been provided to public health laboratories and the Division of Sexually Transmitted Diseases Control of LCDC for policy and decision making by public health officials, especially in regard to recommendations for antimicrobial therapy for *N. gonorrhoeae*.

ACKNOWLEDGMENTS

I would like to thank Annie Savoie, Denise Shewchuk, Diane Ward and Chris Sheardown for their technical support and preparing the figures for this paper. I also wish to thank Louise Cormier and Tom Wong, Division of STD, Laboratory Centre for Disease Control, Health Canada, for providing data on the incidence of gonorrhea in Canada.

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LABORATORY NETWORK FOR MONITORING ANTIMICROBIAL RESISTANCE WHONET-ARGENTINA PROGRAM: RESULTS OF FIVE YEARS OF OPERATION

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In the past two decades there has been a dramatic increase in the proportion of microorganisms resistant to antibacterial drugs, including the causative agents of infections acquired in the community. This phenomenon may be due to the misuse of antimicrobial agents, but there is clear evidence that it is taking place at a much more alarming rate in some regions than in others.

The application of regional strategies to achieve more appropriate use of antimicrobial agents entails, among other things, the development of reliable programs to monitor the degree of bacterial resistance to the drugs of greatest clinical interest. This information also makes it possible to focus attempts to limit development of new resistance mechanisms or large increases in existing resistance *(1, 2)*.

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In Argentina, the national laboratory network began to take shape in 1994. At first, the network consisted of centers in the Federal Capital; today it has 33 participating institutions throughout the country (Figure 1). Coordination has been entrusted to the Antimicrobial Service of the Bacteriology Department, Dr. Carlos G. Malbrán National Institute of Infectious Diseases (ANLIS), with the collaboration of an advisory committee.

In order to standardize the methodology and criteria for the interpretation of results, the coordinating center regularly offers a course on theory and practice, attended by at least one staff member from each participating laboratory, that presents the latest information on antimicrobial agents. In addition, a workshop is held once a year to discuss laboratory results and possible sources of methodological error or quality control problems. All the laboratories use the same manual of procedures, which includes a list of the drugs to be tested on each group of microorganisms.

The coordinating laboratory receives samples of any bacteria that present unusual resistance profiles or require confirmation. These are assayed using quantitative and/or molecular techniques to identify the resistance mechanisms (for example, detection of the *mec*A gene in *Staphylococcus* spp.; identification of extended-spectrum β-lactamases (ESBla) in enterobacteria; detection of *van*A, *van*B, *van*C1, or *van*C2 genes in *Enterococcus* spp.). This review by the coordinating laboratory brings to light new mechanisms of resistance and the spread of existing ones so that appropriate warnings can be issued.

The network laboratories are subject to two external quality control programs. The first is the National Program for Bacteriological Quality Control, coordinated by ANLIS and the Buenos Aires Provincial Public Health Laboratory. This program sends bacterial isolates to participating laboratories for biochemical characterization and determination of antimicrobial susceptibility. Each participating laboratory receives an analysis of their own results, including appropriate pointers on possible sources of error. The program also circulates the latest international recommendations on the interpretation of antimicrobial susceptibility tests, as well as bibliographic information on recent publications in the area of clinical microbiology.

The other quality control program is the External Quality Assurance Scheme in Antimicrobial Susceptibility Testing (EQAS AST), developed by the World Health Organization and the U.S. Centers for Disease Control and Prevention (CDC). Strains for this program are distributed to the participating laboratories, and the results and respective analyses are submitted to WHO and the CDC for review.

Reference strains from the ATCC collection are distributed regularly for internal quality control of antimicrobial susceptibility testing. The results are incorporated into the computerized information system, which uses WHONET, the database management program developed by the World Health Organization.

Each participating laboratory submits, on a monthly basis, the results from all antimicrobial susceptibility tests performed following the recommendations of the United States National Committee for Clinical Laboratory Standards (NCCLS) on agar diffusion testing. The coordinating laboratory analyzes the information; prepares detailed comments on the results, indicating those that need to be confirmed; and analyzes the tests performed for purposes of internal quality control.

Since 1998, the WHONET-Argentina network has participated in two Latin American programs for the monitoring of resistance, namely: Monitoring of Enteropathogen Resistance in Latin America, a project organized by PAHO in collaboration with the Canadian Laboratory Center for Disease Control (LCDC), the latter acting in its capacity as an international reference center, and the Latin American Network for Monitoring Antimicrobial Resistance (RESISTNET) coordinated by the Pan American Association for Infectious Diseases, PAHO, and Pfizer Laboratories International.

FIGURE 1. WHONET Laboratory Network, Argentina, 1998

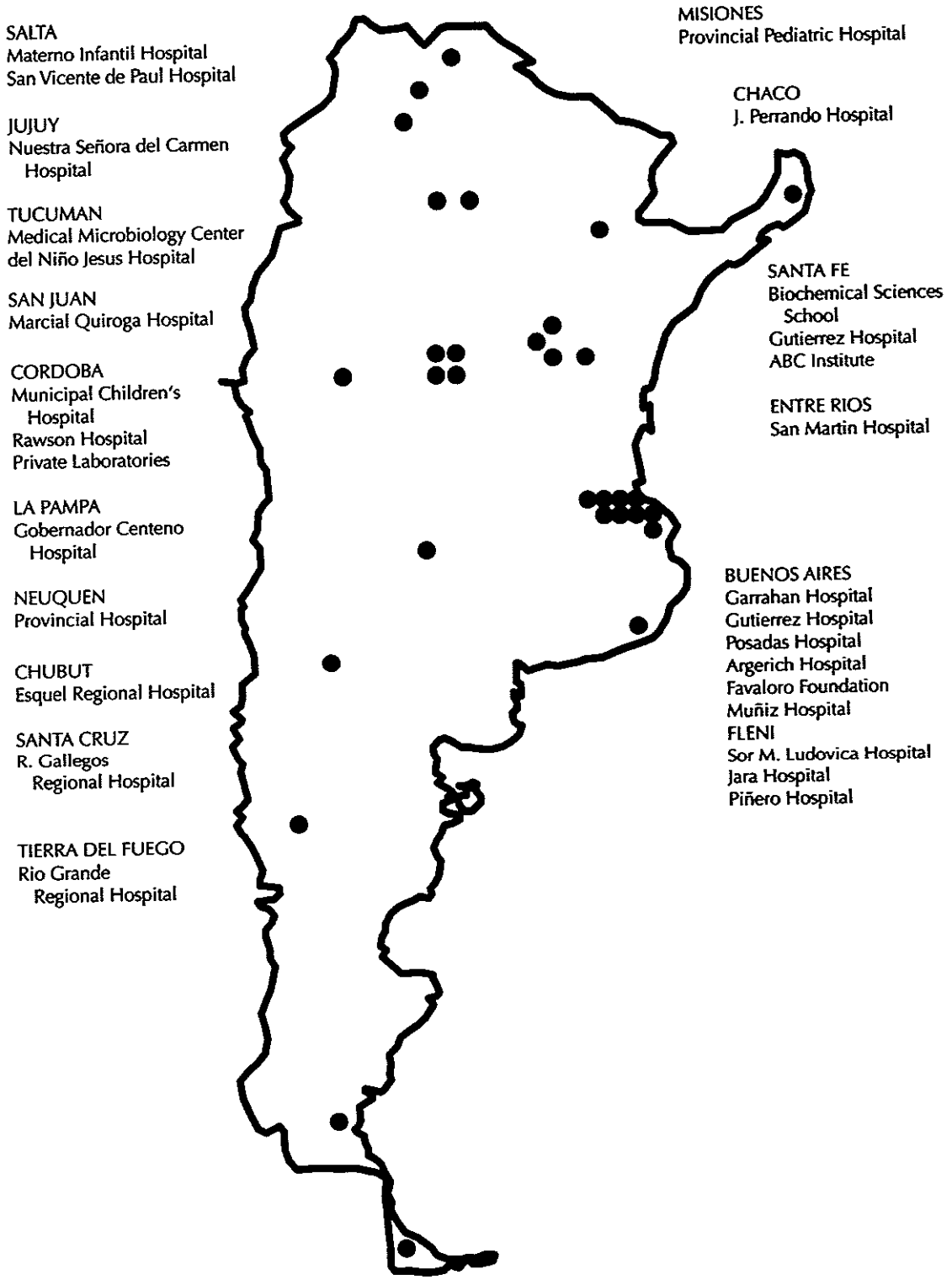


Figure 2 shows the results obtained by the program in 1994–1998 for the microorganisms of greatest clinical interest.

ENTEROBACTERIA

Approximately half the microorganisms studied were from the family *Enterobacteriaceae*. In general, resistance to the β-lactam and aminoglycoside antibiotics was very high, particularly among hospitalized patients (data not shown). In 1997, with the participation of network laboratories, a study was conducted to identify the enzymatic and impermeability mechanisms involved in resistance to third- and fourth-generation cephalosporins and monobactams. A total of 622 enterobacteria isolates were collected, with inhibition area diameters of ≤27 and ≤22 mm produced by cefotaxime and ceftazidime, respectively. Quantitative sensitivity tests were performed on these microorganisms. As part of characterizing the β-lactamases present, the isoelectric point of crude enzymatic extracts was determined for each of the isolates. In addition, hybridization and polymerase chain reaction (PCR) tests were performed using probes and specific nutrients for TEM, SHV, CTX-M, PER, AMP-C, and PSE β-lactamase families. Also, given the suspected existence of an impermeability phenomenon, electrophoresis tests were conducted on outer membrane proteins. The results are presented below in the sections corresponding to each of the species.

During 1994–1997 no resistance to carbapenems was found in this family of microorganisms. In 1998, through the network, the first isolates with reduced susceptibility to these drugs were observed, as follows: one isolate of *Enterobacter cloacae*, three of *Enterobacter aerogenes*, one of *Enterobacter ammenigenus*, one of *Citrobacter freundii*, one of *Serratia marcescens*, and five of *Klebsiella pneumoniae*.

The characteristics of the resistance profiles of several members of this family are described below.

Escherichia coli

The levels of antimicrobial resistance remained almost constant during the first three years, with slight increases in resistance to

FIGURE 2. Percent resistance to antimicrobial drugs among bacterial pathogens of clinical interest, Argentina, 1994–1998

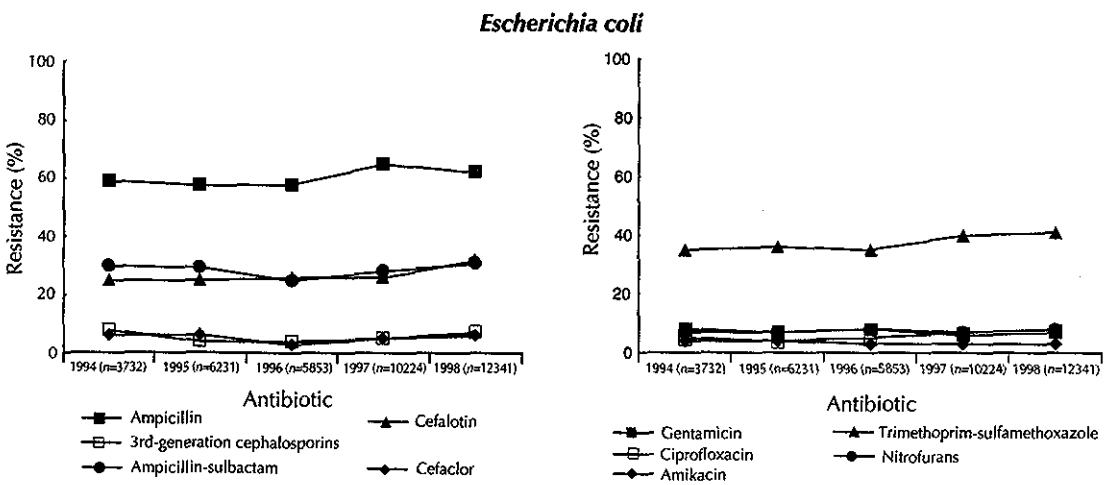
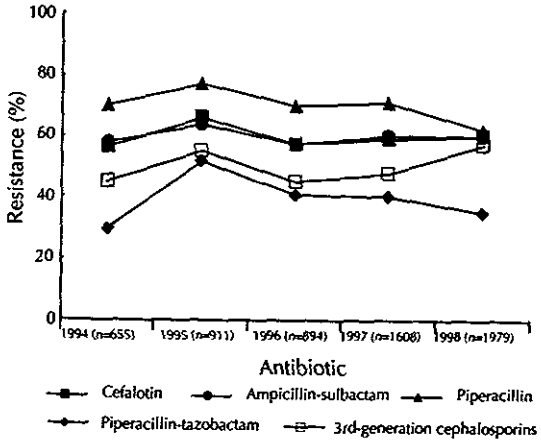
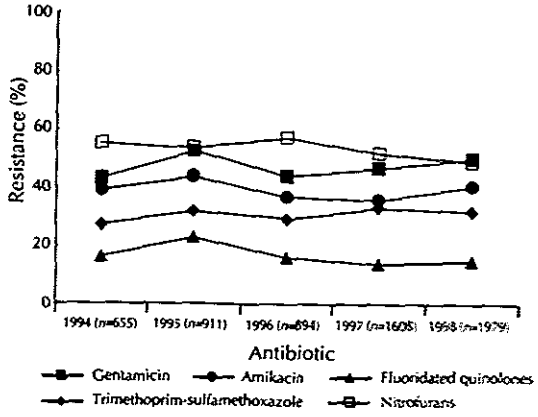


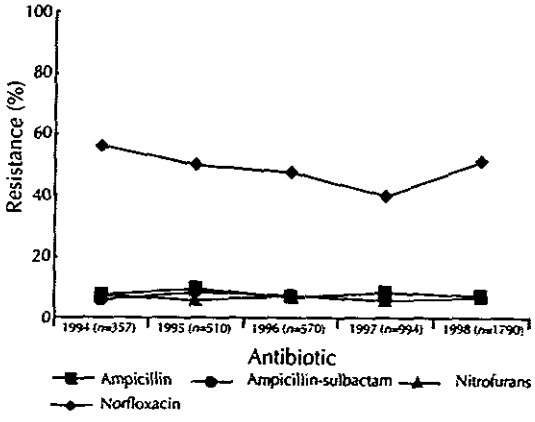
FIGURE 2 *(continued)*

Klebsiella pneumoniae

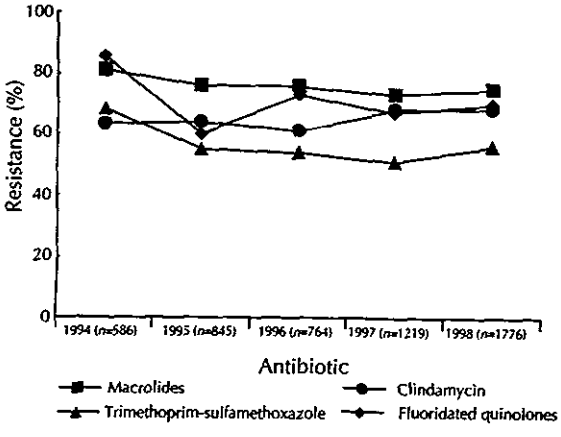




***Enterococcus* spp.**



Methicillin–resistant *Staphylococcus aureus*



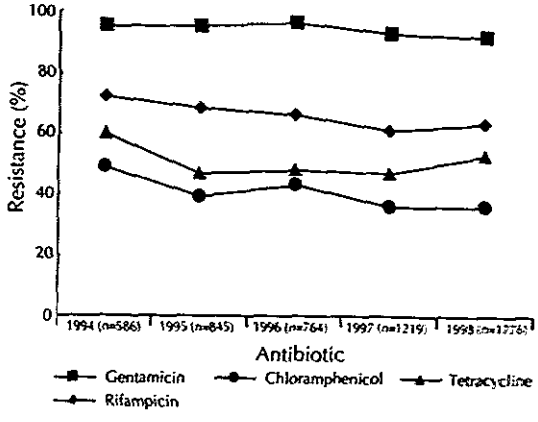
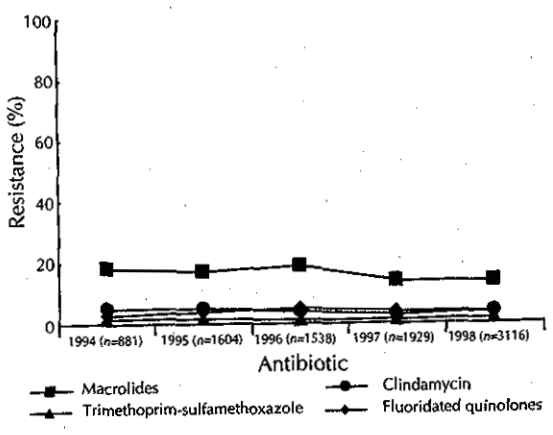
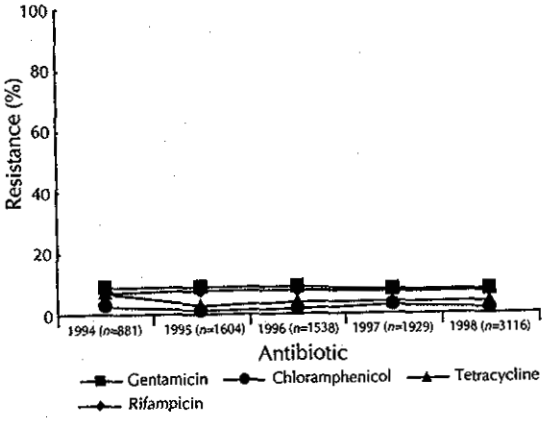


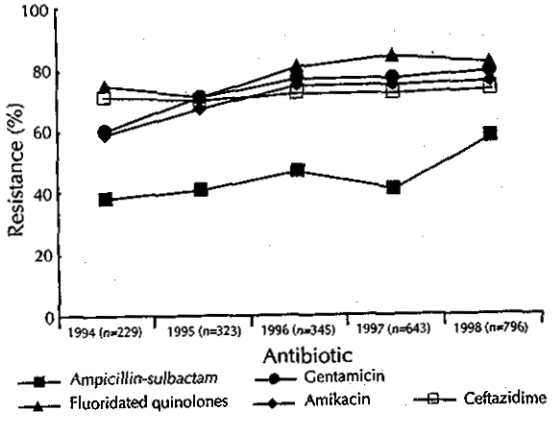
FIGURE 2 *(continued)*

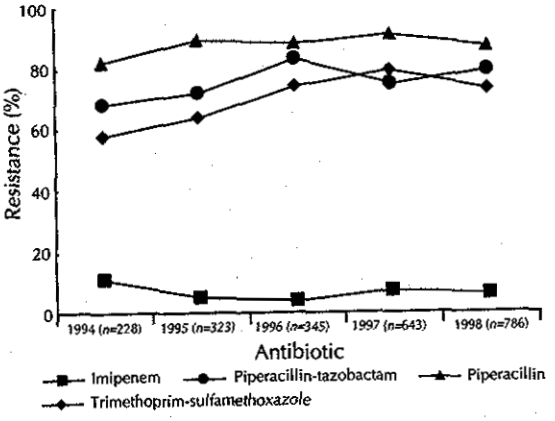
Methicillin-susceptible *Staphylococcus aureus*



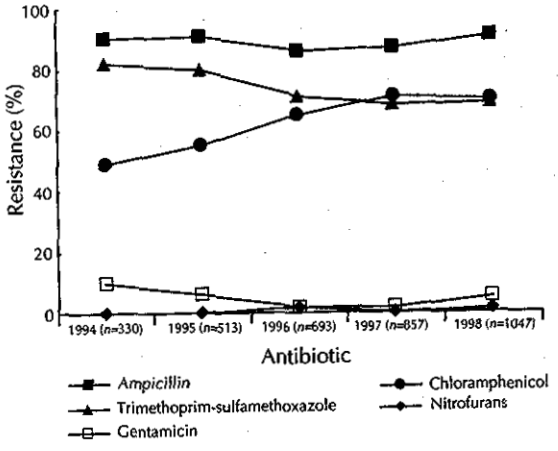


***Acinetobacter* spp.**





Shigella flexneri



Shigella sonnei

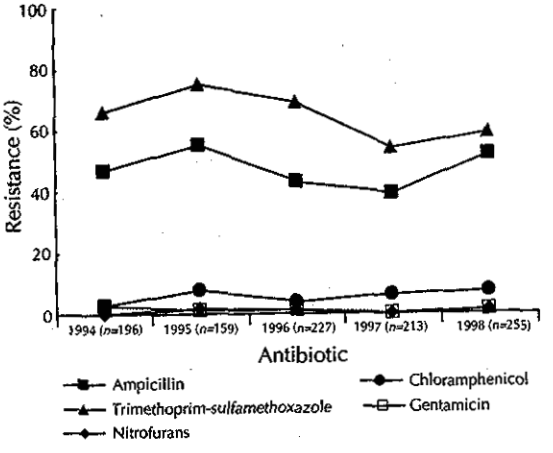
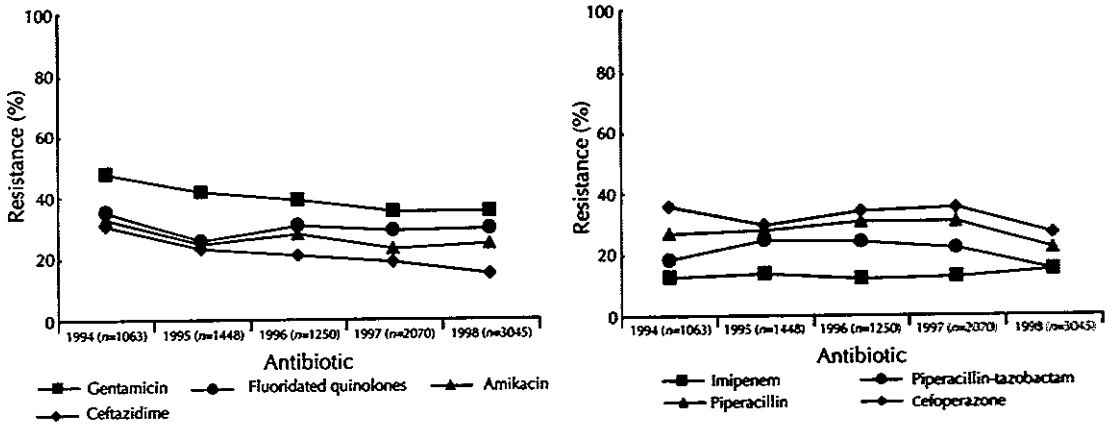
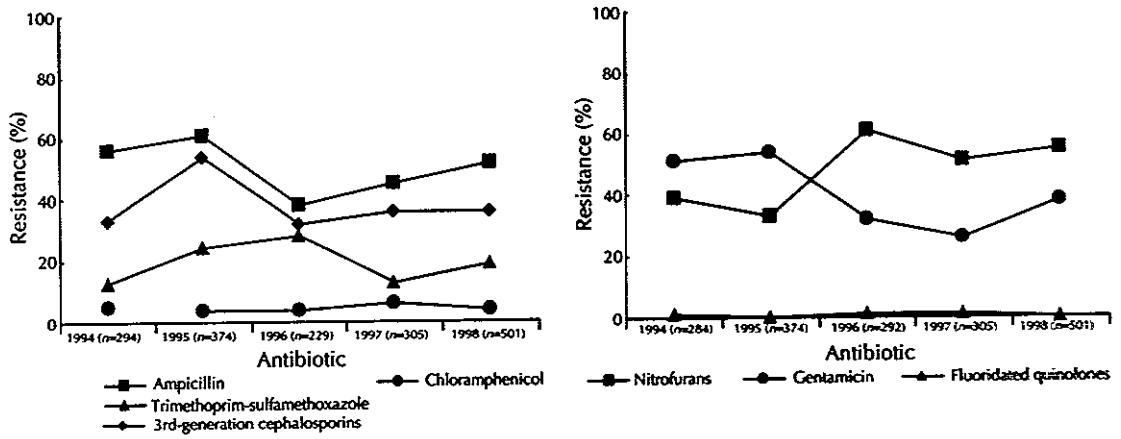


FIGURE 2 *(continued)*

Pseudomonas aeruginosa



***Salmonella* spp.**



Salmonella enteritidis

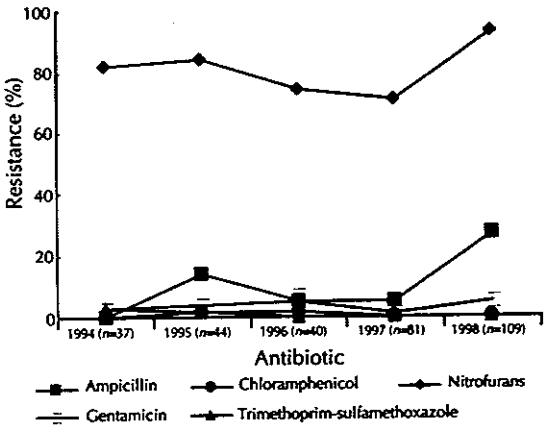
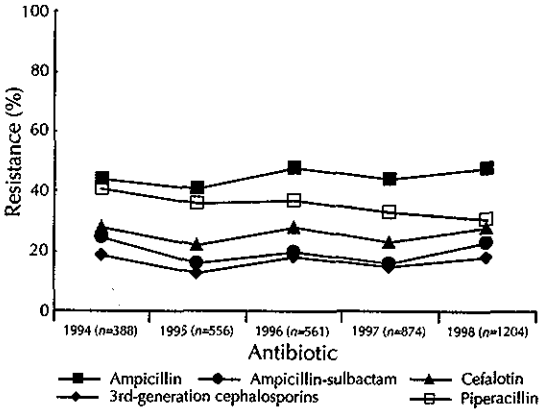
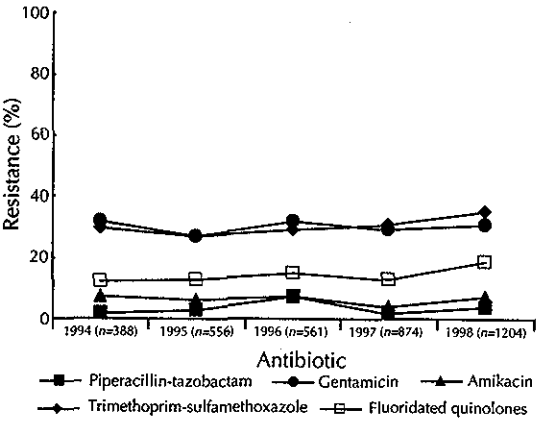


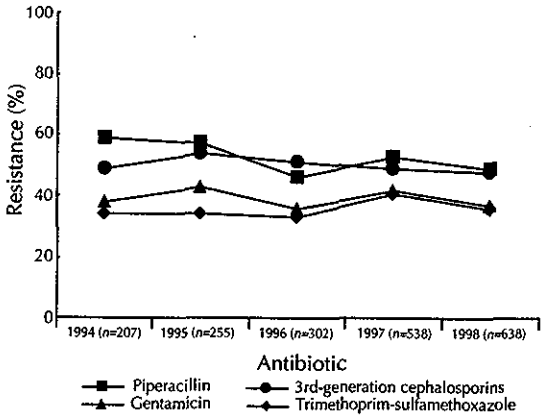
FIGURE 2 *(continued)*

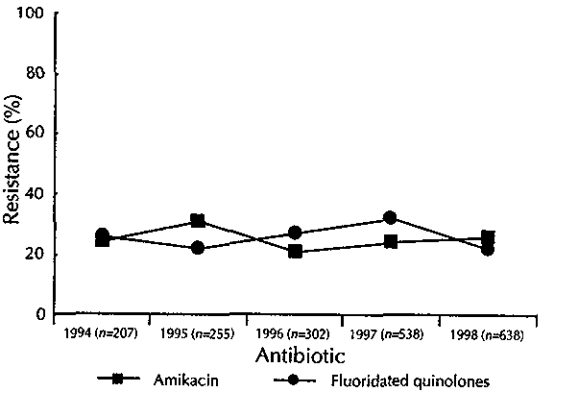
Proteus mirabilis



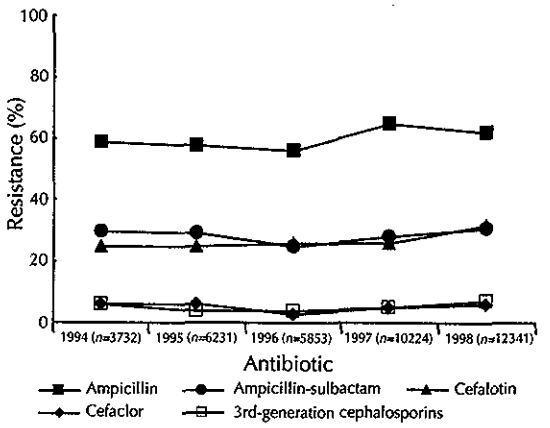


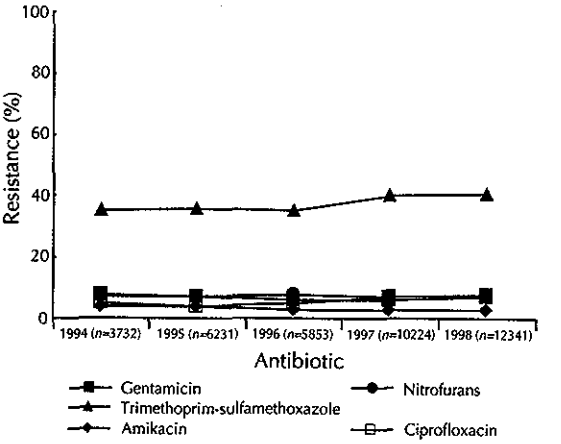
Enterobacter cloacae





Escherichia coli





ampicillin, ampicillin-sulbactam, cefalotin, and cotrimoxazole in 1997 and 1998.

A considerable number of isolates presented resistance to broader-spectrum drugs such as third-generation cephalosporins (TGC) or aminoglycosides. Studies conducted in the United States of America and Canada showed resistance levels of 0.6% and 5.7% to TGC and gentamicin (GEN), respectively *(3)*. A 1997 Argentine study of 80 TGC-resistant isolates revealed that in 19%, the resistance was mediated by overproduction of the chromosomal cephalosporinase (type AMP-C) characteristic of the species, and 81%, by extended-spectrum β-lactamases—specifically, CTX-M-2, PER-2, SHV-2, and SHV5 *(4)*.

Among the oral antibiotics, cefaclor, axetil, cefuroxime, nitrofurans, and fluoridated quinolones retained considerable activity, above 92% in all cases.

Klebsiella pneumoniae

Third-generation cephalosporins and aminoglycosides showed very little activity on *Klebsiella* isolates. Moreover, during 1995 four hospitals (three in Buenos Aires Province and one in Misiones Province) had an increase in the proportion of isolates resistant to both groups of drugs, probably related to outbreaks of nosocomial infections.

Molecular studies conducted on 156 isolates of *K. pneumoniae* indicated that 69% of them carried β-lactamase CTX-M-2, while in the remainder there was a distribution between enzymes such as PER-2 (9%), SHV-2 (11%), and SHV-5 (9%). There was no evidence of ESBla from the TEM family, as has been reported in some of the European and North American countries *(5)*. Although 7% of the isolates analyzed were resistant to cefoxitin, none showed enzymatic activity with that drug. Analysis of outer membrane proteins suggests the existence of impermeability phenomena in these isolates.

Isolates collected from pediatric hospitals showed <5% resistance to fluoridated quinolones, which is very low compared with the levels found in general hospitals (>15%). This difference could be due to the fact that fluoridated quinolones are not approved for use in pediatric practice because of their toxic effects on bone development.

Proteus mirabilis

The aminopenicillins and acylureidopenicillins were more active on *P. mirabilis* than on *E. coli*, whereas with third-generation cephalosporins and fluoridated quinolones, the situation was reversed. First-generation cephalosporins were equally active on both groups of microorganisms, as was amikacin. With gentamicin, however, *P. mirabilis* showed percentages of resistance four times higher than those of *E. coli*.

Piperacillin-tazobactam was the second most active drug against this enterobacterium, after the carbapenems. In this bacterial species, resistance to β-lactam antibiotics conferred by broad- or extended-spectrum plasmid β-lactamases is low, and it can be effectively reversed by the addition of an enzyme inhibitor such as tazobactam.

Enterobacter cloacae

Approximately half the isolates of *E. cloacae* were resistant to third-generation cephalosporins, and about 40% were resistant to gentamicin. In studies of 79 TGC-resistant isolates, derepression of chromosomal β-lactamase was the only mechanism of resistance found in more than half of them. The rest carried plasmid ESBla such as PER-2, CTX-M-2 or SHV-2, and SHV-5. This distribution is atypical since, although ESBla have been described in this species in different regions of the world, the finding continues to be highly unusual *(6)*.

The activity of fluoridated quinolones was also low (lower than for the rest of the enterobacteria), comprising about one-third of the microorganisms.

Salmonella spp.

The activity of antimicrobial agents on this genus varies widely, depending mainly on the serotype and the origin of the isolates—i.e., whether they come from a hospital or the community.

A high proportion of *Salmonella* spp. isolates were resistant to TGC (99% when in association with aminoglycoside resistance), particularly during 1995. These were mainly isolates from nosocomial infections in pediatric hospitals. One group of 160 microorganisms showed, in all cases, the presence of a high-molecular-weight transferable plasmid containing the genes $bla_{CTX-M-2}$, aac(6′)-I, and aac(3)-II, which were responsible for the resistance to third-generation cephalosporins and aminoglycosides (7).

A major loss of activity with nitrofurans began to appear in 1996.

Salmonella enteritidis has been isolated essentially from the adult population in general hospitals, and it rarely requires antimicrobial treatment. It is susceptible to most of the drugs of clinical interest, except for nitrofurans. During 1998 a sharp increase in resistance to ampicillin was observed in this serotype.

There have been a few isolates of *Salmonella* spp. that were resistant to fluoridated quinolones, but these were highly unusual.

Shigella flexneri and *Shigella sonnei*

Ampicillin and cotrimoxazole showed low activity with both species of *Shigella* spp. The isolates of *S. flexneri* were noticeably less susceptible to these two drugs and also to chloramphenicol. For this reason, it is difficult to establish an initial empiric therapy for patients in need of treatment with antimicrobial agents, especially since nitrofurans and fluoridated quinolones may have to be ruled out, the former because of poor patient tolerance and the latter because they have not been approved for pediatric use. However, both of these drugs have excellent levels of activity and have been associated with very few resistant isolates.

The aminoglycosides and first- and second-generation cephalosporins are not effective for the treatment of bacterial diarrheal diseases. It should be pointed out that the frequent appearance of strains resistant to both groups of agents is a sign of the extent to which the corresponding resistant genes have spread.

NONFERMENTING GRAM-NEGATIVE BACILLI

Pseudomonas aeruginosa

Unlike the situation with enterobacteria, in the majority of hospitals that participated in this program there has been a reduction in the resistance of *P. aeruginosa* to most of the drugs in question, particularly gentamicin and ceftazidime. Nevertheless, resistance to imipenem remained constant throughout the period under study.

With variations from year to year, between 8% and 12% of the isolates of *P. aeruginosa* presented simultaneous resistance to ceftazidime, gentamicin, and fluoridated quinolones; between 30% and 50% of these multiresistant isolates were also resistant to imipenem, and it has therefore been very difficult to find therapeutic options.

The drug cefepim, which has been included in the protocol since 1998, and imipenem were the most effective agents against *P. aeruginosa*, with 16% and 14% resistance, respectively.

Acinetobacter spp.

Infections caused by these microorganisms, especially *A. baumannii*, pose a real challenge for hospital health teams, given their growing resistance to antibacterial agents.

Unlike the case of *P. aeruginosa*, these species have been showing a marked rise in resistance to all the drugs tested. During the period in question, the only antimicrobial agent that retained its activity was imipenem.

Aminoglycosides showed very poor activity. Unlike the situation with the other fami-

lies studied, there was no dissociation between the activity of gentamicin and amikacin.

Sulbactam by itself is active in the presence of *Acinetobacter* spp., but, in Argentina, when combined with ampicillin, resistance increased from 40% in 1994 to 60% in 1998. The rest of the β-lactam antibiotics were even less active. The activity of fluoridated quinolones was also low.

GRAM-POSITIVE COCCI

Staphylococcus aureus

Resistance to oxacillin, and thus to the activity of β-lactam antibiotics, varied considerably from one institution to another. This resistance was closely tied to resistance to gentamicin, the macrolides, tetracycline, rifampicin, clindamycin, cotrimoxazole, fluoridated quinolones, and chloramphenicol. As was to be expected, the conventional fluoridated quinolones had low activity on Gram-positive bacteria. Trovafloxacin was tried in 1998 and demonstrated a broader spectrum of activity against these microorganisms; only 5% of the isolates were reported to be resistant.

Through molecular epidemiology studies (PFGE), the prevalence of two methicillin-resistant clones of *Staphylococcus aureus* was detected in Argentina: a multiresistant one previously described in Brazil and another one, prevalent in pediatric hospitals, which was resistant only when methicillin was used in combination with gentamicin *(8)*. Reduced susceptibility to glycopeptides was not detected.

Enterococcus spp.

Probably because of the proportion of isolates of *E. faecium* present in the sample, resistance to ampicillin and ampicillin-sulbactam was consistently lower than 10%. Half the isolates were resistant to the conventional fluoridated quinolones, and 26%, to trovafloxacin, which was tried in 1998. High levels of resistance to gentamicin and streptomycin, evaluated with high-powered disks, was found in 22% and 25% of the isolates, respectively.

Fortunately, vancomycin-resistant isolates of *Enterococcus* spp., causative of infectious processes, were not reported until 1998.

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OVERVIEW OF ANTIMICROBIAL RESISTANCE BY *SHIGELLA* SPP. IN 10 CHILEAN HOSPITALS: THE PRONARES PROJECT[1]

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Shigella *infections are a frequent cause of morbidity and mortality among Latin American children. In Chile,* Shigella *infections are responsible for some 4% to 12% of the cases of acute diarrhea of varying degrees of severity and 22% to 30% of the episodes of bloody diarrhea among children under 5 years of age, depending on the socioeconomic level of the population studied and whether the surveillance is active or passive.*

The emergence and spread of antimicrobial resistance in strains of Shigella *is a real problem that complicates the treatment of serious cases. In order to gain an overview of resistance in* Shigella *strains isolated in Chile, a surveillance program using a common protocol was set up in 10 hospitals in November 1997. The results were analyzed using the WHONET software. A total of 495 isolates were studied in the course of 12 months of surveillance, 395 of them from children.* Shigella flexneri *was the most frequent species in adults and* Shigella sonnei *was the most common in children. In the* Shigella flexneri *isolates the percentages of resistance observed were 87% for ampicillin, 76% for cotrimoxazole, 73% for tetracycline, and 52% for chloramphenicol. These figures compared with only 4% resistance to furazolidone and 0% to ciprofloxacin. The* Shigella sonnei *isolates were highly resistant to ampicillin (91%), tetracycline (75%), and chloramphenicol (81%), compared with 1% resistance to furazolidone and 0% to ciprofloxacin. The resistance patterns were similar in the different regions of the country, which indicates that this is a generalized phenomenon with only slight regional differences. The most frequent pattern was the association of genes resistant to ampicillin, chloramphenicol, and cotrimoxazole. It is very necessary to establish surveillance systems to monitor the resistance of* Shigella *spp. to the clinically useful antimicrobial drugs, which would appear to be the only way to provide guidelines for effective empirical treatment in each situation that may arise.*

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INTRODUCTION

Shigellosis is a major cause of diarrhea in both the developing and industrialized countries *(1–8)*. In Chile, *Shigella* infections are responsible for some 4% to 12% of acute cases of diarrhea and 22% to 30% of bloody diarrheal cases in children under 5 years of age, depending on the socioeconomic situation of the population studied and whether the monitoring is active or passive *(1–3)*.

The clinical shigellosis picture covers a spectrum of symptoms ranging from watery diarrhea with or without fever to the classical dysenteric syndrome, which can entail serious complications *(9, 10)*.

The distribution of *Shigella* species and serotypes varies from one geographical area to another. In Latin America the two predominant species are *Shigella flexneri* and *S. sonnei*, whereas in the industrialized countries *Shigella sonnei* is the species isolated most frequently. *Shigella boydii* remains confined to the region of India and is isolated only sporadically in other areas. *Shigella dysenteriae* type 1, the most virulent of all the serotypes, is rare in the Americas, but there are occasional outbreaks that produce high mortality. The last reported epidemic occurred in Mexico and Guatemala in 1969 and spread rapidly to the rest of Central America and subsequently to Africa and India, where it has become an endemic pathogen. In Chile, *S. sonnei*, *S. flexneri* 2, and *S. flexneri* 6 account for more than 80% of the serotypes isolated *(1–3)*.

The main problem currently associated with shigellosis is undoubtedly its treatment, even though it is one of the enteric infections for which the use of antimicrobials is widely accepted and recommended, since it has been demonstrated that proper antibiotic therapy shortens the duration and severity of diarrheal symptoms, reduces the bacteria-shedding time, and also reduces potential complications of the disease *(9–11)*. Studies conducted in Europe, Africa, and North America have shown high percentages of *Shigella* strains resistant to various, commonly used antimicrobial drugs such as ampicillin, cotrimoxazole, and chloramphenicol, with levels as high as 100%, 96%, and 65%, respectively. In addition, a considerable number of multiresistant strains have been identified *(4, 12–16)*. In Latin America there is only limited information available in this regard, although studies conducted in Bolivia and Brazil already show a high percentage of multiple resistance *(6, 17)*.

In Chile the resistance of *Shigella* was monitored from 1995 until 1997 in a community north of Santiago, where the situation was similar to the one described above, with high percentages of resistance to the antibiotics customarily used in the country. In this study, the discovery of 69% resistance to chloramphenicol, a first-line drug for the treatment of shigellosis, is a significant event affecting the situation of this disease in Chile. Moreover, 49% of the isolates were multiresistant—i.e., resistant to one or more antimicrobial drugs *(18)*.

Given these circumstances, the care and treatment of shigellosis has become considerably more complicated. The present situation reduces the possibility of setting standards for empirical treatment that will ensure effective results. With regard to the resistance of *Shigella* strains, there is no information available in Chile that would allow an overview of the situation nationwide. Although data exist in the files of individual laboratories, since there is no common code, it is difficult to integrate them and compare the results for the entire country. For this reason, through the National Program for the Surveillance of Resistance (PRONARES Project) and using the WHONET software, we undertook to monitor the antimicrobial resistance of *Shigella* in 10 hospitals representing different regions of the country so as to obtain results that would reflect the situation in Chile as a whole. The objectives of the study were:

a) To determine and compare the percentage of antimicrobial resistance in strains of *Shigella flexneri*, *S. sonnei*, and *S. boydii* isolated in 10 hospitals located in different regions of Chile; and

b) To analyze the pattern of resistance in the *Shigella* strains isolated in Chile.

MATERIALS AND METHODS

In November 1997 the PRONARES Project started making arrangements to monitor the antimicrobial resistance of pathogenic bacteria associated with various diseases, including *Shigella*, in 10 general hospitals located in different parts of the country. The hospitals selected were representative of different Chilean provinces. Two were in the northern area (Antofagasta and Iquique Hospitals), four in the central area (Gustavo Fricke Hospital in Viña del Mar and Félix Bulnes Hospital, Roberto del Río Hospital, and the Military Hospital, in Santiago), and four in the southern area (Chillán, Talcahuano, Temuco, and Osorno Hospitals).

The study of antimicrobial susceptibility was conducted locally using the agar gel diffusion technique, Kirby-Bauer method. The inhibition halo of each isolate was tested for the following antimicrobial drugs: ampicillin, tetracycline, ciprofloxacin, chloramphenicol, cotrimoxazole, nalidixic acid, and furazolidone, following the recommendations of the U.S. National Committee for Clinical Laboratory Standards (NCCLS), with some variations in the selection of antibiotics at the local level (*19*). The corresponding halos were entered on an ad hoc form and sent to the Eastern Microbiology Unit at the University of Chile, where they were entered into the WHONET database.

The following steps were taken to standardize the technique and ensure quality control: use of a manual of procedures and standardized rules for measurement, local visits, periodic checks against ATCC reference strains (*Escherichia coli* 25922, *Staphylococcus aureus* 29213, *Pseudomonas* aeruginosa 27853, *Haemophilus influenzae* 49247, *Streptococcus pneumoniae* 49619, *Enterococcus faecalis* 29212). External microbiological control, which consisted of sending unidentified isolates to participating centers, was the responsibility of the Eastern Microbiology Unit.

The strains were classified as sensitive, intermediate, or resistant, following the NCCLS standards published in 1998 and already incorporated in WHONET.

The percentage of resistance to each antibiotic was calculated, and the profiles of resistance of the various *Shigella* species were analyzed.

RESULTS

The results of the first 12 months of surveillance in the 10 Chilean hospitals were studied.

A total of 495 *Shigella* isolates were obtained in the 10 hospitals during the surveillance period, 395 from pediatric patients and 100 from adults. Table 1 shows the distribution of the different types of *Shigella* by age of the patients. *Shigella flexneri* was the species isolated most frequently from adult patients, and *Shigella sonnei* was the one found most often in pediatric patients. There were 30 *Shigella* isolates for which the species could not be determined, and these have been indicated merely as *Shigella* sp.

Of the total of 219 *Shigella flexneri* isolates obtained in the 10 participating institutions during the surveillance period, 215 were studied in vitro to determine their susceptibility to chloramphenicol; 218 were tested against ampicillin; 216 against cotrimoxazole; 198 against furazolidone; 183 against ciprofloxacin, and finally, 59 against tetracycline. The *S. flexneri* isolates showed high percentages of resistance to antibiotics such as ampicillin, cotrimoxazole, tetracycline, and chloramphenicol, with resistance levels of 87%, 76%, 73%, and 52%, respectively. There was very low resistance to furazolidone—only 4% of the isolates were resistant—and no resistance to ciprofloxacin was observed at all (Table 2).

The species *Shigella sonnei* also presented high percentages of resistance to ampicillin

TABLE 1. Distribution of *Shigella* isolates in 10 Chilean hospitals by age of patients. The PRONARES Project, 1997–1998

	Age					
	≤ 15 years		> 15 years		Total	
Species	No.	%	No.	%	No.	%
S. flexneri	156	40	63	63	219	44
S. sonnei	219	55	23	23	242	49
S. boydii	3	1	1	1	4	1
Shigella sp.	17	4	13	13	30	6
Total	395	100	100	100	495	100

(91%), tetracycline (75%), and chloramphenicol (81%). For ampicillin and tetracycline the percentages were similar to those for *S. flexneri*. With chloramphenicol, however, resistance was significantly higher than for the *S. flexneri* isolates. Again, a low percentage of resistance to furazolidone (1%) was observed, and there was no resistance to ciprofloxacin (Table 2).

All four of the *Shigella boydii* isolates obtained during the surveillance period were studied *in vitro* for their susceptibility to chloramphenicol, ampicillin, cotrimoxazole, furazolidone, and ciprofloxacin, and two of the four were studied for sensitivity to tetracycline. Although the number of isolates is very small, it is worth noting that three were resistant to ampicillin and two were resistant to both cotrimoxazole and furazolidone (Table 2).

Figure 1 shows the variations between the hospitals in the northern, central, and southern areas of Chile in percentages of resistance to each antibiotic. Resistance to ampicillin was high in all three areas studied—very high in the northern and central areas (96% and 95%, respectively) and a bit lower in the south (73%). For chloramphenicol, the highest percentage of resistance was seen in the central area (81%), while in the north and south of the country the resistance figures were similar, i.e., 52% and 54%, respectively. For cotrimoxazole, the percentages of resistance ranged from 31% in the central area to 52% in the southern area and 72% in the northern area. For tetracycline, the values were quite high in all three areas: 60%, 85%, and 68% in the north, the central area, and the south, respectively. Finally, resistance to furazolidone was very low in all three areas, ranging from 1% to 4%. No resistance to ciprofloxacin was observed.

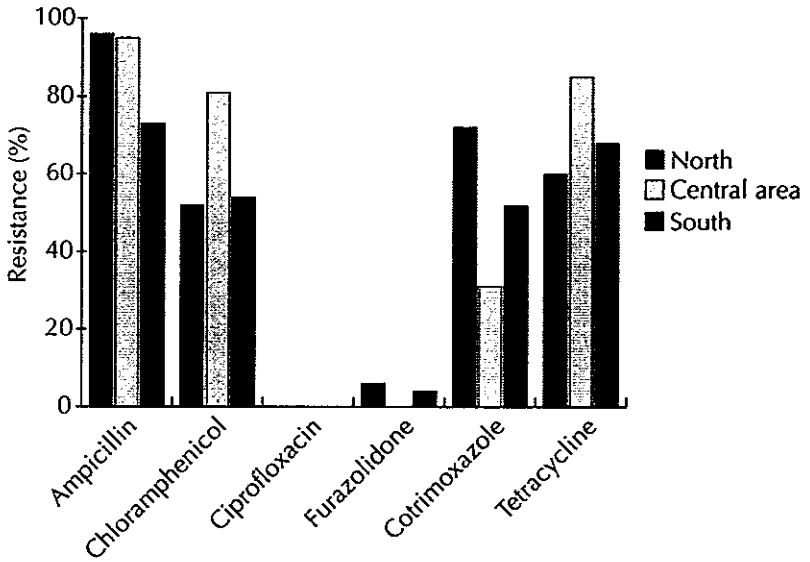
To analyze the patterns of resistance, 396 isolates were used that had been studied in vitro for susceptibility to at least four antibi-

TABLE 2. Antimicrobial resistance of *Shigella* isolates in 10 Chilean hospitals, 12 months of surveillance. The PRONARES Project, 1997–1998

	S. flexneri		*S. sonnei*		*S. boydii*	
Antibiotic	No. of isolates studied	Resistance (%)	No. of isolates studied	Resistance (%)	No. of isolates studied	Resistance (%)
Chloramphenicol	215	52	234	81	4	–
Ampicillin	218	87	241	91	4	75
Cotrimoxazole	216	76	242	21	4	50*
Furazolidone	198	4	181	1	4	50*
Ciprofloxacin	183	–	172	–	4	–
Tetracycline	59	73	55	75	2	–

*Two out of four isolates.

FIGURE 1. Variations in resistance among hospitals in northern, central, and southern Chile, 12 months of surveillance. The PRONARES Project, 1997–1998



otics (ampicillin, cotrimoxazole, chloramphenicol, and furazolidone). Of this total, 381 isolates were resistant to one or more antimicrobial agent. Ten different profiles of resistance were observed. The most frequent pattern for *S. flexneri* was resistance to ampicillin, chloramphenicol, and cotrimoxazole, which was observed in 119 isolates. That pattern was followed in frequency by resistance to ampicillin and chloramphenicol, which was observed in 22 isolates. For *Shigella sonnei*, the most frequent pattern was resistance to ampicillin and chloramphenicol. Finally, of the three *Shigella boydii* isolates, two were resistant only to ampicillin. Table 3 also shows that 44% (176/396) of the selected strains had a pattern of multiple resistance.

DISCUSSION

This study, which monitored *Shigella* antimicrobial resistance in 10 hospitals of Chile, shows us a discouraging and troubling national picture with regard to the resistance of this pathogen to various antimicrobial drugs that are recommended for the clinical treatment of shigellosis. *Shigella* resistance is a universal phenomenon. Many countries have reported high percentages of *Shigella* resistance to commonly used antimicrobial drugs such as ampicillin, cotrimoxazole, and chloramphenicol, which have been employed at different times and in numerous countries as the treatment of choice for this disease. In 1994 chloramphenicol was introduced in Chile as the treatment of choice for shigellosis, in light of increased resistance to ampicillin and subsequently to cotrimoxazole *(20–21)*.

As in the rest of Latin America, there is little information available on this problem in Chile. In a previous study of ours, in which we monitored the antimicrobial susceptibility of *Shigella* isolates in a semirural commune north of Santiago in 1995–1997, we found high percentages of resistance to various antimicrobial drugs, as indicated in the scientific literature published abroad. Resistance to ampicillin ranged between 56% and 100% among the different species; resistance to cotrimoxazole was between 21% and 53%; and resistance to chloramphenicol, between 11% and 69%.

TABLE 3. Patterns of resistance of 381 *Shigella* isolates resistant to one or more antimicrobial drugs. The PRONARES Project, 1997–1998

Antibiotic	*S. flexneri*	*S. sonnei*	*S. boydii*	*Shigella* sp.	Total
Ampicillin	4	3	2	2	11
Cotrimoxazole	10	3	–	1	14
Furazolidone	1	–	–	–	1
Ampicillin-chloramphenicol	22	127	–	4	153
Ampicillin-cotrimoxazole	17	7	–	–	24
Ampicillin-furazolidone	1	–	–	–	1
Chloramphenicol-furazolidone	1	–	–	–	1
Ampicillin-chloramphenicol-cotrimoxazole	119	23	–	19	161
Ampicillin-chloramphenicol-furazolidone	2	3	–	–	5
Ampicillin-cotrimoxazole-furazolidone	–	1	1	–	2
Ampicillin-chloramphenicol-cotrimoxazole-furazolidone	6	1	–	1	8
Total	183	168	3	27	381

There was also a high percentage of multiresistant strains (49%) *(18)*. This study revealed the need for more knowledge about the antimicrobial resistance of *Shigella* strains in the Chilean situation.

The present study has shown that the picture is no more encouraging at the national level, with resistance to chloramphenicol currently appearing in 81% of *Shigella sonnei* isolates and 52% of *Shigella flexneri* isolates. Resistance of *Shigella sonnei* to ampicillin was 91%, and with *S. flexneri* it was 87%. For cotrimoxazole, the degree of resistance was significantly higher in the isolates of *S. flexneri* (76%) than in those of *S. sonnei* (21%). Given the limited number of strains of *S. boydii (4)*, a species that is rarely isolated in Chile, it is not appropriate to draw conclusions about their behavior in terms of antimicrobial resistance.

As in the previous study, no resistance to ciprofloxacin was observed, and there were very low levels of resistance to furazolidone, probably because of the limited use of this drug in the treatment of shigellosis.

With regard to the variations in resistance observed in the different areas of the country, there is high resistance to ampicillin in the northern, central, and southern areas of Chile, with percentages of 96%, 95%, and 73%, respectively. This is an interesting phenomenon because, even though ampicillin has not been used for some 15 years for the treatment of shigellosis, it can be seen that the bacterium has retained the genes that are resistant to that antibiotic. A possible explanation might be selective pressure from the widespread use of ampicillin in Chile for other indications, especially respiratory infections. The same might be said of cotrimoxazole, which is still frequently used in primary care for the treatment of acute diarrhea.

With regard to chloramphenicol, there was rather high resistance in the north (52%) and south (54%) of Chile, and the level was even higher in the central area (81%), probably because of the widespread use of this antimicrobial drug in the capital, Santiago, often without any clear indication. The figures for cotrimoxazole were different in the three areas: 72% in the north, and 31% and 52% in the central area and the south, respectively. These variations in resistance may reflect different policies regarding the use of antibiotics in the various regions of the country. Thus we see that the northern area has the highest percentage of resistance to cotrimoxazole (72%) but less for chloramphenicol (52%), unlike the central area, where resistance to chloramphenicol is very high (81%) and not so high for cotrimoxazole (31%).

Despite these variations between the different areas of the country, because resistance hovers at such high levels, none of the aforementioned antimicrobial agents can be used

empirically for the treatment of *Shigella* infections. Moreover, we believe it important to emphasize the high number of multiresistant isolates, which accounted for 44% (176/396) of the isolates selected for analysis of the resistance profile.

This rapid acquisition of resistance to antimicrobial drugs on the part of *Shigella* strains has led to the search for other drugs, such as the third-generation cephalosporins and the new quinolones, for the treatment of *Shigella* infections.

According to our study and others that have been done abroad, ciprofloxacin, as well as other new quinolones, show excellent *in vitro* activity against the several *Shigella* species. It has also proven to be effective in clinical and bacteriological treatment of the disease, achieving high concentrations in serum and stool. Its disadvantage, however, is that its use in children is restricted because it has the potential to damage growth cartilage, according to observations in small rodents exposed to high concentrations of antibiotic over an extended period of time.

In this regard, a great deal of experience has been gained using ciprofloxacin in children for specific indications—mainly for the treatment of infections due to multiresistant *Pseudomonas aeruginosa* in flare-ups of cystic fibrosis. In these experiences with prolonged treatment, the appearance of reversible arthralgias has been documented in a small proportion of patients, but no disorders of growth cartilage have been observed, even after evaluation with sensitive diagnostic imaging methods. A clinical trial in which ciprofloxacin was used for the treatment of children with shigellosis, published recently by Salam M. et al. *(22)*, showed good clinical and bacteriological cure of the disease and was unable to establish a link between the use of ciprofloxacin and the development of arthropathy in the children. For these reasons, we believe that, given the multiple resistance observed in *Shigella* strains, consideration might well be given to the use of ciprofloxacin, an antimicrobial drug that has not been proven to have any more adverse effects in children than those observed in adults *(23–27)*.

In the search for effective antimicrobial agents against shigellosis, third-generation cephalosporins *(28–30)* have been used. However, it is important to save these drugs of last resort for the treatment of other serious infections, such as meningitis and septicemia, in order to avoid the emergence of bacterial resistance. The use of third-generation cephalosporins should be restricted to serious cases of shigellosis caused by multiresistant strains. Moreover, it is important to keep in mind that studies in other countries have shown that, even though the third-generation cephalosporins have excellent *in vitro* sensitivity and are very effective clinically in the treatment of shigellosis, they do not reduce bacteria-shedding time, which is a key consideration in the treatment of this enteric infection that is so readily transmitted between children *(28–30)*.

The present study showed low levels of resistance to furazolidone—4% for *Shigella flexneri* and 1% for *Shigella sonnei*. A clinical trial conducted in Bangladesh in 1995, in which furazolidone was used for the treatment of bloody diarrhea, showed good clinical and bacteriological improvement of shigellosis patients *(31)*. Although in clinical practice furazolidone is used in many Latin American countries with apparently good results in cases of shigellosis, there have been few controlled treatment studies to evaluate this use of the drug. For this reason, only light to moderate use in cases of mild shigellosis could be recommended. Furthermore, a technical problem for evaluating the susceptibility of furazolidone in vitro is the absence of cut-off values for enteric infections; this has made it necessary to use the values corresponding to urinary infections *(19)*.

Given the rapid acquisition of resistance by *Shigella* strains, coupled with the lack of regulations governing the use of antimicrobial agents by either physicians or the general public, the developing countries need to have safe and effective vaccines—ideally oral vaccines—that will protect against shigellosis. As long

as that goal is not achieved, projects like the PRONARES Program are an excellent means of monitoring the resistance of different pathogens at the country level. For this reason, it is important to maintain and enhance these programs.

With regard to the monitoring of *Shigella*, it would be important in the future to control the selection of antibiotics in all participating centers in such a way that they make use of all the antibiotics recommended in the protocol, thus allowing for better analysis of the results. This was an obstacle in analyzing the *in vitro* susceptibility results for nalidixic acid, which was used in a very small number of cases.

Earlier Chilean studies and foreign studies of *Shigella* strains show that, in addition to presenting good in vitro activity, nalidixic acid has demonstrated good clinical efficacy in the treatment of shigellosis. It has therefore been used as the drug of choice in some countries, such as Brazil and Bangladesh, given the increase in resistance to ampicillin and cotrimoxazole, the original first-line antibiotics in these and other regions of the world *(31–33)*. It will be important in the future to have information on the behavior of *Shigella* strains with nalidixic acid in Chile.

Bearing in mind the ease with which *Shigella* strains acquire resistance, as well as the variations that exist in different geographical areas, we consider it of the utmost importance to conduct susceptibility studies in each country to determine the degree of bacterial resistance and also provide the basis for orienting clinical physicians with regard to appropriate empirical treatment. This should help to prevent the selection and spread of resistant strains.

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SURVEILLANCE OF ANTIMICROBIAL SUSCEPTIBILITY OF STRAINS RESPONSIBLE FOR INVASIVE INFECTIONS AT 11 HOSPITALS IN CHILE[1]

NATIONAL PROGRAM FOR MONITORING ANTIMICROBIAL RESISTANCE (PRONARES)

Olivia Trucco A.,[2] Valeria Prado J.,[1] Francisco Valdivieso R.,[1] María Cristina Díaz J.,[1] Alicia Ojeda S.,[1] and the PRONARES Group[3]

Invasive infections require early and effective treatment, which must often be initiated empirically. In order to improve the chances for success, it is essential to have information about the prevalent agents and their local patterns of resistance to antibiotics.

PRONARES, Chile's program for monitoring antimicrobial resistance, was instituted at 11 of the country's hospitals in November 1997. With the use of WHONET software, analyses and comparisons were made of the resistance patterns observed over a 12-month period throughout the country in 1,305 isolates of strains responsible for invasive infections (20 isolates from sterile body fluids per month, per center). Antimicrobial susceptibility was determined using the disk diffusion method, following the guidelines of the U.S. National Committee for Clinical Laboratory Standards (NCCLS).

Most of the isolates were obtained by hemoculture (63.1%). Of the bacteria isolated, 57% were Gram-negative bacilli and 43% were Gram-positive cocci. The most prevalent species was Staphylococcus aureus *(355): 40% of the isolates were resistant to methicillin and 38.3% and 37.6% to cefazolin and gentamicin, respectively; 17% to trimethoprim-sulfamethoxazole; and 10.5% to rifampicin. The coagulase-negative species of staphylococci had the highest indexes of resistance.*

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With Streptococcus pneumoniae, *24.2% of isolates were resistant to penicillin, whereas none of them were resistant to cefotaxime or vancomycin. Among the Gram-negative bacilli,* Escherichia coli *(320 isolates) had high levels of resistance to ampicillin (58.3%), aztreonam (47.2%), trimethoprim-sulfamethoxazole (40.3%), and ceftriaxone and ampicillin-sulbactam (18.4%), but less than 5% showed resistance to gentamicin, ciprofloxacin, amikacin, or imipenem.*

Among the nonfermenting bacteria, 100% of Pseudomonas *and* Acinetobacter *(151 isolates) proved to be resistant to ampicillin, while 59.1% were resistant to ampicillin-sulbactam in combination, 35.9% to ceftazidime, and 41.4% to cefoperazone. Half the isolates were resistant to gentamicin and 32.7%, to amikacin.*

The high levels of resistance observed in our environment, as well as the variations from one region to another, bear out the need to maintain a surveillance program for monitoring the resistance of prevalent pathogens.

INTRODUCTION

Bacterial resistance to antimicrobial agents develops when these drugs are introduced for the treatment of infectious diseases. In spite of the efforts that have been made to create new and better molecules, the bacteria continue to find ways to elude their action.

Over the years, antimicrobial resistance has extended among a growing number of pathogens that plague humankind, thus posing a serious problem for the therapeutic management of many prevalent infectious diseases.

The literature has described many new mechanisms through which bacteria have managed to thwart the bactericidal or bacteriostatic action of antibiotics, and some antimicrobial agents can induce resistance even during the course of treatment (*1–3*).

The resistance phenomenon is of particular concern in the case of life-threatening conditions such as invasive infections, when the antimicrobial management of patients becomes critical. The complexity of progress in modern medicine is such that patients in intensive care units, already weak and suffering from disease, are especially at risk of developing serious systemic infections of hospital origin because of resistant pathogens or opportunistic infections, or both (*4, 5*).

Life-threatening bacterial infections are a growing cause of morbidity and mortality, especially during the beginning and final periods of life. They contribute significantly to higher mortality from infectious diseases, as well as to the cost of caring for such infections (*6*).

Microorganisms associated today with invasive infections can vary in frequency from hospital to hospital and include numerous bacterial species, both Gram-positive and Gram-negative. The most common ones are the enterobacteria, *Staphylococcus aureus* and coagulase-negative staphylococci (CNS), nonfermenting bacilli such as *Pseudomonas* and *Acinetobacter*, and, finally, *Enterococcus* spp. (*7–9*).

At the present time, the most serious problems in Chilean hospitals are the increasingly broad profile of resistance to third-generation cephalosporins in Gram-negative bacilli, the growing resistance to methicillin in *Staphylococcus aureus* and CNS, and the recent emergence of *Enterococcus* strains resistant to vancomycin (*10–14*).

In this group of microorganisms, antimicrobial resistance is the rule rather than the exception. For this reason, it is just as important to study the in vitro behavior of these bacteria vis-à-vis the various antimicrobial agents as it is to characterize the disease-causing strains themselves. The problem becomes even more important with life-threatening infections in which the prognosis depends largely on the speed with which the condition is clinically recognized and appropriate and timely antibiotic treatment (usually empirical)

can be initiated. There is no time to wait for the results of microbiological studies and, in any event, antimicrobial treatment should be based on local epidemiological information about the agents and the resistance that has developed against them—a phenomenon that is highly dynamic.

When resistance is high, it is imperative to monitor the behavior of the pathogens vis-à-vis specific antimicrobial agents. Such an investigation should not only test the agents routinely used in the clinic but also look at new molecules that may be more active, cover a broader spectrum, or have particular pharmacokinetic advantages, so that up-to-date information will be available to help in making treatment decisions.

PRONARES is a longitudinal program for monitoring antimicrobial resistance in various sentinel centers throughout Chile. The program evaluates infectious pathogens using standardized study and analysis methods (WHONET Program).

The present article reports on the in vitro susceptibility of bacteria associated with invasive infections at 11 Chilean hospitals over a one-year period.

MATERIALS AND METHODS

A surveillance system was implemented using a common study protocol and manual of procedures. The antimicrobial agents to be used with each pathogen were defined. Eleven hospitals, located throughout Chile, were invited to participate—two in the northern area (Antofagasta and Iquique), five in the central area (Viña del Mar and Santiago), and four in the southern area (Chillán, Talcahuano, Temuco, and Osorno).

Each participating center contributed up to 20 isolates a month, taken consecutively from hospitalized patients with invasive infections, one isolate per patient, over the period from 1 November 1997 through 30 October 1998.

"Invasive infection" was considered to exist whenever a pathogenic or opportunistic bacterial strain was isolated from a sterile site (blood, cerebrospinal fluid, or other organic fluids) from a patient presenting a compatible clinical picture.

Antimicrobial susceptibility was studied in each laboratory using the Kirby-Bauer agar disk diffusion method. The inhibition halo of each isolate studied was measured for each antibiotic. The antibiotics were selected taking into account the particular pathogen and the study protocol, based on the U.S. National Committee for Clinical Laboratory Standards (NCCLS) recommendations (*15*). The halos were recorded in an ad hoc manner and sent to the Microbiology Unit–East, where they were entered in the WHONET software database.

For purposes of internal quality control, the following ATCC reference strains were included: *Escherichia coli* 25922, *S. aureus* 29213, *Pseudomonas aeruginosa* 27853, *Haemophilus influenzae* 49247, *S. pneumoniae* 49619, and *Enterococcus faecalis* 29212. External microbiological control was the responsibility of the Microbiology Unit–East, which submitted unknown isolates every four months to each participating center.

The isolates were automatically classified as susceptible, intermediate, or resistant when their inhibition halos were recorded, based on the cut-off values established in the NCCLS 98 guidelines, which have been incorporated into the WHONET software.

The data analysis was presented in the form of histograms (a graph showing the distribution of the inhibition halos observed) and resistance profiles for the different bacterial species with respect to each antimicrobial agent included in the study. The reports were sent regularly to each participating center for use at the local level.

RESULTS

During the 12-month surveillance period, information was gathered on 1,305 isolates taken from sterile sites from hospitalized pa-

tients. The most frequent source of these isolates was hemocultured (63.1%). Less frequent sites were the abdomen (13.6%), central venous catheter (9.3%), pleural effusion (5.3%), cerebrospinal fluid (2.4%), tissues (1.5%), articular fluid (1.2%), and others (bone marrow, gastric fluid).

Of the bacteria isolated, 57% were Gram-negative bacilli and the remaining 43% were Gram-positive cocci. Table 1 shows the species isolated and their frequency. Three patterns of prevalence were observed for the pathogens in the participating hospitals: in six of the hospitals, *E. coli* and *S. aureus* were responsible for similar numbers of cases and were by far the most prevalent species (60% or more); in two other hospitals, *S. aureus* was the most frequent pathogen, accounting for more of half the isolates; and, finally, in the three remaining hospitals, four bacteria were almost equally prevalent: *S. aureus, E. coli, Pseudomonas* spp., and *Klebsiella* spp.

In the overall analysis of susceptibility, the Gram-positive cocci showed high profiles of resistance in the cases of *Staphylococcus aureus*, CNS, and *Streptococcus pneumoniae*. Of the 355 *S. aureus* isolates, 40% were resistant to methicillin; 38.3%, to first-generation cephalosporins (cefazolin); and 37.6%, 43%, 50.9%, and 38%, to gentamicin, erythromycin, chloramphenicol, and clindamycin, respectively. Only the trimethoprim-sulfamethoxazole combination and rifampicin showed good in vitro activity, with resistance levels of 17% and 10.5%, respectively (Figure 1). A pattern of multiple resistance (resistance to three or more antibiotics) was observed in 28% of the isolates.

Staphylococcus spp., *S. aureus*, and CNS all presented a similar profile of antibiotic resistance, with somewhat higher levels in CNS (Figure 2).

Of the 64 isolates of *Streptococcus pneumoniae*, 24.2% were resistant to penicillin, whereas none of them was found to be resistant to cefotaxime or vancomycin.

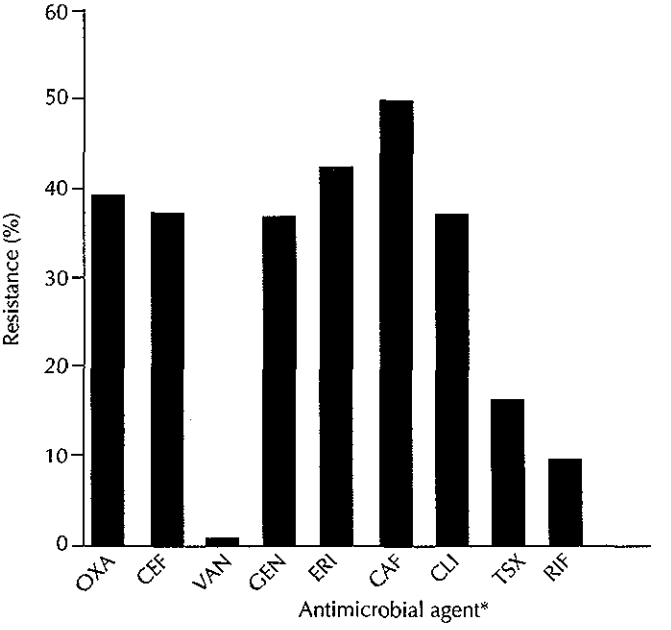
Among the Gram-negative bacilli, *Escherichia coli* (320 isolates) had high levels of resistance to ampicillin (58.3%), aztreonam (47.2%), trimethoprim-sulfamethoxazole (40.3%), and cefotaxime (24.7%). Better activity was demonstrated by the ampicillin-sulbactam combination, to which only 18.4% of the isolates were resistant. Gentamicin, ciprofloxacin, amikacin, and imipenem had good in vitro activity, with resistance found in less than 5% (Figure 3).

When the resistance profiles of other fermenting Gram-negative bacilli, such as *Klebsiella* spp. (124 isolates), were compared with the isolates of *E. coli*, *Klebsiella* was the most resistant of all: 62.2% of the isolates showed resistance to aztreonam, 57.2% to cefotaxime, 34.5% to gentamicin, 16.7% to ciprofloxacin,

TABLE 1. Frequency of pathogenic bacteria isolated from systemic infections at 11 Chilean hospitals. PRONARES Program, November 1997 through October 1998

Bacterial species	Isolates (No.)	Frequency (%)
Staphylococcus aureus	355	27.2
Escherichia coli	320	24.6
Klebsiella spp.	124	9.5
Coagulase-negative *Staphylococcus*	103	7.9
Pseudomonas	97	7.5
Streptococcus pneumoniae	64	4.9
Salmonella	63	4.9
Other enterobacteria	52	4.0
Acinetobacter	50	3.8
Other *Streptococcus* spp.	30	2.3
Enterococcus	24	1.8
Haemophilus influenzae	12	0.9
Miscellaneous	11	0.8
Total	1,305	100

FIGURE 1. Antimicrobial resistance of 355 *Staphylococcus aureus* isolates causing invasive infections in 11 Chilean hospitals, by antimicrobial drug. PRONARES Program, 1997–1998



*OXA: oxacillin; CEF: cefazolin; VAN: vancomycin; GEN: gentamicin; ERI: erythromycin; CAF: chloramphenicol; CLI: clindamycin; TSX: trimethoprim-sulphamethoxazole; RIF: rifampicin.

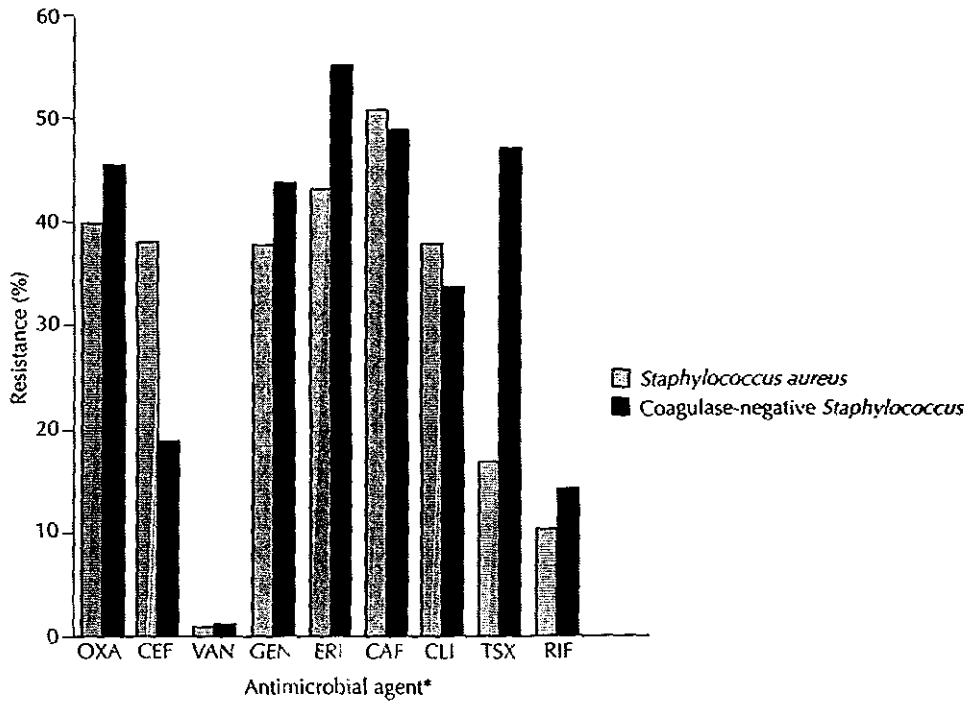
and 43.9% to the ampicillin-sulbactam combination (Figure 4).

Figure 5 shows the histogram generated by the distribution of *E. coli* inhibition halos in the presence of aztreonam. In this case the histogram makes it possible to predict the presence of an enzymatic resistance mechanism in some of these isolates—namely, the production of extended-spectrum β-lactamases (ESBL)—inasmuch as 38% of the isolates had an inhibition halo equal to or smaller than 27 mm, the cut-off value for suspecting the production of this type of inactivating enzymes in *E. coli* isolates (*15*).

Among the 151 isolates of nonfermenting Gram-negative bacilli, those of *Pseudomonas* and *Acinetobacter* were 100% resistant to ampicillin. When a β-lactamase inhibitor (sulbactam) was added, the combination did not show any improved activity against *Pseudomonas;* however, resistance in the *Acinetobacter* isolates dropped to 13%. Ceftazidime and cefoperazone showed moderate activity against *Pseudomonas* (resistance levels of only 19.5% and 23.3%, respectively), but their activity was very low in the presence of *Acinetobacter* (70.7% and 84% resistance). With gentamicin, 33.7% of the *Pseudomonas* and 79.6% of the *Acinetobacter* isolates were resistant. Amikacin was more active with both species than its predecessor, gentamicin. It performed better against *Pseudomonas,* in which resistance was 19.4%, than against *Acinetobacter,* in which it was 57.1%.

Aztreonam also showed better activity against *Pseudomonas* than against *Acinetobacter* (18.5% and 70% resistance, respectively). The antimicrobial agent that was most active against both genera turned out to be imipenem, for which the resistance levels were only 6.8% in *Pseudomonas* and 2.2% in *Acinetobacter* (Figure 6).

FIGURE 2. Antimicrobial resistance (%) of 355 *Staphylococcus aureus isolates* and 101 isolates of coagulase-negative Staphylococcus causing invasive infections in 11 Chilean hospitals, by antimicrobial drug. PRONARES Program, 1997–1998

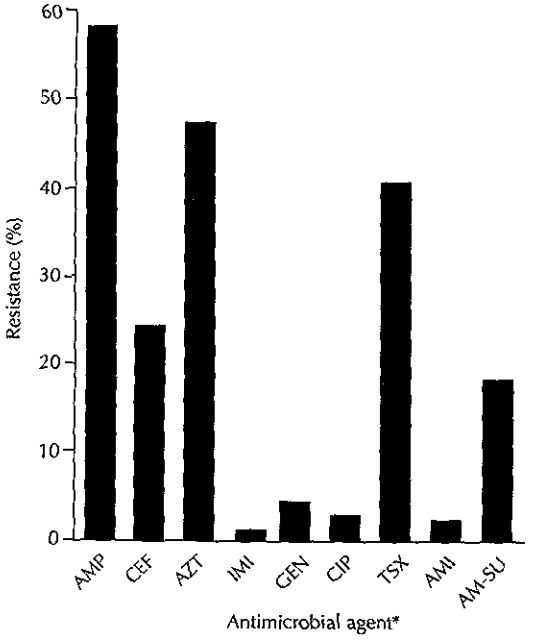


*OXA: oxacillin; CEF: cefazolin; VAN: vancomycin; GEN: gentamicin; ERI: erythromycin; CAF: chloramphenicol; CLI: clindamycin. TSX: trimethoprim-sulphamethoxazole; RIF: rifampicin.

Another contribution of the WHONET system was to show differences in the resistance mechanisms of bacteria, isolated from different hospitals, to the same antibiotic. Figures 7 and 8 show two different histograms for *Acinetobacter baumannii* in two different hospitals. In one hospital, all the isolates of this species were resistant to ampicillin, and 100% were susceptible to ampicillin-sulbactam given in combination. This would indicate that the β-lactamases produced by these isolates had a great affinity for the inhibitor. In contrast, at another hospital, the isolates of this species produced β-lactamases that were not recognized by sulbactam and failed to have any susceptibility to the combination, which was mirrored by small inhibition halos at left of center on the graph.

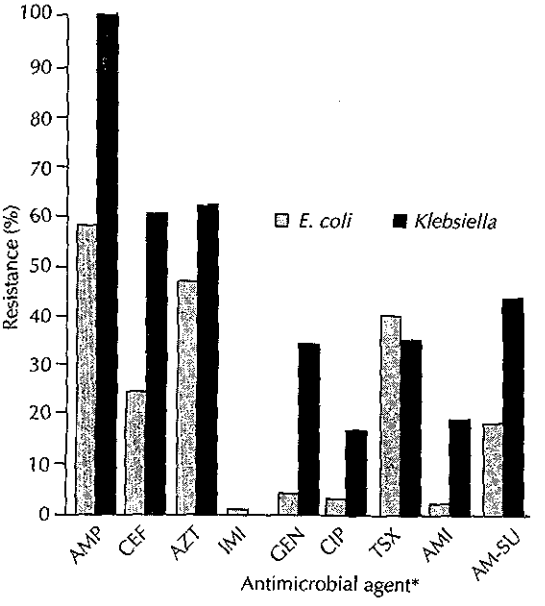
With the surveillance system in place in a number of centers throughout Chile, we were able not only to evaluate the first-line antimicrobial agents already in use for the treatment of invasive infections but also to assess new therapeutic options. One of these was the new fluoroquinolone grepafloxacin, which, consistent with reports from other countries, showed little activity against *S. aureus* isolates (37.5%), many of which were resistant to methicillin. This agent also showed poor activity against nonfermenting bacilli (ranging from 35.2% to 80%), but it showed good activity against fermenting Gram-negative bacilli such as *E. coli*

FIGURE 3. Antimicrobial resistance (%) in 320 isolates of *Escherichia coli* causing invasive infections in 11 Chilean hospitals, by antimicrobial drug. PRONARES Program, 1997–1998



*AMP: ampicillin;. CEF: ceftriaxone; AZT: aztreonam; IMI: imipenem; GEN: gentamicin; CIP: ciprofloxacin; TSX: trimethoprim-sulphamethoxazole; AMI: amikacin; AM-SU: ampicillin-sulbactam.

FIGURE 4. Antimicrobial resistance (%) in 320 isolates of *Escherichia coli* and 124 isolates of *Klebsiella spp.* causing invasive infections in 11 Chilean hospitals, by antimicrobial drug. PRONARES Program, 1997–1998



*AMP: ampicillin; CEF: ceftriaxone; AZT: aztreonam; IMI: imipenem; GEN: gentamicin; CIP: ciprofloxacin; TSX: trimethoprim-sulphamethoxazole; AMI: amikacin; AM-SU: ampicillin-sulbactam.

and *Klebsiella* spp. (resistance of 5% and 10%, respectively).

DISCUSSION

The high proportion of isolates from invasive infections that currently exhibit resistance to the antimicrobial agents in use at Chilean health care centers can be attributed to a number of factors. Among these, as pointed out earlier, is technological progress itself. The many advances in the care of seriously ill patients has led to the concentration of patients at high risk for life-threatening infections in hospitals—patients who are being subjected to invasive procedures and broad-spectrum combination therapies. This situation exerts selective pressure on bacteria in the hospital environment and facilitates the selection and subsequent spread of resistant strains.

Having a system in place to monitor resistance in the prevalent pathogens has turned out to be very useful for modifying the empirical therapeutic practices. The PRONARES program made it possible to obtain information on a sizable number of isolates (1,305) from invasive infections in our environment within the relatively brief period of 12 months.

The results have clearly shown that in Chile two organisms, *S. aureus* and *E. coli*, are chiefly responsible for invasive infectious processes. Together, they accounted for 51.7% of the strains associated with these infections.

FIGURE 5. Inhibition halos for *Escherichia coli* isolates tested with aztreonam in a study conducted in 11 Chilean hospitals. PRONARES Program, 1997–1998

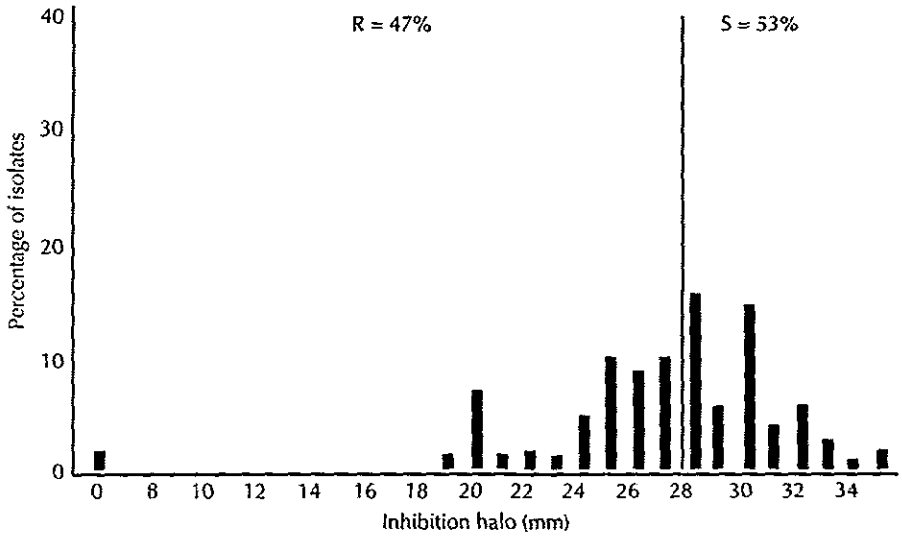
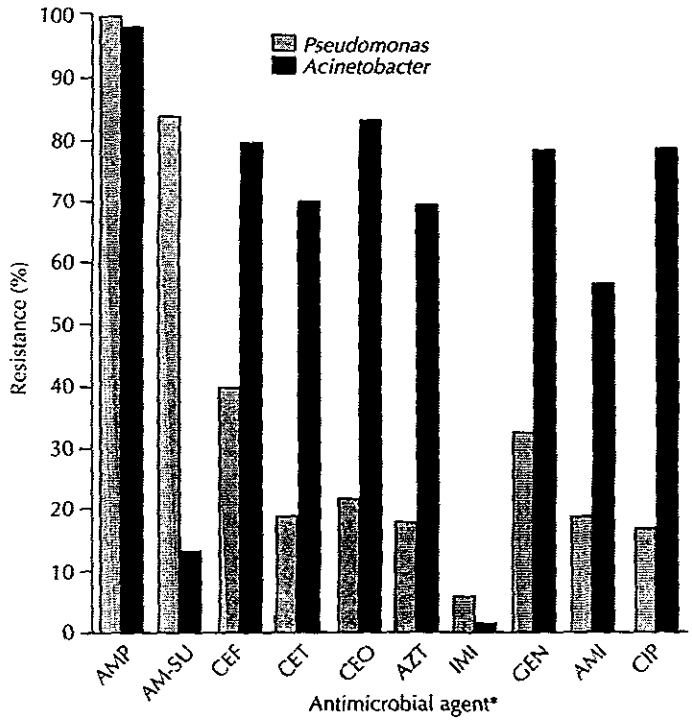
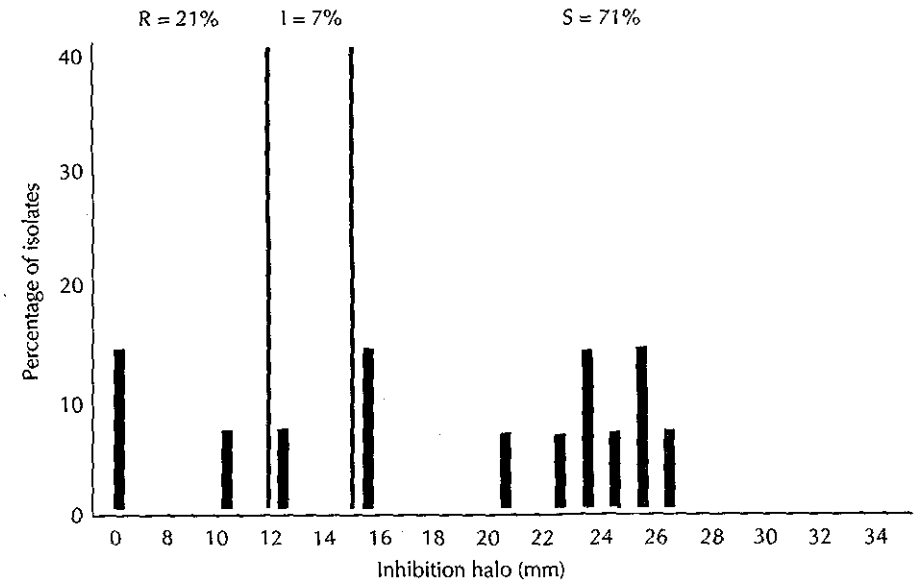


FIGURE 6. Comparative antimicrobial resistance (%) in isolates of *Pseudomonas* spp. and *Acinetobacter* spp., by antimicrobial drug, in a study conducted in 11 Chilean hospitals. PRONARES Program, 1997–1998



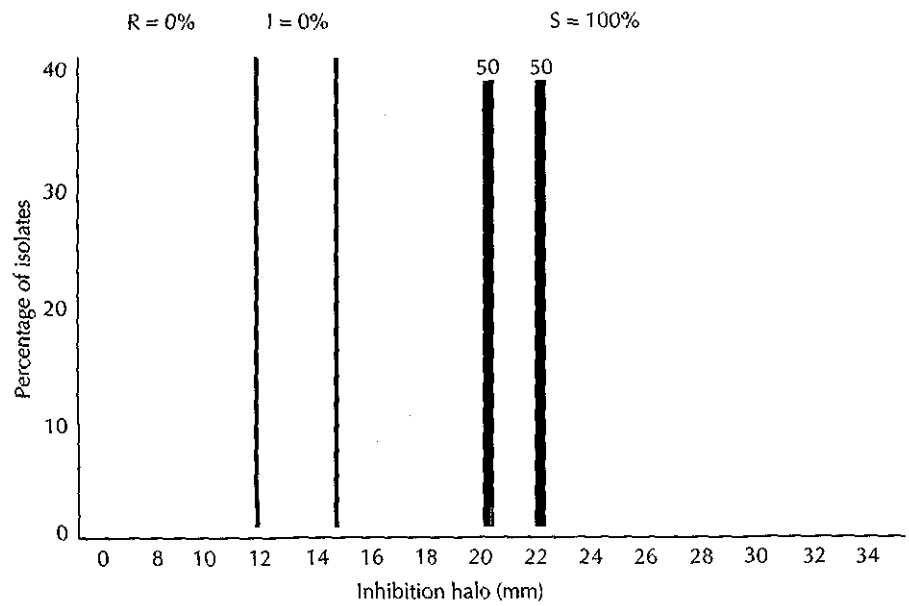
*AMP: ampicillin; AM-SU: ampicillin-sulbactam; CEF: ceftriaxone; CET: ceftazidime; CEO: cefoperazone; AZT: aztreonam; IMI: imipenem; GEN: gentamicin; AMI: amikacin; CIP: ciprofloxacin.

FIGURE 7. Inhibition halos (mm) for isolates of *Acinobacter baumannii* tested with ampicillin-sulbactam combination in hospital A. PRONARES Program, Chile, 1997–1998



R = resistant; I = intermediate; S = susceptible.

FIGURE 8. Inhibition halos (mm) for isolates of *Acinobacter baumannii* tested with ampicillin-sulbactam combination in hospital B. PRONARES Program, Chile, 1997–1998



R = resistant; I = intermediate; S = susceptible.

These findings are consistent with reports by such authors as Pfaller et al. (*16*), who, in a monitoring study of resistance in the United States of America, showed that these two organisms were also the most prevalent. In other countries, however, monitoring studies of nosocomial infections have indicated significant involvement of Gram-positive cocci such as CNS (33.5%), as well as *Enterococcus* spp. (*16, 17*).

We observed a high frequency of *S. aureus* isolates resistant to methicillin (40%), and resistance to this drug was even more frequent in CNS isolates (45.4%). These levels are higher than previously reported in our environment, which suggests a rising trend. On the other hand, according to published reports (*18, 19*), *Staphylococcus* isolates from community-acquired infections are still showing relatively little resistance to methicillin in Chile.

High levels of resistance to antimicrobial agents were demonstrated by all the Gram-negative bacilli. For both the fermenting and the nonfermenting bacilli, the percentages of resistance to the majority of antibiotics were high.

The ampicillin-sulbactam combination is effective against *Acinetobacter* as has been previously seen. This combination therefore represents a good option for the treatment of infections caused by these pathogens (*20*).

Comparison of resistance levels in the various participating centers revealed differences for some of the pathogens; thus, it is indispensable to have a national network where local results can be generated. The processing of such results as a whole helps in the formulation not only of recommendations or standards for the treatment of invasive infections at the local level, but also of broader guidelines that are valid for the entire country.

Maintaining a national surveillance system on antimicrobial susceptibility also makes it possible, as in this study, to evaluate new therapeutic options that might offer solutions to the problems of resistance we are facing today.

The study of resistance is particularly important in the case of life-threatening infections. In our environment, the incidence of bloodstream infections is 1.8 per 1,000 hospital discharges. This figure suggests how vitally important it is to have adequate information for the management of patients with these very serious infections.

Through use of the WHONET computer software for compiling and analyzing information, we have been able to establish a central database that will assist in the selection of antibiotics for the empirical treatment of our patients. WHONET has also enabled us to spot quality control problems in the laboratories and to deal with them, identify bacterial subpopulations on the basis of their resistance profile, and predict the appearance of new resistance mechanisms.

Maintenance of the surveillance system over time will make it possible to evaluate trends in antimicrobial resistance and to make recommendations regarding the best therapeutic options for empirical use, as well as strategies for controlling the spread of resistance.

The surveillance exercise—which has been conducted with great success in other Latin American countries such as Argentina and Venezuela—has shown that, in some cases, the development and spread of antimicrobial resistance has remained constant year after year, while at the same time resistance among nosocomial bacteria is steadily rising. This reinforces our interest in maintaining this system of antimicrobial surveillance in Chile.

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ANTIMICROBIAL RESISTANCE AND MICROBIOLOGICAL SURVEILLANCE IN CUBA[1]

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A retrospective study (1989–1998) was conducted in Cuba to determine the antimicrobial resistance in six bacteria that have been the object of microbiological surveillance: Streptococcus pneumoniae, Haemophilus influenzae *b*, Shigella *spp.*, Salmonella typhi, Neisseria meningitidis *B and* Neisseria gonorrhoeae. *A total of 5,305 strains were studied, 4,523 of them from patients with invasive processes, and the rest from carriers of* S. pneumoniae *and* H. influenzae. *The strains originated in isolates obtained from hospital patients throughout the country and from studies conducted in the community, and were processed at the National Microbiology Reference Laboratory, Pedro Kouri Institute of Tropical Medicine. Minimum inhibitory concentrations were determined for all of them, and some were also subjected to the β-lactamase test. The results presented are compared with findings from the national and international literature.*

A comparison of the patterns of resistance encountered in the Cuban strains with those reported in other countries indicate that Cuba's policy regarding the use of antibiotics has been influenced by economic factors and drug availability.

INTRODUCTION

Antimicrobial resistance has become an increasingly important problem throughout the world. An essential component of any program for controlling antimicrobial resistance is the appropriate use of these drugs. The indiscriminate use of antibiotics in humans, animals, and agriculture exerts selective pressure and leads to the development of resistance. It is therefore essential to devise better strategies for controlling the problem *(1)*.

New aspects of antimicrobial resistance and the consequent spread of the respective bacteria in the environment and the community should be closely watched by epidemiologists, clinicians, and microbiologists, since resistance is a health problem for both developed and developing countries. Many authors have sug-

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gested that the prime factor leading to the spread of resistant bacteria in the community is the indiscriminate and rising use of antibiotics *(2–4)*.

Various underlying mechanisms are involved in the development of antibiotic resistance *(5)*.

In order to achieve more effective control, it is essential to know how the patterns of bacterial resistance evolve over time in a given region or country.

In Cuba, the microbiological laboratory network of the National Health System comprises 160 units; 89 of them in hospitals and the rest in province and municipality health units. The microbiology laboratory at the Pedro Kouri Institute of Tropical Medicine (PKI) serves as the national reference laboratory. There, all microbiological surveillance for the National Health System is unified.

The problem of emerging resistance is being recognized throughout the world. Its magnitude and extension make it everyone's problem. One of the most important elements in addressing the problem is microbiological surveillance of drug resistance. In the present report we will review the historical patterns of antimicrobial resistance for the following microorganisms that are the object of national microbiological surveillance: *Streptococcus pneumoniae, Haemophilus influenzae* type b, *Shigella* spp., *Salmonella typhi, Neisseria meningitidis* B, and *Neisseria gonorrhoeae.*

MATERIALS AND METHODS

At the request of the Pan American Health Organization, a retrospective study was undertaken by the National Microbiology Reference Laboratory (NMRL) of the PKI. The study began by bringing together the data on antimicrobial resistance that had been gathered over many years by the NMRL-PKI on the following microorganisms:

	Years studied
• *Streptococcus pneumoniae*	(1992–July 1998)
• *Haemophilus influenzae* b	(1989–July 1998)
• *Shigella* spp.	(1990–1997)
• *Salmonella typhi*	(1995–1997)
• *Neisseria meningitidis* B	(1989–1997)
• *Neisseria gonorrhoeae*	(1995–July 1998)

In addition, strains from carriers of *S. pneumoniae* and *H. influenzae,* isolated between January 1997 and July 1998, were also studied.

a) Strains. For each of the microorganisms, the laboratory procedures used to identify the strains were reviewed in order to assure adherence to the guidelines of the NMRL-PKI Manual of Operations and Procedures (MOP) *(6)*.

All specimens had been sent to the NMRL-PKI by laboratories in the network. There were 4,523 specimens isolated from invasive processes and 782 obtained from carriers, for a total of 5,305 isolates.

b) In vitro tests for antimicrobial susceptibility. In all cases, minimum inhibitory concentrations (MIC) were determined for each of these microorganisms using the established tests for the respective infectious agents *(6)* and following the guidelines set by the U.S. National Committee on Clinical Laboratory Standards (NCCLS) *(7)*.

c) The β-lactamase test. This test was applied to *Shigella* spp., *N. meningitidis* B, and *N. gonorrhoeae* using nitrocefin oxoid.

d) Plasmid profile. Also in the cases of *Shigella* spp., *N. meningitidis* and *N. gonorrhoeae,* a plasmid profile was determined using the techniques of Maniatis *(8)*.

The accumulated data from each laboratory were examined, and a new database was developed for the specific purposes of the study. Published national scientific information was also reviewed.

Pearson's correlation coefficient (R) was calculated for each antibiotic, based on length of time in years and the percentage of resistance found for each microorganism.

RESULTS AND DISCUSSION

Streptococcus pneumoniae is one of the most important causative agents of acute in-

fectious bacterial disease. High on the list of emerging—and nowadays increasingly common—infectious diseases, this organism was found to be very resistant. It is associated with rising morbidity and mortality at the two extremes of life, especially mortality in children under 5 years of age. In the monitoring process, the microbiology laboratory should regard sterile body fluids indicative of invasive processes as pathological products, and therefore, important clinical findings. *S. pneumoniae* has been systematically monitored in Cuba since 1992.

Table 1 shows the results obtained from 359 isolates of *S. pneumoniae* from invasive processes, 285 of them from patients under 5 years of age and 74 from adults living in various regions of the country. Overall resistance to penicillin rose steadily from 1992, when it was 7%, until 1998, when it reached 10.8% (R=0.96; P>0.01). The study also revealed intermediate susceptibility in 32.1%. If the latter group is also regarded as resistant, then the total proportion of resistance to penicillin would be 42.9%. This is demonstrated (except for cotrimoxazole and chloramphenicol, which had $R = 0.96$ and P>0.05) by the high positive correlation coefficients, all of which were statistically significant (ranging from $P = 0.01$ to $P = 0.05$).

A study conducted in 1997–1998 in three Cuban kindergartens with populations of nasopharyngeal carriers under 5 years of age yielded the following results. In a sample of 195 isolates in the city of Havana, resistance to oxacillin was 61.4% and to cotrimoxazole, 59.3%; chloramphenicol, erythromycin, and tetracycline had lower levels that were very close to the critical value of P (P = 0.0519). Resistance to ofloxacin was not observed. Resistance in the isolates obtained from carriers (1998), compared with those from patients suffering from invasive processes, showed statistically significant values for penicillin-oxacillin and ofloxacin, at $P < 0.01$. The difference was not statistically significant for chloramphenicol, cotrimoxazole, tetracycline, or erythromycin ($P > 0.05$).

Low and Scheld *(1)* have recently contended that, based on the data available for *Streptococcus pneumoniae*, not only the exposure to antimicrobial agents is important, but also the concentration at which these drugs are used.

In the case of Cuba, apart from any misuse of antibiotics that may exist, the high levels of resistance to oxacillin and cotrimoxazole that were found in the specimens isolated from nasopharyngeal carriers should be taken into account. They may be indicative of the extent to which these drugs are used because of their availability in primary health care facilities.

Haemophilus influenzae **b** becomes more important every day because of its impact in terms of morbidity from acute respiratory and neurological infections (33% of all bacterial meningitides) *(9)* and of mortality in the early ages, especially in children under 5 years of age, and above all in the first year of life. The progressive changes observed in the patterns of resistance make *H. influenzae* b one of the emerging resistant bacteria of greatest concern

TABLE 1. Antimicrobial resistance (MIC) of *Streptococcus pneumoniae* in 359 isolates from invasive processes, Cuba, 1992–1998

Antimicrobial agent	1992 (n = 17)	1993 (n = 43)	1994 (n = 45)	1995 (n = 56)	1996 (n = 24)	1997 (n = 81)	July 1998 (n = 93)
Penicillin	7.0	7.0	7.2	8.2	8.8	9.4	10.8
Chloramphenicol	12.3	12.2	13.4	13.8	14.6	13.1	13.9
Cotrimoxazole	45.6	47.4	45.4	42.3	46.4	48.5	49.3
Tetracycline	11.3	11.4	12.4	12.1	13.5	14.4	14.4
Ofloxacin	12.2	13.3	12.6	14.6	16.4	18.2	19.8
Erythromycin	2.3	2.2	3.4	3.8	4.6	5.1	6.2

TABLE 2. Antimicrobial resistance (MIC) of *Haemophilus influenzae* type b in 856 isolates from invasive processes, Cuba, 1989–1998

Antimicrobial agent	1989 (n = 156)	1990 (n = 139)	1991 (n = 97)	1992 (n = 130)	1993 (n = 88)	1994 (n = 61)	1995 (n = 50)	1996 (n = 18)	1997 (n = 59)	July 1998 (n = 58)
Ampicillin	40.3	40.5	41.1	42.3	42.6	43.2	45.0	47.5	52.4	55.6
Chloramphenicol	41.4	41.7	40.4	43.8	44.0	45.3	52.2	56.6	55.6	62.7
Cotrimoxazole	42.4	43.5	42.4	45.6	48.4	49.4	52.3	56.4	68.5	74.3
Tetracycline	10.2	10.3	10	10.3	11.4	13.4	14.1	17.5	19.4	21.2
Ofloxacin	–	–	–	–	–	–	–	–	–	–
Ceftriaxone	–	–	–	–	–	–	–	–	3.2	6.4

– = zero.

to pediatricians the world over, and Cuba has not been spared.

This makes microbiological surveillance and the monitoring of resistance patterns even more important, in order to acquire a solid scientific foundation for orienting therapies. By the late 1980s, studies of the resistance of *H. influenzae* b to antimicrobial drugs had already begun. Table 2 summarizes the patterns of in vitro resistance to antibiotics for 856 isolates of *H. influenzae* b obtained from cerebrospinal fluid and blood between 1989 and July 1998. Resistance to ampicillin ranged from 40.3% to 55.6% (R = 0.91 and $P > 0.01$). Increased resistance was also observed with cotrimoxazole (R = 0.92), chloramphenicol, and tetracycline ($R = 0.94$ and $P > 0.01$). Resistance to ceftriaxone was observed for the first time in 1997 (R = 0.68 and $P > 0.05$), and by July 1998 it had doubled. *H. influenzae* has not shown resistance to ofloxacin for almost 10 years. Evidence of β-lactamase production was seen in 40% of the isolates.

Reporting on a study of strains isolated in 1992, Valdivia et al. *(10)* call attention to the need to modify the treatment of *H. influenzae* b, since 49% of the 55 isolates that they studied already showed resistance to ampicillin, which at the time was the drug of choice. The authors recommend the use of third-generation cephalosporins. Earlier, in 1992, the same authors had reported finding 42.5% resistance to ampicillin. At the time scientists were already aware of what was happening in other countries, such as Spain, Taiwan, South Africa, and Chile *(11, 12)*, where 64%, 36%, 25%, and 24%, respectively, of different serotypes of *H. influenzae* b had been found to exhibit resistance to this drug.

In the study mentioned previously, conducted in Cuba from 1997 to July 1998 among children under 5 years of age at three kindergartens in Havana, 587 specimens of acapsular *H. influenzae* were isolated from the nasopharynx. According to the MIC for these strains, the resistance patterns were different from those for strains isolated from invasive processes: resistance was lower for ampicillin and chloramphenicol and slightly higher for tetracycline, and it was double for cotrimoxazole and ceftriaxone.

Comparison for each of the antibiotics found significant differences of the percentages of *H. influenzae* resistance found in 1998 with those for acapsular *H. influenzae* isolated from the carriers, for ampicillin and chloramphenicol ($P = 0.05$), while for tetracycline and ceftriaxone, although not actually significant, Pearson values were borderline ($P = 0.0505$ and $P = 0.5114$).

***Shigella* spp.** Resistance of *Shigella* to various antibiotics has been reported from many countries for over 10 years. In a study by Shagid and O'Briend *(12)*, the status of *Shigella* resistance in various parts of the world was updated as of 1989. In that same year, Albert *(13)* reported multiple resistance of *S. dysenteriae* type 1.

In light of the virulence shown by *Shigella* strains circulating in Cuba, microbiological surveillance was established in 1990 to monitor the circulating strains and assess their re-

sistance (Table 3). At that time, the World Health Organization (WHO) warned countries about the importance of treating every laboratory-confirmed case of shigellosis on the basis of the results of in vitro sensitivity tests.

Shigella continues to be one of the primary etiologic agents associated with acute diarrheal disease in Cuba.

The MIC determinations for the 2,250 isolates studied over 8 years of ongoing microbiological surveillance (partially published by Ramírez et al.) *(14)* showed a statistically significant increase in resistance to nalidixic acid ($R = 0.84$) between 1990 and 1997. This resistance developed later in Cuba than in some other countries *(15–17)*, but it is statistically significant and is on the rise. Surveillance must therefore be maintained, since nalidixic acid is the drug of choice for treating this infectious agent. On the other hand, there has been a decline in the resistance of *Shigella* to gentamicin ($R = 0.94$; $P>0.01$) (see Table 3). Trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, ceftriaxone, and cefotaxime all show a correlation between the percentage of resistance and length of time.

In 1994, Ramírez et al., at the Pedro Kouri Institute *(15)*, studied plasmids in 40 isolates of *Shigella flexneri* from a single outbreak among children interned in a pediatric hospital. For ampicillin, the MIC indicated 82.5% resistance to concentrations above 8 mg to 16 mg/mL, while seven isolates, i.e. 18.5%, proved to be sensitive to concentrations of 4 mg/mL. A common plasmid was found, which was purified and transferred to *E. coli* HB 101, thus confirming the transmission of resistance.

In many countries *Shigella* has exhibited increasing virulence, which has made it necessary to step up surveillance *(12, 13)*.

Given the significance of the research demonstrating the transfer of plasmids from one bacterial species to another *(18)*, it is essential to remain on the alert for all enterobacteria.

Salmonella typhi is not a health problem in Cuba. It continues to be the object of microbiological surveillance, and its susceptibility to antimicrobial drugs has been monitored for years. Typhoid fever has occurred in isolated cases or small outbreaks. *S. typhi* has only shown resistance to ampicillin in 1996 and 1997 (10% and 8%, respectively). Resistance to any of the other drugs in use (chloramphenicol, tetracycline, cotrimoxazole, and nalidixic acid) has not appeared. In Cuba, unlike other tropical countries, no strains resistant or multiresistant to chloramphenicol *(19, 20)* have been detected. Data on antibiotic resistance, based on laboratory surveillance and patient clinical progress, point to chloramphenicol as the classical drug of choice for the treatment of typhoid fever in Cuba.

***Neisseria meningitidis* B.** The book *Cronología de una epidemia* [Chronology of an Epidemic] *(21)* recounts the history of the epidemic of meningococcal meningitis in Cuba that began in the mid-1970s. The highest incidence rate of meningococcal meningitis B was reported in 1983, when it peaked at 14.4 per 100,000 population. Microbiological monitoring of meningococcus isolates from invasive

TABLE 3. Antimicrobial resistance (MIC) of *Shigella* spp. in 250 isolates from patients, Cuba, 1990–1997

Antimicrobial agent	1990 (*n*=250)	1991 (*n*=250)	1992 (*n*=300)	1993 (*n*=300)	1994 (*n*=250)	1995 (*n*=300)	1996 (*n*=300)	July 1997 (*n*=300)
Ampicillin	90.0	95.5	98.2	90.1	85.0	80.0	96.0	98.0
Cotrimoxazole	90.5	90.8	91.9	86.5	66.5	85.0	85.0	85.0
Tetracycline	70.5	65.0	70.0	76.0	75.2	70.9	68.0	70.0
Nalidixic acid	2.0	1.5	2.0	5.0	8.0	10.0	8.0	7.0
Gentamicin	10.0	10.0	8.0	8.0	8.0	7.0	7.0	5.0
Chloramphenicol	75.0	70.0	74.0	77.0	73.0	71.0	83.0	80.0
Ceftriaxone	50.9	51.0	55.0	50.0	49.0	50.0	55.0	50.0
Cefotaxime	60.4	65.0	60.0	61.0	60.8	65.0	65.0	50.0

processes (22) was started that year. Monitoring included not only characterization of strains but studies of their behavior when challenged with the antibiotics most often used in clinical practice, especially penicillin, which has been and continues to be the antibiotic of choice (23).

Between 1989 and 1993, Martínez et al. (22) at the Pedro Kouri Institute conducted a longitudinal study using MIC to determine the sensitivity of 682 isolates of *Neisseria meningitidis* B and found that 24% of them had intermediate susceptibility to penicillin, while susceptibility to chloramphenicol, ampicillin, and rifampicin was 16.65%, 19.85%, and 33.95%, respectively. None of the strains produced β-lactamase or were plasmid carriers. In 1989, the Cuban antimeningococcal BC vaccine began to be applied throughout the country. By 1997 the incidence rate of meningococcal meningitis was 0.49 per 100,000 population.

By the mid-1980s, several countries had reported strains that showed intermediate susceptibility, but without β-lactamase activity or the presence of plasmids (24). In Cuba the first strains to show intermediate susceptibility (16.3%) appeared in 1986 (25). After that, there was a rising trend in strains with these characteristics, which reached 42.2% in 1996 (Llanes, unpublished data). In 1997, 56% of 166 isolates presented intermediate susceptibility to penicillin (25). Intermediate susceptibility increased from 24% to 88.9% in a 10-year period. None of the strains studied by the Cuban authors produced β-lactamase, nor were any penicillin-resistant strains reported until 1997.

In 1994, Sosa et al. published a study of strains that showed intermediate susceptibility to penicillin, but none of them were found to have resistance plasmids (23).

Neisseria gonorrhoeae. The Nordic countries have seen a sizable drop in the incidence of gonorrhea in the past decade, but at the same time there has been an increasing proportion of resistance to treatment with the drugs indicated for uncomplicated cases.

Epidemiologically, the gonorrhea picture in Cuba is quite different from the situation in developed countries (26, 27). A total of 31,700 cases were reported in 1997, but with very low coverage as to laboratory culture.

The MIC determinations for 106 Cuban isolates studied between 1995 and July 1998 (Table 4) showed that resistance to penicillin rose steadily, going from 54% to 63.6% during that period. The situation is even more serious if intermediate susceptibility is taken into account (12%, 10%, and 13.6% in 1995, 1996 and 1997, respectively). Resistance to tetracycline was 28%, 35%, and 60% those same years, with intermediate susceptibility at 20% in 1995, 10% in 1996, and 20% in 1997. There was no resistance to cefuroxime, ceftriaxone, cefotaxime, or ciprofloxacin. The production of β-lactamase was 58%.

In Cuba the pressure exerted by penicillin and tetracycline for more than a decade is one of the factors that led to clear in vitro resistance to these two agents (28–30).

CONCLUSIONS

The findings show the need to improve systematic microbiological monitoring of bacterial resistance to antimicrobial drugs.

A steady increase in the resistance of *S. pneumoniae* to antibiotics was found in both invasive processes and carriers. *H. influenzae* b showed high resistance to antimicrobial agents and 40% of the invasive strains exhibited β-lactamase activity.

Shigella spp. showed a steady increase in resistance to nalidixic acid.

In 1996 the emergence of *S. typhi* resistance to ampicillin was confirmed.

No penicillin-resistant strains of *N. meningitidis* B have been reported as yet, but intermediate susceptibility to that antibiotic has been on the rise.

There has been a steady rise in the resistance of *N. gonorrhoeae* to penicillin and tetracycline, with 58% of the strains exhibiting β-lactamase activity.

TABLE 4. Antimicrobial resistance (MIC) in 106 isolates of *Neisseria gonorrhoeae*, Cuba, 1995–1998

	1995 (*n* = 60)			1996 (*n* = 21)			1997–1998 (*n* = 25)		
Antimicrobial agent	S	I	R	S	I	R	S	I	R
Penicillin	34	12	54	30	10	60	22,7	13,6	63,6
Tetracycline	52	20	28	55	10	35	22	20	60
Cefuroxime	100	–	–	100	–	–	100	–	–
Ceftriaxone	100	–	–	100	–	–	100	–	–
Cefotaxime	100	–	–	100	–	–	100	–	–
Ciprofloxacin	100	–	–	100	–	–	100	–	–

– = zero
S = susceptible
I = intermediate resistance
R = resistance

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ANTIMICROBIAL RESISTANCE IN THE CARIBBEAN

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Resistance to antimicrobials is increasing among microbial pathogens causing nosocomial and community-acquired infections in the Caribbean. The Caribbean Antimicrobial Resistance Surveillance (CAARS), a laboratory-based surveillance program, is being piloted to measure and review the baseline data on resistance patterns of hospital and community acquired infections in the English- and Dutch-speaking Caribbean. The data on antimicrobial resistance of Gram-negative bacteria isolated in six laboratories during a six-month period in 1998 and pathogens isolated in community-acquired infections during 1994–1997 were reviewed. The overall resistance of Gram-negative aerobic bacilli (excluding Salmonella spp. *and* Shigella spp.*) to ampicillin, gentamicin and trimethoprim-sulphamethoxazole did not differ significantly among laboratories and countries. Resistance to third generation cephalosporins is high in Trinidad. Multiple antibiotic-resistant* Salmonella typhimurium, S. heidelberg, Shigella flexneri *type 2/2a and ampicillin- and gentamicin-resistant* S. enteritidis *strains causing community-acquired enteric infections were observed in the Caribbean. The rate of penicillin-resistant pneumococci, cefotaxime-ceftriaxone-resistant* Haemophilus influenzae, *ceftriaxone- and spectinomycin-resistant gonococci and rifampicin- and isoniazid-resistant* Mycobacterium tuberculosis *causing community-acquired infections are very low in the Caribbean. However, the potential for introduction and spread of resistant strains in the Caribbean is very high. Hence, ongoing surveillance of antimicrobial resistance of nosocomial and community pathogens is essential in this area.*

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INTRODUCTION

The growing use of antibiotics has induced a widespread dissemination of resistant genes among bacterial populations in developed and developing countries *(1, 2)*. Multiple drug-resistance is becoming more common in community-acquired infections due to *Shigella* spp. *Streptococcus pneumoniae, Mycobacterium tuberculosis* and *Neisseria gonorrhoeae*, as well as nosocomial pathogens such as *Staphylococcus aureus*, enterococci and Gram-negative bacilli. The global spread of resistant genes poses a threat to advanced antimicrobial drug regimens being used for the management of both community-acquired and nosocomial infections. The estimated cost of antimicrobial resistance ranged from as low as US$ 100 million to as high as $30 billion per year in hospitals in the United States of America *(3)*. Although data on the prevalence of antibiotic resistance are fragmentary and underutilized, several trends in resistance have emerged in many countries. Based on those findings, the United States Institute of Medicine emphasized the need for surveillance to monitor trends in antimicrobial resistance patterns in hospital- and community-acquired infections *(4)*.

Data on antibiotic resistance are limited in the Caribbean. A few studies have been conducted on the prevalence of antibiotic-resistant organisms in selected institutions during the past three decades *(5–8)*. None of the countries had a monitoring program to assess the trends in antibiotic resistance in order to institute policies to prevent and control the spread of pathogens in communities and hospitals. In this paper, we present the resistance patterns of pathogens responsible for important community- and hospital-acquired infections in selected countries of the Caribbean.

MATERIALS AND METHODS

An antimicrobial resistance surveillance program to monitor the emergence of resistance in pathogens causing nosocomial and community-acquired infections is being established through a network of hospital, and public health and private laboratories in the Caribbean. All laboratories were informed of the objectives of the regional network and were encouraged to participate in this collaborative program. The monitoring program includes nosocomial pathogens isolated from blood stream, urinary tract and wound infections, and respiratory pathogens *(Streptococcus pneumoniae, Haemophilus influenzae* and *M. tuberculosis)*, enteric pathogens *(Salmonella* spp. and *Shigella* spp.) and gonococci responsible for community-acquired infections.

Six participating laboratories contributed the results of identification, quality control of antimicrobial susceptibility testing (AST) and the profile on all clinically significant bacterial pathogens isolated between January 1998 and June 1998. Antibiotic susceptibility testing was done by the disk diffusion method (Kirby-Bauer).

Tests for antimicrobial resistance were conducted on 1,653 isolates of *Salmonella* spp. and 162 of *Shigella* spp. causing enteric infections. The Caribbean Epidemiology Center (CAREC) performed the tests by the disk diffusion method (Kirby-Bauer), as described by the U.S. National Committee for Clinical Laboratory Standards (NCCLS). Strains of *S. pneumoniae* ($n = 85$) and *H. influenzae* ($n = 94$) isolated from invasive community-acquired infections (septicemia, meningitis, and pneumonia) in three sentinel laboratories were tested by the E-test method (AB BIODISK, Solna, Sweden).

Similarly, 204 isolates of *M. tuberculosis* from five countries were tested for sensibility to antituberculosis drugs by the indirect proportion method or radiometric method *(9–11)* at the WHO Collaborating Center for Tuberculosis Bacteriology Research, at the Laboratory Centre for Disease Control (LCDC) Ottawa, Canada.

All *N. gonorrhoeae* isolates were tested for β-lactamase production by the chromogenic cephalosporin method using cefinase disks (BBL, Cockeysville, MD) in all laboratories participating in the Gonococcal Antimicrobial Surveillance Program (GASP) in the Carib-

bean. Minimum inhibitory concentrations (MIC) to penicillin, tetracycline, ciprofloxacin, ceftriaxone, and spectinomycin were determined at CAREC by the agar dilution method, using gonococcal medium base supplemented with 1% isovitalex, and serial twofold dilutions of antibiotic. MIC interpretive criteria for each antibiotic were those recommended by the NCCLS.

Quality Control

Quality control was performed by testing *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 29213, *Staphylococcus aureus* ATCC 25913, *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247 and ATCC 49766, *Neisseria gonorrhoeae* ATCC 49226 and WHO III, WHO V and WHO VII, applying interpretive criteria as described by the NCCLS.

RESULTS

Frequency of Occurrence of Pathogens in Participating Laboratories

During the six-month study period (January to June 1998), six laboratories (four public and two private) in four countries provided data to CAREC on 2,099 isolates of bacterial pathogens and their antimicrobial susceptibility results. The most common site of these isolates was urine, followed by wound infections, blood, and others. The predominant Gram-negative (nonfastidious) pathogens isolated in participating laboratories during the study period and the number and proportion of each organism were as follows: *Escherichia coli*, 718 (34.2%); *Klebsiella* spp., 472 (22.5%); *Pseudomonas* spp., 281 (13.4%); *Enterobacter* spp., 267 (12.7%); *Proteus* spp., 245 (11.5%); others, 116 (5.5%).

Gram-negative Pathogens

Table 1 lists the results for 13 antimicrobial agents tested against Gram-negative pathogens isolated, and resistance rates seen in the Caribbean. The antimicrobial resistance rate for ampicillin in this study ranged from 51% in Barbados to 85% in Bahamas. Interestingly, the amoxicillin-clavulanate resistance rate was higher in Grand Bahamas (59%) compared to other laboratories (23%–32%) in the Caribbean. Piperacillin resistance rates ranged from 27% to 33% in this study. Aminoglycoside resistance rates were higher in hospital laboratories (8%–11%) compared to isolates tested in two private laboratories (3%–8%).

The overall rate of 9% resistance to third-generation cephalosporins was seen in pathogens isolated in a tertiary hospital laboratory in Trinidad. The resistance rates for trimethoprim-sulphamethoxazole ranged from 27% in the British Virgin Islands to 47% in Barbados. Quinolone resistance was higher in the British Virgin Islands (32%) than in other countries in this study.

Table 2 shows the resistance rates for *E. coli* and *Klebsiella* spp. isolated in laboratories participating in this program. *E. coli* was the pathogen most commonly isolated in this study, and it showed a resistance rate of 6% to quinolones (ofloxacin) and aminoglycosides. Resistance to third-generation cephalosporins among *E. coli* isolates was 2% in Trinidad. The resistance rate to third-generation cephalosporins was 19% among *Enterobacter* spp. isolates, followed by *Klebsiella* spp. (10%), *Pseudomonas* (10%), and *Proteus* spp. (4%). Twelve to sixteen percent of *Pseudomonas* spp. isolates were found to be resistant to tobramycin and gentamicin. Resistance rates for piperacillin and ofloxacin were 9% and 8%, respectively.

Antimicrobial Resistance of *Salmonella* Other than *S. typhi*

Tests for resistance to a variety of antibiotics were conducted on 1,653 strains of *Salmonella* (Table 3). The overall prevalence of ampicillin resistance was 18.3%, followed by chloramphenicol (15%), amoxycillin-clavulanate (12.6%), trimethoprim-sulphamethoxazole

TABLE 1. Resistance to antibiotics (%) in Gram-negative facultative anaerobic bacilli in the Caribbean, according to laboratory and antimicrobial agent, 1998

Antimicrobial class and agent tested	Resistance (%) – Laboratory					
	TT-03	TT-06	TT-07	BV-01	BH-02	BR-01
Penicillins and β-lactam inhibitors						
• Ampicillin	74	72	63	71	85	51
(*n* resistant/total)	(969/1,306)	(55/76)	(25/40)	(51/66)	(297/351)	(27/53)
• Amoxicillin-clavulanate	24	32	23	NT	59	NT
(*n* resistant/total)	(314/1,294)	(33/71)	(9/40)		(135/332)	
• Piperacillin	27	–	NT	29	33	NT
(*n* resistant/total)	(277/1,010)	(0/7)		(11/38)	5/15)	
Cephalosporins						
• Cephazolin-cephalothin	45	NT	67	50	64	49
(*n* resistant/total)	(346/765)		(9/28)	(33/66)	(101/284)	(28/66)
• Cefuroxime	17	19	8	21	43	NT
(*n* resistant/total)	(182/1,056)	(9/47)	(2/24)	(14/66)	(46/105)	
• Cefoxitin	19	NT	NT	NT	NT	NT
(*n* resistant/total)	(30/160)					
• Cefotaxime-ceftriaxone-ceftazidime (*n* resistant/total)	9 (134/1,432)	16 (7/44)	NT	NT	NT	NT
Aminoglycosides						
• Gentamycin	10	8	3	7	11	10
(*n* resistant/total)	(133/1,353)	(5/66)	(1/32)	(6/86)	(42/386)	(11/51)
• Nalidixic acid	NT	38	25	NT	NT	NT
(*n* resistant/total)		(5/33)	(10/40)			
Quinolones						
• Norfloxacin	NT	7	8	25	9	NT
(*n* resistant/total)		(2/28)	(3/40)	(22/88)	(23/261)	
• Ciprofloxacin-ofloxacin	7	7	8	47	NT	NT
(*n* resistant/total)	(54/774)	(2/27)		(18/38)		
Others						
• Furadantin	22	38	35	28	NT	NT
(*n* resistant/total)	(103/461)	(5/13)	(14/40)	15/53		
• Trimethoprim-sulphamethoxazole	29	29	29	27	37	47
(*n* resistant/total)	(373/1,265)	(20/68)	(9/31)	18/66	(143/386)	(36/76)

NT = not tested.

(8.1%), and gentamicin, (2.1%). *S. typhimurium* strains were responsible for most of the increase in antibiotic resistance seen in the Caribbean during the study period. Of *S. typhimurium* strains, 49% were multiresistant (>2 antibiotics). *S. heidelberg* was the second most common serotype, showing resistance to ampicillin, (15.7%), chloramphenicol (11.4%), amoxycillin-clavulanate (10%) and gentamycin (10%). The resistance of *S. agona* and *S. javiana* ranged from 1.6% to 5.8% against ampicillin, gentamicin and trimethoprim-sulphamethoxazole. Resistance among *S. enteritidis* strains was very low (0.6% to 2.3%).

Table 4 shows the resistance rates in different *Salmonella* phage types (PT). *S. typhimurium* PT 104b was the most resistant in this study. Of 128 *S. typhimurium* (PT104 b) strains tested, 95% were resistant to ampicillin and chloramphenicol, followed by amoxycillin-clavulanate (90%) and trimethoprim-sulphamethoxazole (49%) as compared to PT 49 (2%–3%) and PT 135 (0%). No resistance was observed for PT 4 and PT 1.

Shigella spp.

Regarding *Shigella* spp., 162 isolates were tested for antimicrobial resistance to common

TABLE 2. Resistance to antibiotics of *Escherichia coli* and *Klebsiella* sp. in the Caribbean, 1998

Antimicrobial class and agent tested	Resistance (%) *Escherichia coli* (*n* = 718)	*Klebsiella* sp. (*n* = 472)
Penicillins and β-lactam inhibitors		
• Ampicillin	59 (424/718)	98 (464/472)
• Amoxicillin-clavulanate	14 (78/577)	31 (126/403)
• Piperacillin	36 (171/479)	29 (109/379)
Cephalosporins		
• Cefazolin-cefalotin	46 (311/682)	33 (147/453)
• Cefuroxime-cefoxitin	5 (26/558)	9 (37/420)
• Cefotaxime-ceftriaxone-ceftazidime	4 (18/479)	10 (36/379)
Aminoglycosides		
• Gentamicin	5 (38/677)	6 (30/470)
• Tobramycin	5 (26/479)	7 (25/379)
• Nalidixic acid	NT	NT
Quinolones		
• Norfloxacin	12 (3/25)	11 (3/27)
• Ciprofloxacin-ofloxacin	6 (31/498)	2 (10/420)
Others		
• Furadantin	21 (108/522)	25 (102/407)
• Trimethoprim-sulphamethoxazole	33 (236/718)	25 (117/471)

NT = not tested.

TABLE 3. Resistance to antibiotics (%) in *Salmonella* isolates in the Caribbean, 1994–1997

Antimicrobial class and agent tested	*Salmonella* *typhimurium* (*n* = 528)	*enteritidis* (*n* = 350)	*heidelberg* (*n* = 70)	*javiana* (*n* = 62)	*agona* (*n* = 52)	*Salmonella* spp. (*n* = 591)	Total (*n* = 1,653)
Penicillins and β-lactam inhibitors							
• Ampicillin	49.4	2.3	15.7	1.6	3.8	3.4	18.3
• Amoxicillin-clavulanate	35.2	1.1	10	–	3.8	2.2	12.6
Cephalosporins							
• Cefazolin	0.1	0.6	1.4	–	1.9	0.2	0.3
• Cefotaxime	–	–	–	–	–	–	0
Aminoglycosides							
• Gentamicin	1.3	0.9	10	1.6	5.8	3.0	2.1
Quinolones							
• Norfloxacin	–	–	–	–	–	–	0
• Ciprofloxacin	–	–	0	0	0	0	0
Others							
• Chloramphenicol	47	–	11.4	–	–	0.7	15.0
• Trimethoprim-sulphamethoxazole	23.1	0.9	–	–	5.8	1.2	8.1

– = zero.

TABLE 4. Phage types and antibiotic resistance in *Salmonella* spp. isolated between 1994–1997 in selected countries of the Caribbean

Antimicrobial class and agent tested	*Salmonella typhi* PT 104 b (*n* = 128)	PT 49 (*n* = 44)	PT 135 (*n* = 14)	*Salmonella enteritidis* PT 8 (*n* = 17)	PT 4 (*n* = 19)	PT 1 (*n* = 5)
Penicillins and β-lactam inhibitors						
• Ampicillin	122 (95%)	1 (2.3%)	–	–	–	–
• Amoxicillin-clavulanate	115 (90%)	1 (2.3%)	–	–	–	–
Cephalosporins						
• Cephazolin	–	–	–	–	–	–
• Cefotaxime	–	–	–	–	–	–
Aminoglycosides						
• Gentamycin	–	–	–	2 (11.8%)	–	–
Quinolones						
• Norfloxacin	–	–	–	–	–	–
• Ciprofloxacin	–	–	–	–	–	–
Others						
• Chloramphenicol	122 (95%)	2 (4.6%)	–	–	–	–
• Trimethoprim-sulphamethoxazole	63 (49%)	1 (2.3%)	–	–	–	–

– = zero.

antibiotics. Of those, 83% of *Sh. flexneri* isolates were resistant to trimethoprim-sulphamethoxazole, followed by resistance to ampicillin (78.5%), chloramphenicol (55.5%), and amoxycillin-clavulanate (54%). All strains were susceptible to quinolones and gentamycin.

Streptococcus pneumoniae and *Haemophilus influenzae*

Of 86 *S. pneumoniae* isolates tested, one was resistant to penicillin, cefaclor, amoxycillin-clavulanate and erythromycin. However, 7% of *S. pneumoniae* isolates presented intermediate resistance (0.12 to 1 μg/mL) to penicillin. All isolates were sensitive to trimethoprim-sulphamethoxazole and ceftriaxone.

Of all *H. influenzae* isolates tested in Trinidad, 18% were β-lactamase positive. Resistance noted was 20% to ampicillin, 4% to chloramphenicol, and 7.4% to trimethoprim-sulphamethoxazole. The rate of beta-lactamase producing *H. influenzae* was 5% in St. Vincent. Also, 5% resistance to ampicillin and chloramphenicol was observed there. All isolates were sensitive to cefuroxime and ceftriaxone. Of 203 *M. tuberculosis* isolates profiled for antimicrobial susceptibility, three isolates (3/203) were isoniazid- and rifampicin-resistant. The overall resistance rate for any antibiotic was 9.8% (Table 5).

N. gonorrhoeae

Among 1,944 isolates tested, the rates of penicillinase-producing *N. gonorrhoeae* (PPNG) ranged from 4% in Trinidad, to 72% in Bahamas, Guyana, and Suriname. The lowest rates were observed in St. Lucia (5%–6%) and Antigua (12 %). Between 1994 and 1997, PPNG rates decreased in Suriname (from 72% to 50%), St. Vincent (38% to 10 %), Cayman Islands (56% to 23%), Barbados (37% to 14%), and Belize (63% to 53%).

In Guyana, 57% of N. *gonorrhoeae* were PP/TRNG (penicillinase-producing and plasmid-mediated tetracycline resistant), 15% PPNG alone, and 20% presented chromosomally-mediated tetracycline resistant (CMTR). The total burden of resistant *N. gonorrhoeae* (penicillin and tetracycline) was 91% in Guyana as

TABLE 5. Antimicrobial resistance patterns in community-acquired respiratory pathogens in selected Caribbean countries, 1994–1997

Antibiotic	*Mycobacterium tuberculosis* (*n* = 203)	*Streptococcus pneumoniae* (*n* = 86)	*Haemophilus influenzae* (*n* = 74)
Any antibiotic	20 (9.8%)	NA	NA
Isoniazid	4 (2%)	NA	NA
Rifampicin	2 (1%)	NA	NA
Streptomycin	10 (4.7%)	NA	NA
Isoniazid plus rifampicin	3 (1.5%)	NA	NA
Penicillin G	NA	1%*	NA
Ampicillin	NA	NA	23.5
Amoxicillin-clavulanate	NA	1%	–
Cefaclor	NA	1%	–
Ceftriaxone	NA	NA	–
Chloramphenicol	NA	NA	3.4
Trimethoprim-sulphamethoxazole	NA	–	7.6
Erythromycin	NA	1%	NA
β-lactamase	NA	NA	23.5

NA = not applicable.
* 7% of *S. pneumoniae* presented intermediate resistance to penicillin.
– = zero.

compared to 37% (22% CMTR, and 9% TRNG) in St. Vincent and 43% (34% CMTR and 9% TRNG) in Trinidad. All isolates were susceptible to ceftriaxone (MIC_{90} 0.004 μg/mL), ciprofloxacin (MIC_{90} 0.01 μg/mL) and spectinomycin (MIC_{90} 32 μg/mL). Sporadic strains of reduced susceptibility to ciprofloxacin (>0.0125 μg/mL) and ceftriaxone were observed in Guyana, Suriname, St. Vincent and Trinidad. The MIC_{50} and MIC_{90} to ciprofloxacin and ceftriaxone were two- to fourfold higher in PPNG, CMRNG, CMTR and TRNG strains, as compared to sensitive gonococcal isolates from Guyana and Trinidad.

DISCUSSION

It is very clear that an effective surveillance system is needed in the Caribbean to monitor trends in antimicrobial resistance patterns of pathogens causing both nosocomial and community-acquired infections. The information on resistance patterns provided by surveillance programs is crucial to the development of empirical approaches for the management of serious infections, as well as for policy formulation for the control of antimicrobial-resistant organisms. In the United States, the National Nosocomial Infection Surveillance system (NNIS) *(12)*, the Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) *(13)*, and the Intensive Care Antimicrobial Resistance Epidemiology (ICARE) *(14)* projects have been providing data on antimicrobial resistance of nosocomial pathogens. No such programs exist in the Caribbean. As a cornerstone to improving national sexually-transmitted disease control programs, a laboratory-based surveillance program was set up for monitoring the trends in gonococcal resistance and detection of new types of resistance in the Caribbean. This program linked to the WHO/PAHO-supported Gonococcal Antimicrobial Surveillance Program (GASP). As part of the laboratory-based Caribbean Antimicrobial Resistance Surveillance project, a pilot study was carried out in four countries in 1998, to establish the baseline resistance rates in common bacterial pathogens. This was done through the application of standardized protocols, quality control, and

reporting of antibiotic resistance results, which are presented in this paper and aim to emphasize the need for ongoing surveillance of antibiotic resistance.

During the last three decades, several institutional studies to establish the baseline data on antibiotic resistance rates in common bacterial pathogens have been conducted in the Caribbean *(5–8, 15)*. Data on resistance rates for ampicillin, trimethoprim-sulphamethoxazole, first-generation cephalosporins and gentamicin in pathogens isolated between 1975 and 1992 in selected countries have been published elsewhere *(5, 7, 16)*. French et al. *(5)* reported a multidrug-resistant rate of 44% among Gram-negative bacilli in 1975 at the University Hospital of the West Indies, Jamaica. Multidrug-resistant rates during 1992 were 55%, 51% and 43% in public hospital laboratories in Jamaica, Trinidad, and Barbados, respectively.

Of 544 Gram-negative bacterial isolates tested, less than 1% resistance to third-generation cephalosporin was observed in three Caribbean islands, including Barbados, in 1992 (6). Bodonaik et al. *(17)* reported an overall rate of 18% resistance to third-generation cephalosporins in the University Hospital of the West Indies in Jamaica. Higher rates were observed in *Enterobacter* spp. (20%) and *Acinetobacter* spp. (28%) in that study. Similar rates were found in a tertiary care hospital in Trinidad in 1998. Levett et al. *(18)* reported few isolates of Gram-negative bacilli resistant to third-generation cephalosporins in 1993. However, no prevalence studies on extended spectrum β-lactamase (ESBL)-producing Gram-negative bacilli were conducted in Barbados. In Trinidad, the prevalence of resistance to third-generation cephalosporins ranged from 9% in a general hospital to 18% among pathogens isolated in a private laboratory. No published reports of ESBL prevalence are available from other countries of the Caribbean.

Several outbreaks of multidrug resistant *Salmonella* spp. and antimicrobial resistance patterns in nontyphoidal *Salmonella* spp. and *Shigella* spp. in the Caribbean were reported between 1980 and 1995 *(19–23)*. Antimicrobial resistance in enteric pathogens is increasing in the United States of America *(24)*. The risk factors for increased resistance in *Salmonella* spp. were shown to be the use of antibiotics one month prior to infection, and the patient's age (under 1 year) *(24)*. In this study, resistance to ampicillin, chloramphenicol, and trimethoprim-sulphamethoxazole was high in *S. typhimurium*, followed by *S. heidelberg* and *S. agona*. The occurrence of multidrug resistant *S. typhimurium* PT104 b is a cause for concern in the Caribbean (Table 4). It appears that resistant genes are being transferred to other nontyphoidal *Salmonella*, namely, *S. heidelberg* and *S. agona*. The resistance rate among *S. enteritidis* strains is low when compared to other *Salmonella* spp. in our study. Interestingly, antibiotic resistant *Sh. flexneri* serotype 2/2a strains are common in this study. This strain was responsible for two outbreaks (Suriname and Barbados) in the Caribbean. Multidrug resistant *Sh. flexneri* strains have also been reported in other countries in the Caribbean. It appears that this strain is spreading to a number of Caribbean islands.

The low level of resistance seen in *H. influenzae* and *S. pneumoniae* strains responsible for serious community-acquired infections is surprising. Although the rate of penicillinase-producing *H. influenzae* (5%–20%) is comparable to that of the United States, the prevalence of resistance to chloramphenicol and trimethoprim-sulphamethoxazole was very low in the present study. No second- or third-generation cephalosporin resistance was found among strains of *H. influenzae*. Only 1% of *S. pneumoniae* isolates were found to be resistant to penicillin and erythromycin. Resistant strains of *S. pneumoniae* are still uncommon in the Caribbean despite two published reports in the last few years *(25, 26)*. Importation of penicillin-resistant strains of *S. pneumoniae* by visitors to the Caribbean from other countries of the Americas, Europe, and elsewhere warrants ongoing surveillance in certain key islands.

The global rate of resistance in tuberculosis has recently been reviewed by Cohn et al. (27) Even though the rate of multidrug-resistant tuberculosis is high in the Americas, the prevalence of resistance in the present study was only 9.4%. Resistance to isoniazid-rifampicin was seen in 1.5% (3/203) of *M. tuberculosis* isolates. However, only a fraction of those strains were tested for antibiotic susceptibility in this series; therefore, the data should be interpreted with caution. The possibility of TB cases with primary and acquired resistant *M. tuberculosis* strains exists in CAREC member countries. This means there is an urgent need for a comprehensive study to determine the true prevalence of resistance in *M. tuberculosis* in the Caribbean.

The continued high prevalence of plasmid-mediated penicillin- and tetracycline-resistance among isolates of *N. gonorrhoeae* in the Caribbean and other parts of the world precludes the use of these antibiotics for the treatment of gonococcal infections (28–31). However, *N. gonorrhoeae* remains highly susceptible to ceftriaxone, spectinomycin and fluoroquinolones, drugs which have been shown to be effective in the treatment of gonorrhea in the Caribbean. Only a few strains having intermediate susceptibility to ceftriaxone and quinolones have been identified in Caribbean countries. To ensure that these drugs are effective, ongoing surveillance of gonococcal resistance is needed.

In conclusion, the baseline data on antimicrobial resistance of selected pathogens have been established in the Caribbean. Ongoing national and regional surveillance of emerging pathogens and their resistance mechanisms would enhance our efforts to contain the spread of antibiotic resistant organisms in the Caribbean.

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TRENDS IN THE ANTIMICROBIAL SUSCEPTIBILITY OF STRAINS ISOLATED FROM A HIGH-COMPLEXITY HOSPITAL IN CHILE FROM 1991 TO 1998

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In view of the fact that local information on antimicrobial resistance helps to improve clinical decision-making and supports policies on the utilization of antimicrobial drugs, the objective of this study was to determine trends in the antimicrobial resistance of the principal bacteria in Carlos Van Buren Hospital in Valparaiso, Chile. Data on the antimicrobial susceptibility of all the bacteria isolated in the Microbiology Laboratory of Van Buren Hospital from 1991 to 1998 were analyzed.

Substantial increases were observed in antimicrobial resistance, especially in Gram-negative bacilli, which means that the usefulness of many of these agents has decreased.

INTRODUCTION

Since the incorporation of antimicrobial drugs as therapeutic tools, it has been observed that certain microorganisms present natural resistance to antibiotics, or acquire it through various genetic mechanisms. In the hospital and the community a link has been found between the increase in the resistance to antimicrobials and the increase in their use. This phenomenon has accelerated in hospitals, to a large extent because of the greater dissemination of resistant strains in those institutions, the result of poor practices for the prevention of infection, and the intensive use of antimicrobials, which in turn is the consequence of the lack of effective policies to rationalize their use or the ineffectiveness of such policies, when they exist.

With some frequency, multiresistant bacteria are isolated for which there are no effective antimicrobials in the hospital environment. Although this phenomenon has not resulted in a demonstrated increase in mortality (1–3), it has led to concern among practicing physicians about the treatment of cer-

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tain severe infections. Furthermore, the clear impact of antimicrobial resistance has been observed in the cost of health care, since the need for treating infections due to bacteria resistant to certain antibiotics has given rise to the empirical use of third generation drugs, which cost 20 times as much or even more than that of traditional therapies (Eliana Rojas, personal communication). For these reasons, epidemiology surveillance of resistance to antimicrobials has become a useful tool in the guiding of clinical patient care and in the adoption and strengthening of policies for the utilization of antimicrobial drugs.

MATERIALS AND METHODS

Carlos Van Buren Hospital is located in the city of Valparaiso, Chile. It is a tertiary level public hospital with 650 beds and approximately 30,000 admissions per year. It treats critical adult, pediatric, and neonatal patients. Bacterial identification was carried out in the hospital's microbiology laboratory using standard microbiology techniques; the antibiograms were performed using the Kirby-Bauer technique, following the recommendations of the U.S. National Committee for Clinical Laboratory Standards (NCCLS) (4). Internal and external quality controls are exercised periodically by the Institute of Public Health of Chile. Susceptibility results are reported on the basis of the halo readings from the WHONET software (5).

The bacteria selected for epidemiological analysis were those most frequently isolated in Carlos Van Buren Hospital, where a minimum of 40 such isolates were available per year. This information was obtained from the microbiology laboratory database and corresponds to the percentage of bacteria isolated from hospital patients (one isolate per patient) between 1991 and 1998 that were susceptible to antimicrobials. To determine the differences between the percentages of susceptibility observed in 1991 and in 1998, the χ^2 test was performed.

RESULTS

During the period analyzed, data on susceptibility to antimicrobials were obtained for 30,191 bacterial isolates from the hospital. These came for the most part from urine samples, surgical wounds, bronchia, and blood. The bacteria most frequently isolated were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* coagulase-negative *Staphylococcus, Proteus mirabilis, Acinetobacter baumannii,* and *Enterobacter* spp. (see Table 1).

In 1998, 97% of the isolates of the species *Acinetobacter baumannii* were sensitive to imipenem, half of the isolates were sensitive to ampicillin-sulbactam, and less than 40% to other antibiotics evaluated. Of the isolates of *Pseudomonas aeruginosa*, 95% were sensitive to imipenem and 91% to ceftazidime. Sensitivity to the rest of the drugs was less than 20%. Of the isolates of the *Klebsiella pneumoniae* spp., more than 90% were sensitive to imipenem, but less than 65% were sensitive to the other antimicrobial drugs. All the isolates of *Escherichia coli* were sensitive to imipenem and 90% to the majority of the antimicrobials commonly used in the hospital environment. Of the *Proteus mirabilis* isolates, 99% were sensitive to imipenem and more than 70% to cefotaxime and amikacin. There was no resistance to imipenem among the isolates of *Enterobacter cloacae,* and 80% were sensitive to

TABLE 1. Distribution of the bacterial strains isolated in the Carlos Van Buren Hospital, Valparaiso, Chile, in 1998

Bacterial species	Number of isolates	Percentage
Escherichia coli	1,801	43.5
Staphylococcus aureus	580	14.0
Pseudomonas aeruginosa	347	8.4
Klebsiella pneumoniae	333	8.0
Coagulase-negative *Staphylococcus*	229	5.5
Proteus mirabilis	202	4.9
Acinetobacter baumannii	136	3.3
Enterobacter spp.	119	2.9
Enterococcus spp.	24	0.6
Other	371	8.9
Total	4,142	100

the rest of the antimicrobials (see Table 2). All the isolates of *Staphylococcus aureus* were sensitive to vancomycin and 68% to oxacillin. Similarly, 60% of the isolates of coagulase-negative staphylococci were sensitive to oxacillin. Isolates of *Enterococcus* spp. were found very infrequently; 90% of them were sensitive to ampicillin and 100% to vancomycin (see Table 3).

Changes in the sensitivity of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, coagulase-negative *Staphylococcus*, *Proteus mirabilis*, *Acinetobacter baumannii*, and *Enterobacter* spp. between 1991 and 1998 can be seen in Table 4. There were significant reductions in the sensitivity of the isolates of *A. baumannii* to amikacin (49.9%), ampicillin-sulbactam (49.2%), and ciprofloxacin (~41.7%). In addition, the sensitivity of *K. pneumoniae* isolates to cefotaxime decreased by 42.2%, to ceftazidime by 58.6%, and to ciprofloxacin by ~34.9%. The sensitivity of *P. aeruginosa* to amikacin also decreased, by 18.1%, and to ceftazidime by 3.2%. With regard to the isolates of *P. mirabilis*, the most notable change was the decrease in the sensitivity to ciprofloxacin by 34.5%. In general, the sensitivity of the *E. coli* strains has held steady; however, a ~4% reduction in its response to ciprofloxacin was observed. The sensitivity of *E. cloacae* to cefotaxime was reduced by 22.1%, to ceftazidime by 9.6%, to ciprofloxacin by 7.2%, and to ampicillin/sulbactam by 57.4%. The isolates of *S. aureus* showed a decrease of 8.4% in sensitivity to oxacillin.

COMMENTS AND CONCLUSION

Sensitivity to quinolones, represented by ciprofloxacin, has declined substantially among the majority of the Gram-negative bacilli subject to surveillance. There has also been a decline in the sensitivity of nonfermenting Gram-negative bacilli and in the strains of *P. mirabilis* to amikacin. Almost all the Gram-negative bacilli, except for *E. coli*, have shown reduced sensitivity to third-generation cepha-

TABLE 2. Sensitivity of the most frequently isolated Gram-negative bacilli to various antimicrobials, Carlos Van Buren Hospital, Valparaiso, Chile, 1998

Bacterial species (number of isolates)	Sensitivity (%)							
	Amikacin	Ampicillin	Ampicillin-sulbactam	Cefotaxime	Ceftazidime	Imipenem	Gentamicin	Ciprofloxacin
Escherichia coli (1,801)	96	29	76	96	96	100	92	93
Klebsiella pneumoniae (333)	57	1	30	42	37	98	44	56
Pseudomonas aeruginosa (347)	64	0	6	24	91	95	46	73
Acinetobacter baumannii (136)	36	2	50	3	26	97	14	14
Proteus mirabilis (202)	73	35	84	92	99	99	53	60
Enterobacter cloacae (47)	89	17	57	38	85	100	87	89

TABLE 3. Sensitivity of the most frequently isolated Gram-negative coccaceous bacteria to various antimicrobials, Carlos Van Buren Hospital, Valparaiso, Chile, 1998

Bacterial species (number of isolates)	Sensitivity (%)						
	Oxacillin	Ampicillin	Vancomycin	Cefazolin	Erythromycin	Clindamycin	Ciprofloxacin
Staphylococcus aureus (580)	68	5	100	72	47	71	66
Coagulase-negative *Staphylococcus* (229)	60	12	100	86	52	78	71
Enterococcus spp. (24)	N/A	90	100	N/A	N/A	N/A	29

N/A = not applicable; these antibiotics were not evaluated.

TABLE 4. Sensitivity of certain Gram-negative bacilli to various antimicrobials, Carlos Van Buren Hospital, Valparaiso, Chile, 1991 and 1998

Bacterium (number of isolates, 1991 and 1998)	Sensitivity (%)											
	Amikacin		Gentamicin		Ampicillin-sulbactam		Cefotaxime		Ceftazidime		Ciprofloxacin	
	1991	1998	1991	1998	1991	1998	1991	1998	1991	1998	1991	1998
Klebsiella pneumoniae (394 and 333)	63	57	40	44	33	30	73	42*	89	37*	86	56*
Acinetobacter baumannii (292 and 136)	72	36*	9	14	98	50*	1	3	24	26	24	14
Pseudomonas aeruginosa (366 and 347)	78	64*	48	46	3	6	8	3	94	91*	73	73
Enterobacter cloacae (136 and 47)	78	89	62	87*	54	23	86	67*	94	85	96	89
Escherichia coli (1,734 and 1,801)	94	96	93	92	72	76	92	96*	98	96	97	93*
Proteus mirabilis (232 and 202)	77	73	49	53	51	84	91	92	94	99	91	60*

*Statistically significant change.

losporins, such as ceftazidime and cefotaxime, as illustrated by the sensitivities of the *K. pneumoniae* isolates presented in Figure 1. The reduction in the sensitivity of *A. baumannii* to ampicillin-sulbactam in the last two years must be considered serious, especially since this drug is one of the two effective options for treating infections by this agent (see Figure 2). With respect to *S. aureus*, the loss of sensitivity to oxacillin detected in the hospital at the beginning of the 1980s has slowed in recent years (see Figure 3). On the other hand, the increase in the sensitivity observed among some bacteria, such as *E. cloacae* or *P. mirabilis*, is of greater interest from the microbiological standpoint than from the practical one, since it concerns less frequently isolated bacterial types and involves antimicrobials that are not the first indicated, especially because of their cost (third-generation cephalosporins or ampicillin-sulbactam). The sensitivity of Gram-negative bacilli to imipenem has remained high and 100% of the strains of *Staphylococcus aureus* and *Enterococcus* spp. remain susceptible to vancomycin.

FIGURE 1. Susceptibility of *Klebsiella pneumoniae*, Hospital Carlos Van Buren, Valparaiso, Chile, 1991 to 1998

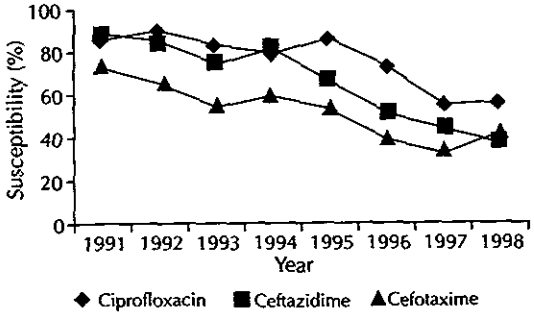


FIGURE 2. Susceptibility of *Acinetobacter baumannii*, Hospital Carlos Van Buren, Valparaiso, Chile, 1991 to 1998

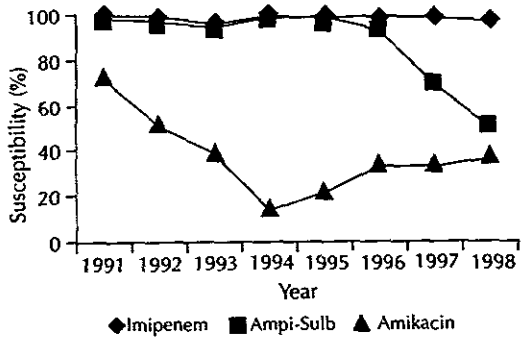
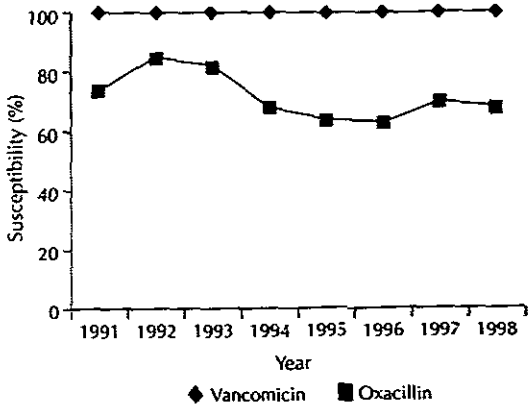


FIGURE 3. Susceptibility of *Staphylococcus aureus*, Hospital Carlos Van Buren, Valparaiso, Chile, 1991 to 1998



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OVERVIEW OF ANTIBIOTIC RESISTANCE IN COLOMBIA[1]

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To determine the status of antibiotic resistance in Colombia, data on hospital bacterial infections and bacteria in the community were gathered from recent epidemiological reports.

The most significant findings were the following: Regarding the bacteria in the community, 20% of the Streptococcus pneumoniae *isolates had reduced susceptibility to penicillin, and 8.3% were highly resistant. In the cases of* Haemophilus influenzae, *β-lactamase production was 22% in the isolates from hemocultures. In the isolates of* Salmonella *spp., 76% were resistant to ampicillin and trimethoprim-sulfamethoxazole, while resistance in the* Shigella *spp. was 80% for ampicillin and chloramphenicol and 93% for trimethoprim-sulfamethoxazole, with marked variations between regions. Resistance in* Vibrio cholerae *was 21% for trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol; in* Neisseria meningitidis, *8.5% had reduced susceptibility to penicillin and 43% to rifampicin. Among the isolates of* N. gonorrhoeae, *51% were β-lactam-positive.*

Among the isolates from hospitals, Staphylococcus aureus *presented very low susceptibility to oxacillin (66% of isolates in university hospitals). The first cases of vancomycin-resistant* Enterococcus *spp. were reported. Resistance of* Enterobacter *spp. and* Klebsiella pneumoniae *to third-generation cephalosporins was also observed. Ceftazidime and imipenem were more active against* Pseudomonas aeruginosa. *The sensitivity of* Acinetobacter *spp. to amikacin, aztreonam, ciprofloxacin, cefoperazone, and ceftazidime was less than 50%. The data show that antimicrobial resistance in Colombia is a major problem, whose recognition should lead to the strengthening of surveillance systems and the formulation of national policies that will make for better control.*

INTRODUCTION

Antibiotic resistance is a worldwide phenomenon that respects neither countries nor national borders. A current example is the spread of penicillin-resistant pneumococci, which went from sporadic events in a few countries in the 1970s to a frequency today of more than 20% in almost all areas of the world *(1)*. Other examples of this problem are methicillin-resistant *Staphylococcus aureus*, already a common finding in nosocomial

[1] Source: *Revista Panamericana de Infectología* 1999 (suppl. 1 May):S18–S25. Published by permission of the Asociación Panamericana de Infectología [Pan American Association of Infectious Diseases].

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infections; the appearance of vancomycin-resistant enterococci; and, in Gram-negative bacteria, the spread of extended-spectrum β-lactams, which is being described with increasing frequency (2). These examples show that it is increasingly important to monitor antibiotic resistance in order to understand and anticipate the trends and thus promote actions to improve management of the problem (3).

Colombia shares with many other countries the factors that contribute to the growing resistance to antibiotics. Among these are the lack of clear policies for controlling and governing the production of antibiotics, the precarious economic situation of the health systems, and the almost total lack of systematic surveillance programs at either the institutional or national level.

Colombia also has other special circumstances that have stood in the way of gaining an adequate understanding of the problem: meager research and published literature on the topic, the need for more research and reference centers devoted to basic aspects of resistance, lack of standardized methods, and inadequate quality assurance systems in many Colombian laboratories.

Despite these circumstances, however, in recent years, thanks to efforts by public and private institutions, external quality control programs have been strengthened and internal quality control has assumed greater importance as a fundamental aspect of laboratory performance. This has permitted not only the generation of more reliable data on the susceptibility and resistance of infectious agents to antibiotics, but also the possibility of compiling these data to give a current view of the status of antibiotic resistance in Colombia, as in the present article.

MATERIALS AND METHODS

Recent data were collected on the bacteria that cause infections in the community and in hospitals.

From the bacteria in the community, information was obtained on the following pathogens of special importance: *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella* spp., *Shigella* spp., *Vibrio cholerae, Neisseria meningitidis,* and *Neisseria gonorrhoeae.* The data came from national programs coordinated by the Microbiology Group at the National Institute of Health and had been published in biweekly national epidemiological reports or various national and international scientific publications.

To obtain data on hospital bacterial isolates, four third-level hospitals were selected, each of which has ongoing quality control programs, offers routine systematic management of data on susceptibility, and applies internationally standardized methods in its testing.

Two of these institutions were university hospitals: the University del Valle Evaristo García Hospital in Cali, and the St. Vincent de Paul University Hospital in Medellín. Both of them are recognized for their strong teaching component. For the most part they serve low-income communities. The other two hospitals that contributed data are private: the Santafé de Bogotá Foundation Hospital in Bogotá, and the Pablo Tobón Uribe Hospital in Medellín. They both have teaching functions, but are not considered university hospitals. They tend to serve communities that are in a better economic situation.

Susceptibility data for 1997 were collected from these hospitals in electronic or written form. The Pablo Tobón Uribe Hospital and St. Vincent de Paul University Hospital routinely use WHONET as a tool for the collection and management of data on susceptibility.

RESULTS

Streptococcus pneumoniae. The available data in Colombia on this microorganism are part of a study promoted by the Pan American Health Organization and the Regional System for Vaccines (SIREVA) in six Latin American countries. In Colombia, the study

was coordinated by the Microbiology Group at the National Institute of Health. As of 1998, a total of 543 isolates had been studied, all of them from children under the age of 5, and 73.7% from children under 2. The breakdown by diagnosis was as follows: meningitis, 255 isolates (47.1%); pneumonia, 215 (39.6%); sepsis, 43 (7.9%); other invasive processes, 30 (5.5%).

Of the 543 isolates studied, 110 (20.3%) showed reduced susceptibility to penicillin: 65 of these (12%) had intermediate resistance (MIC 0.15–1.0 μg/mL) and 45 (8.3%) were highly resistant (MIC >2.0 μg/mL). The frequency of highly resistant isolates rose significantly between 1994 and 1998 (Table 1).

Resistance to penicillin was accompanied by resistance to other antibiotics, particularly ceftriaxone: 32 (71.1%) of the 45 isolates that were highly resistant to penicillin also showed intermediate resistance to ceftriaxone, and 6 (13.3%) were highly resistant (MIC >2.0 μg/mL) to this drug. Moreover, 54 (49.1%) of the isolates with reduced susceptibility to penicillin were resistant to a third and fourth antibiotic *(4)*.

Haemophilus influenzae. A total of 264 isolates from different hospitals in Colombia were processed using the broth microdilution technique in the Microbiology Laboratory of the National Institute of Health between May 1994 and April 1997. With variations depending on where the isolates came from, their susceptibility to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole ranged from 5.8% to 87%, with some 5.3% to 18% of the isolates clearly resistant. On the other hand, only 1% of the isolates showed intermediate susceptibility to cefuroxime, and all of them were sensitive to ceftriaxone.

In these same isolates the production of β-lactamase was detected in 6.5% of the samples from cerebrospinal fluid and 22% of those from hemoculture *(5)*.

Staphylococcus. In Colombia it is common to find methicillin-resistant *Staphylococcus aureus* (MRSA) in hospital isolates. To illustrate this situation, data were compiled for the year 1997 from two university hospitals, St. Vincent de Paul University Hospital (SVPUH) in Medellín and University del Valle Evaristo García Hospital in Cali, both of which have more than 500 beds, and two private hospitals, Pablo Tobón Uribe Hospital in Medellín and Santafé de Bogotá Foundation Hospital in Bogotá, with less than 500 beds *(6–9)*.

Susceptibility to oxacillin was 92% to 93% in the private hospitals, whereas the levels at the university hospitals were considerably lower: 66% and 68%, respectively, for St. Vincent de Paul and Evaristo García (Table 2). Resistance to vancomycin has not been reported to date.

An example of the presence and spread of MRSA in a university hospital can be seen at the SVPUH in Medellín, where the frequency of MRSA increased from 5% in 1972 to 41% in 1998. In 1994, when 64 of isolates of this pathogen were studied by pulsed-field gel electrophoresis, 90% of them were shown to be ge-

TABLE 1. Annual increase in penicillin-resistant *Streptococcus pneumoniae*, Colombia, 1994–1998.*

		Isolate resistance					
	Number of	Intermediate		High		Total	
Year	isolates	No.	%	No.	%	No.	%
1994	140	9	6.4	5	3.6	14	10.0
1995	167	18	10.8	5	3.0	23	13.8
1996	100	17	17.0	6	6.0	23	23.0
1997	99	20	20.2	17	17.2	37	37.4
1998	37	1	2.7	12	32.4	13	35.1
Total	543	65	12.0	45	8.3	110	20.3

* Modified from *(4)*.

TABLE 2. Susceptibility of *Staphylococcus aureus* hospital isolates to various antibiotics, Colombia, 1997

		Antibiotic–Susceptibility (%)						
Hospital	No. of isolates	Oxacillin	Ciprofloxacin	Cefalotin	Gentamycin	Erythromycin	Trimethoprim-sulfamethoxazole	Clindamycin
SVPUH	1,117	66	–	–	63	56	99	–
PTUH	241	92	90	92	87	–	–	–
UVH	1,093	68	75	–	66	69	–	72
SBF	377	93	93	93	25	82	97	89

SVPUH: St. Vincent de Paul University Hospital, Medellín; PTUH: Pablo Tobón Uribe Hospital, Medellín; UVH: University del Valle Hospital, Cali; SBF: Santafé de Bogotá Foundation Hospital, Bogotá.

netically related, which suggests the dissemination and intrahospital transmission of a clone. More than 90% of these same isolates were susceptible to glycopeptides, rifampicin, trimethoprim-sulfamethoxazole, and fusidic acid, whereas fewer than 10% were susceptible to erythromycin, clindamycin, or the aminoglycosides *(10)*.

The isolates of coagulase-negative *Staphylococcus* showed the following rates of susceptibility to oxacillin: 68% at the SVPUH, 44% at the Pablo Tobón Uribe Hospital, 55% at the University del Valle Hospital, and 51% at the Santafé de Bogotá Hospital. Susceptibility to ciprofloxacin ranged from 67% to 88%, depending on the hospital *(6–9)*.

Enterococcus. The most significant situation with regard to enterococcus resistance in Colombia is the appearance of isolates resistant to vancomycin. In 1997, four cases were reported at a third-level clinic in Bogotá—two of them *E. faecalis*, which had intermediate susceptibility to vancomycin, and two of them *E. faecium*, which were resistant to vancomycin *(9)*. In 1998 there was an outbreak of nosocomial infections at a university hospital in Medellín, in which the first six cases were vancomycin-resistant *E. faecium* (MIC >128 μg/mL); the isolates were also resistant to penicillin, ampicillin, gentamicin, streptomycin, and ciprofloxacin *(11)*.

Enterobacteria. The information compiled from the hospitals came from isolates taken from the urine of hospitalized patients. Of the most commonly isolated enterobacteria, *Escherichia coli* continues to have good levels of susceptibility: approximately 90% of the specimens were sensitive to amikacin, ceftazidime, ceftriaxone, ciprofloxacin, aztreonam, and imipenem. With cefoperazone and cefotaxime there were differences between hospitals, with susceptibility ranging from 54% to 87% and from 77% to 97%, respectively, for these two antibiotics (Table 3) *(6–9)*.

With the third-generation cephalosporins and aztreonam, slightly lower susceptibility was observed for the isolates of *Enterobacter* spp. than for *E. coli*—namely, 56% vs. 77%. Except for the data from one of the hospitals, susceptibility to ciprofloxacin and amikacin was good, ranging from 85% to 99%. Imipenem continued to be very active on these isolates, with an activity of 93%, except at the University del Valle Hospital, where susceptibility was 65% (Table 4) *(6–9)*.

With regard to *Klebsiella pneumoniae*, susceptibility to third-generation cephalosporins, amikacin, and aztreonam was highly variable. Imipenem, however, was very active in all the hospitals surveyed (>98% susceptibility). Sensitivity to ciprofloxacin was equally good, except at one of the university hospitals (Table 5) *(6–9)*.

Pseudomonas aeruginosa. Ceftazidime and imipenem were the most active antibiotics against this bacterial species, with susceptibility ranging from 77% to 87% and from 60% to 98%, respectively. Other antibiotics—aztreonam, ciprofloxacin, cefoperazone, and amikacin—yielded less than 80% susceptibility (Table 6) *(6–9)*.

***Acinetobacter* spp.** Unlike the pathogens mentioned so far, these bacteria had suscepti-

TABLE 3. Susceptibility of *Escherichia coli* hospital isolates to various antibiotics, Colombia, 1997

		Antibiotic–Susceptibility (%)								
Hospital	No. of isolates	Amikacin	Aztreonam	Ampicillin-sulbactam	Cefotaxime	Cefoperazone	Ceftriaxone	Ceftazidime	Imipenem	Ciprofloxacin
SVPUH	682	87	89	64	90	87	91	89	98	85
PTUH	231	95	92	38	94	54	93	92	95	–
UVH	2,320	89	83	41	77	69	94	91	100	89
SBF	1,337	96	94	–	97	80	97	96	99	88

SVPUH: St. Vincent de Paul University Hospital, Medellín; PTUH: Pablo Tobón Uribe Hospital, Medellín; UVH: University del Valle Hospital, Cali; SBF: Santafé de Bogotá Foundation Hospital, Bogotá.

bility levels of less than 50% with amikacin, aztreonam, ciprofloxacin, cefoperazone, and ceftazidime, but higher than 77% with imipenem. Nevertheless, the isolates from one of the hospitals showed 96% susceptibility for amikacin, ceftazidime, and ciprofloxacin (Table 7) *(6–9)*.

***Salmonella* spp. *and Shigella* spp.** In 1996 the Pan American Health Organization launched a program for monitoring the antimicrobial susceptibility of enteropathogens in the Americas. In Colombia, the program is coordinated by the Microbiology Laboratory at the National Institute of Health.

During 1997, 17 public health laboratories in Colombia submitted 92 isolates of *Salmonella* spp., 68 (74%) of which had been obtained from stool, 7 (7.6%) from blood, 4 (4.4%) from cerebrospinal fluid, and 13 (14%) from other specimens. The species detected most frequently were *S. enteritidis*, 44 isolates (47.8%); *S. typhimurium*, 25 (27.1%); and *Salmonella* Group E1, 16 (17.4%). There were two isolates of *S. typhi* (2.2%) and five of various other serotypes (5.5%). Depending on the geographical area of the country from which the isolates had come, the percentages of resistance ranged from 6% to 76% for ampicillin and trimethoprim-sulfamethoxazole, and from 0% to 9% for chloramphenicol *(12)*.

Of the 55 isolates of *Shigella* spp. studied, 52 (94.5%) were taken from stool and 3 (5.5%) from other samples. The species identified were *Sh. flexneri*, 31 (56%); *Sh. sonnei*, 22 (40%); and *Sh. dysenteriae*, 2 (4%). Resistance to ampicillin and chloramphenicol ranged from 40% to 80%, and to trimethoprim-sulfamethoxazole, from 33% to 93% *(12)*.

***Vibrio cholerae* 01.** As part of the national epidemiological surveillance program, the Microbiology Laboratory at the National Institute of Health received 4,000 isolates during the period 1991–1997. Their susceptibility pattern was determined using the agar gel diffusion technique. The isolates recovered between 1991 and 1996 were fully sensitive to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and norfloxacin or cipro-

TABLE 4. Susceptibility of *Enterobacter* spp. hospital isolates to various antibiotics, Colombia, 1997

		Antibiotic–Susceptibility (%)								
Hospital	No. of isolates	Amikacin	Aztreonam	Ampicillin-sulbactam	Cefotaxime	Cefoperazone	Ceftriaxone	Ceftazidime	Imipenem	Ciprofloxacin
SVPUH	136	85	74	41	73	72	71	77	92	93
PTUH	66	95	68	27	76	63	68	68	–	97
UVH	426	65	60	28	62	56	72	68	65	99
SBF	100	87	73	–	76	72	73	73	93	96

SVPUH: St. Vincent de Paul University Hospital, Medellín; PTUH: Pablo Tobón Uribe Hospital, Medellín; UVH: University del Valle Hospital, Cali; SBF: Santafé de Bogotá Foundation Hospital, Bogotá.

TABLE 5. Susceptibility of *Klebsiella pneumoniae* hospital isolates to various antibiotics, Colombia, 1997

Hospital	No. of isolates	Antibiotic–Susceptibility (%)								
		Amikacin	Aztreonam	Ampicillin-sulbactam	Cefotaxime	Cefoperazone	Ceftriaxone	Ceftazidime	Ciprofloxacin	Imipenem
SVPUH	476	64	66	58	78	68	72	69	95	99
PTUH	86	90	81	56	80	61	83	91	–	98
UVH	965	68	64	38	81	58	90	73	68	100
SBF	215	78	70	57	83	67	72	72	94	98

SVPUH: St. Vincent de Paul University Hospital, Medellín; PTUH: Pablo Tobón Uribe Hospital, Medellín; UVH: University del Valle Hospital, Cali; SBF: Santafé de Bogotá Foundation Hospital, Bogotá.

floxacin. In 1997, however, of the 176 isolates recovered and confirmed, 37 (21%) were resistant to trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol *(13)*.

Neisseria meningitidis. To determine the minimum inhibitory concentration with penicillin and rifampicin, studies were done on 249 isolates from the bacterial collection that the Microbiology Laboratory at the National Institute of Health had received from various public health laboratories in Colombia between 1987 and 1997. Using the agar dilution technique, it was determined that 21 isolates (8.5%) had reduced susceptibility to penicillin (MIC 0.125 μg/mL) and 107 (43%), to rifampicin *(14)*.

Neisseria gonorrhoeae. As part of the program on sexually transmitted diseases, the microbiology laboratory at the National Institute of Health studied 100 isolates submitted by various health services in Colombia between 1984 and 1994, using the agar dilution technique. The MIC was ≥ 2 μg/mL for penicillin in 5 isolates of non-β-lactamase-producing strains and 47 of β-lactamase-producing strains. The MIC for tetracycline was ≥ 2 μg/mL in 78 isolates. The presence of β-lactamase was determined in 51% of the isolates *(15)*.

More recently, the susceptibility findings from a study of 29 gonococcus isolates were presented by the Public Health Department Laboratory of the Sectional Health Bureau in the department of Antioquia. Of 10 isolates, 6 were resistant to penicillin and 12 were resistant to tetracycline. Of 5 isolates studied for their susceptibility to spectinomycin, all were sensitive, and the 12 isolates studied for ceftriaxone were also sensitive. However, this is the first report on a gonococcus isolate resistant to ciprofloxacin *(16)*.

DISCUSSION

The figures reported on antibiotic resistance in Colombia are a local manifestation of a growing world problem, as evidenced by data on the resistance of *Streptococcus pneumoniae* to penicillin, the appearance of vancomycin-

TABLE 6. Susceptibility of *Pseudomonas aeruginosa* hospital isolates to various antibiotics, Colombia, 1997

Hospital	No of isolates	Antibiotic–Susceptibility (%)					
		Amikacin	Aztreonam	Cefoperaxone	Ceftazidime	Ciprofloxacin	Imipenem
SVPUH	418	64	66	63	79	63	60
PTUH	120	75	55	63	87	70	98
UVH	695	56	79	–	77	63	97
SBF	273	93	68	76	78	75	84

SVPUH: St. Vincent de Paul University Hospital, Medellín; PTUH: Pablo Tobón Uribe Hospital, Medellín; UVH: University del Valle Hospital, Cali; SBF: Santafé de Bogotá Foundation Hospital, Bogotá.

TABLE 7. Susceptibility of *Acinetobacter* spp. isolates to various antibiotics, Colombia, 1997

		Antibiotic-Susceptibility (%)						
Hospital	No. of isolates	Amikacin	Aztreonam	Cefoperazone	Ceftazidime	Ciprofloxacin	Imipenem	Cefoperaxone-sulbactam
SVPUH	347	46	16	15	41	53	77	58
PTUH	24	32	19	6	42	53	100	–
UVH	557	41	32	15	75	41	100	99
SBF	26	96	20	25	96	96	96	–

SVPUH: St. Vincent de Paul University Hospital, Medellín; PTUH: Pablo Tobón Uribe Hospital, Medellín; UVH: University del Valle Hospital, Cali; SBF: Santafé de Bogotá Foundation Hospital, Bogotá.

resistant strains of *Enterococcus*, the resistance of enteric bacilli to third-generation cephalosporins, and data on oxacillin-resistant *S. aureus*. This alone justifies launching surveillance programs that will make it possible to develop adequate control strategies.

A number of studies have been undertaken in Colombia—for example, on the surveillance of susceptibility to enteropathogens in the Americas, and on the resistance and serotyping of pneumococci—which have been promoted by PAHO and directed in our country by the National Institute of Health. These are good examples of coordinated efforts to determine the real situation of pathogen resistance in the community. However, in the area of hospital infections, where the indiscriminate use of antibiotics also has a greater impact in terms of the development of resistance, there have been few national efforts, and these have been sporadic, sustained only by the individual work of several institutions and professionals.

Publication of these data gives an overview of current developments in Colombia with respect to antibiotic resistance. It is important that this effort be the basis not only for the coordinated monitoring of resistance, but also for the development of national policies that will be directly used to curtail its increase.

It is urgent that the recommendations issued by the various meetings organized by WHO and PAHO be applied in Colombia. The joint involvement of government agencies, research centers, teaching institutions, and health service providers will strengthen the implementation of these policies and help to reduce the impact of this problem on health.

The publication of this information is also expected to foster the creation of epidemiological surveillance and hospital infection committees and strengthen those already in place.

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ANTIMICROBIAL SUSCEPTIBILITY SURVEILLANCE IN ECUADOR

Jeannete Zurita[1] and the REDNARBEC Group[2]

Bacterial resistance has become a serious public health problem and currently is one of the greatest obstacles to be overcome in the field of health care (1–3). One of the strategies to contain this problem consists of determining the magnitude of the resistance and monitoring the behavior of the pathogens in the presence of antimicrobials. In Ecuador few data were available, and they were mostly very local (4, 5), which made obtaining countrywide data indispensable. In April 1999 the National Network for Surveillance of Bacterial Resistance in Ecuador (REDNARBEC) was created, grouping together 10 hospital centers in the country. All of them have internal quality control systems and use the WHONET software.[3] This article presents the data obtained in the first eight months of network operations (May to December 1999).

INTRODUCTION

In April 1999, the bacterial resistance surveillance system called *Red Nacional de Vigilancia de Resistencia Bacteriana en el Ecuador* (REDNARBEC) was implemented in Ecuador. The objectives of the network were to reach an agreement on the standardization of microbiological procedures, cultures, and antibiograms and obtain information on the pathogens and their profiles of resistance. Ten hospitals were invited to participate: seven in the central area (Quito), one in the western area (Manta), and two in the southern area (Azogues and Cuenca). The WHONET software was installed at each hospital, and a protocol for conducting the sensitivity tests was provided.

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MATERIALS AND METHODS

All the laboratories used the Kirby-Bauer disk diffusion method to test for sensitivity (6). After correct identification of the genus and species of the bacterial strain (manual system, bioMérieux API, or BBL-Crystal system), the antibiogram was performed according to an established protocol, following the standards established by the U.S. National Committee for Clinical Laboratory Standards (NCCLS) (7). For internal quality control, ATCC reference strains of *Escherichia coli* 25922, *Staphylococcus aureus* 29213, *Pseudomonas aeruginosa* 27853, and *Enterococcus faecalis* 29212 were included.

The strains were automatically classified as sensitive, intermediate, or resistant on the basis of the inhibition halos, using the cut-off values established in the NCCLS standards, which are included in the WHONET program. Each participating center sent data on bacteria isolated consecutively from the patients served in each institution. The monthly submission was made by diskette to the coordinating center in the Vozandes Hospital. One isolate was entered per patient. Bacteria from hospitals and the community (outpatient and emergency consultation) were recorded, since the software allows such a breakdown.

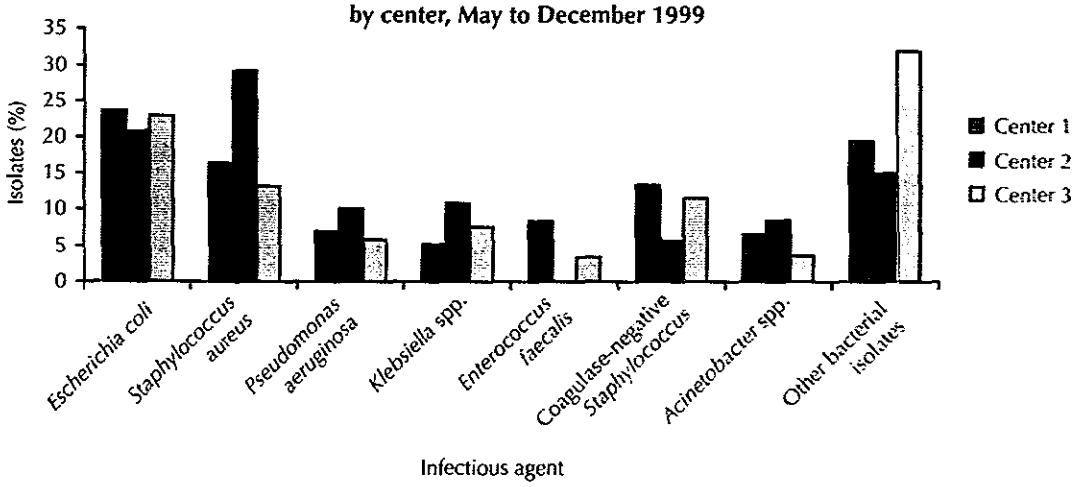
The analyzed data, using both histograms and resistance profiles, was sent monthly to each participating center. Each hospital center analyzed the information for its own use.

RESULTS

During the eight months of surveillance, data were obtained from 7,682 isolates taken from the infectious processes of patients seen at the participating centers. Of those isolates, 4,421 (57.6%) were obtained from hospitalized patients, and 3,261 (42.4%) from outpatient and emergency services (community isolates). *Escherichia coli*, constituting 32% of the isolates, was the pathogen found most frequently, followed by *Staphylococcus aureus*, with 20%. In third place were the isolates of *Pseudomonas aeruginosa* and *Klebsiella*, both with 7%. The distribution of hospital pathogens in three centers are shown in Figure 1.

In the investigation of Gram-positive coccaceous bacteria, it was found that of the 1,530 isolates of *Staphylococcus aureus*, 21% were resistant to oxacillin and a similar pro-

FIGURE 1. Percentages of isolates of specific strains (infectious agents) obtained from hospitalized patients in three centers of the National Network for Monitoring Bacterial Resistance in Ecuador, by center, May to December 1999



portion to trimethoprim-sulfamethoxazole (23%) and clindamycin (22%). With regard to gentamicin, 22% were resistant. For erythromycin, 19% showed resistance, while for ciprofloxacin the figure was 17.6%. Tetracycline and penicillin were the drugs to which the greatest resistance was shown, 30% and 90%, respectively. To date, vancomycin-resistant strains of *Staphylococcus* have not been detected.

The coagulase-negative strains of *Staphylococcus* presented greater resistance than those of *S. aureus*, as can be observed in Figure 2, which shows a comparison of the two bacteria.

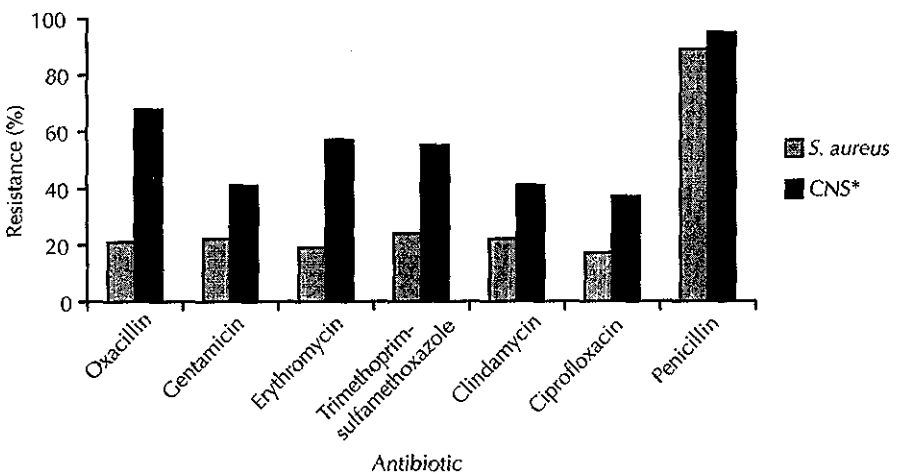
Among the 119 isolates of *Streptococcus pneumoniae* studied, 8.3% were resistant to penicillin (1 µg oxacillin disk); no strains resistant to cefotaxime, imipenem, clindamycin, or vancomycin were detected. With regard to ciprofloxacin, 41.2% of the isolates presented intermediate sensitivity and 58.8% were sensitive.

Enterococci infections are becoming increasingly difficult to treat, since they present multiple resistance and the available therapeutic arsenal is limited. In the REDNARBEC, resistance to ampicillin was detected in only 2% of the isolates of *Enterococcus faecalis* and in 8% of those of *E. faecium*. These resistances are low in comparison to those found in the Vozandes Hospital in 1997 and 1998, which reported resistance to ampicillin of 8% in *E. faecalis* and 30% in *E. faecium* (5). The first report of vancomycin-resistant *E. faecium* came from the Hospital of the Armed Forces of Quito. Vancomycin-resistant strains have not been detected in the other centers.

Gram-negative bacilli present increasing resistance to third-generation cephalosporins and quinolones in many places. Among the 2,455 isolates of *Escherichia coli* studied, there were high levels of resistance to ampicillin (63%), trimethoprim-sulfamethoxazole (52%) ampicillin sulbactam (27%), and ciprofloxacin (23%). However, the levels of resistance to ceftriaxone and cefepime were low, 6% and 1%, respectively (see Figure 3).

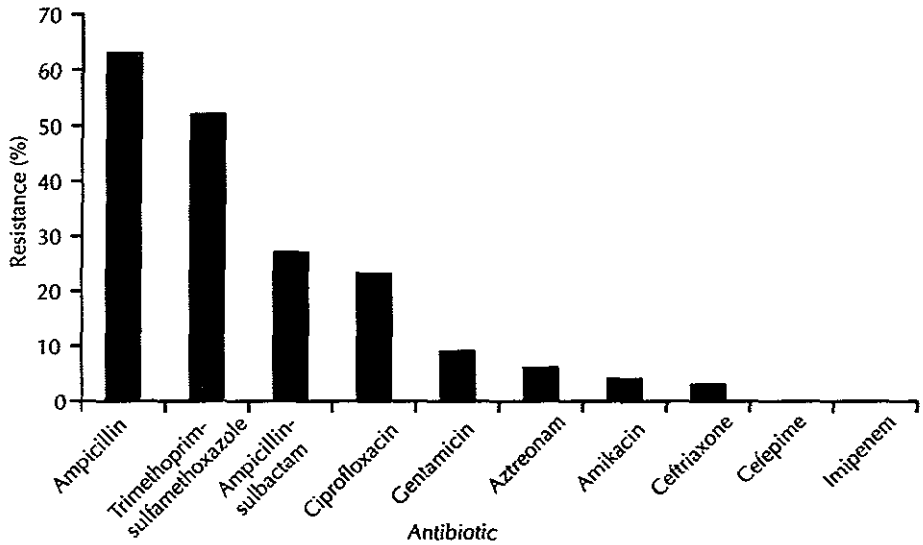
With respect to the sensitivity profile of *Klebsiella* spp., it was observed that 95% of the iso-

FIGURE 2. Resistance of 1,530 isolates of *Staphylococcus aureus* and 496 isolates of coagulase-negative *Staphylococcus* to various drugs. National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999



*Coagulase-negative *Staphylococcus*.

FIGURE 3. Resistance of 2,455 isolates of *Escherichia coli* to various drugs. National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999



lates were resistant to ampicillin, 46% to ampicillin-sulbactam, and 45% to gentamicin. The degrees of resistance to the quinolones (10.7%), imipenem (0%), and cefepime (3%) remain acceptable (see Figure 4).

Figure 5 shows that 14% of the isolates of *Pseudomonas aeruginosa* were resistant to aminoglycosides, 14% to netilmicin, 33% to amikacin, and 40% to gentamicin. For the antipseudomonal drugs such as ticarcillin-clavulanic acid, resistance was 54%. The lowest resistance was observed for imipenen and cefepime (15%), ceftazidime (20%), and aztreonam (21%).

FIGURE 4. Resistance of 538 isolates of *Klebsiella* spp. to various drugs. National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999

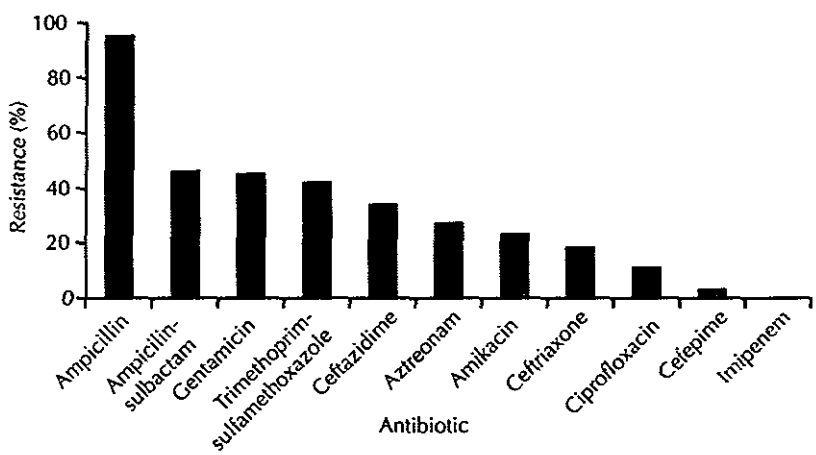
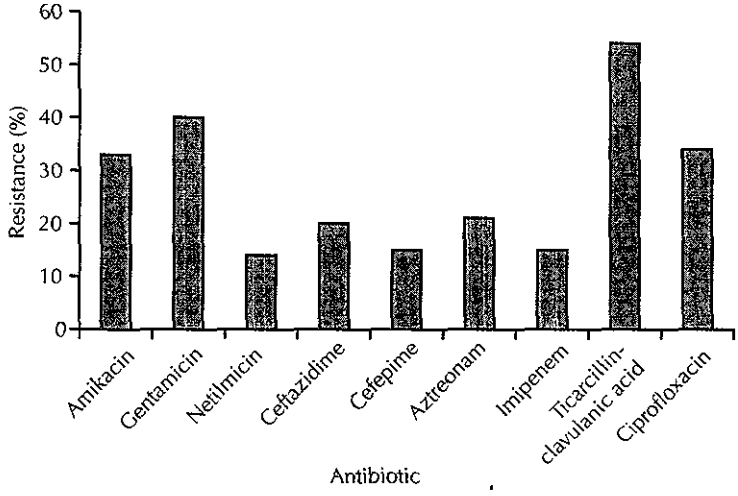


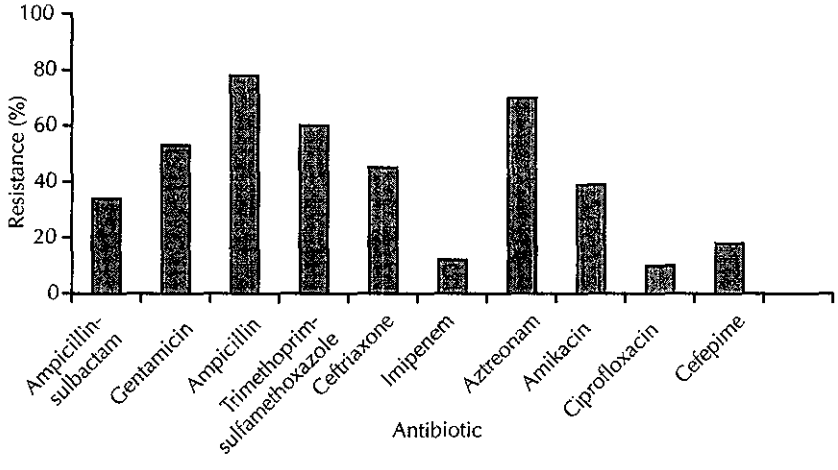
FIGURE 5. Resistance of 537 isolates of *Pseudomonas aeruginosa* to various drugs. National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999



The 316 isolates of *Acinetobacter* spp. were highly resistant to antibiotics: 78% to ampicillin, 60% to trimethoprim-sulfamethoxazole, 70% to aztreonam, 45% to ceftriaxone, and 53% to gentamicin. Lower percentages were resistant to amikacin (39%), ampicillin-sulbactam (34%), cefepime (18%), imipenem (12%) and ciprofloxacin (10%). The observed resistances are presented in Figure 6.

The resistance of *Shigella* strains is a worldwide phenomenon. In many countries, very high resistance has been reported to the anti-

FIGURE 6. Resistance of 316 isolates of *Acinetobacter* spp. to various drugs. National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999



microbial drugs in everyday use, such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol *(8)*. This microorganism continues to be an important etiologic agent for diarrheal syndrome in Ecuador and presents notable multiple resistance *(9)*. The species isolated most frequently in this study was *S. flexneri*, followed by *S. sonnei*; very few strains of *S. boydii* and *S. dysenteriae* were found. According to the data from REDNARBEC the problem in Ecuador is similar to that in the rest of the world, since 96% of the strains are resistant to tetracycline, 77% to ampicillin, 70%, to trimethoprim-sulfamethoxazole, and 72% to chloramphenicol. In this study, the quinolones continued to be a very good therapeutic option for adults, with the data indicating that the resistance to these drugs is nil (see Figure 7).

To date there has been no reported resistance to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, quinolones, and ceftriaxone by strains of *Salmonella* spp.

Among the isolates of *Haemophilus influenzae* obtained from the respiratory tract, 10% were resistant to ampicillin, while 5% of biotype b strains isolated from cerebrospinal fluid were resistant to this β-lactamic drug. Resistance has remained at this level since 1994, when it was first reported in Ecuador (see Figure 8).

Figure 9 shows the sensitivity of *Klebsiella pneumoniae* isolates in three different hospital centers. Center No. 2 presents a large number of BLEA-producing isolates of *Klebsiella* with high resistance to third generation cephalosporins. Significant variations in the sensitivity patterns of *Staphylococcus aureus* in the three hospital centers can also be observed in Figure 10.

DISCUSSION

The data on bacterial resistance to antimicrobials in Ecuador indicate that this is a serious public health problem in the country. Data presented here show oxacillin-resistant *S. aureus* and penicillin-resistant pneumococci, a growing increase in the resistance of Gram-negative bacilli to quinolones and cephalosporins, and the appearance of vancomycin-resistant enterococci.

The establishment of a system for monitoring the resistance of the prevalent pathogens in Ecuador has been very useful in determining not only the degree of resistance but also which bacterial strains are most often involved

FIGURE 7. Resistance of 41 isolates of *Shigella* sp. to various drugs. National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999

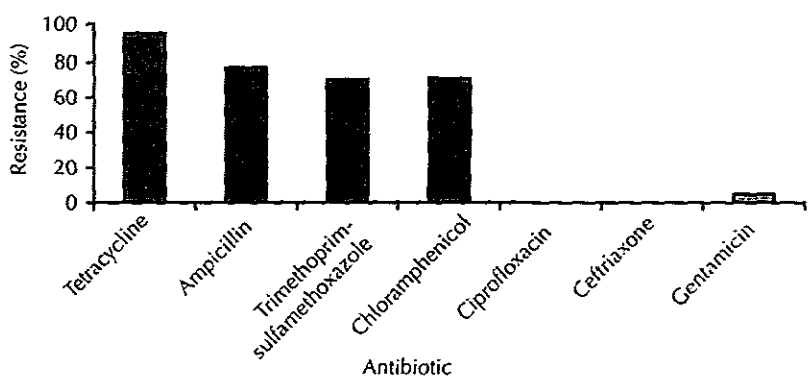
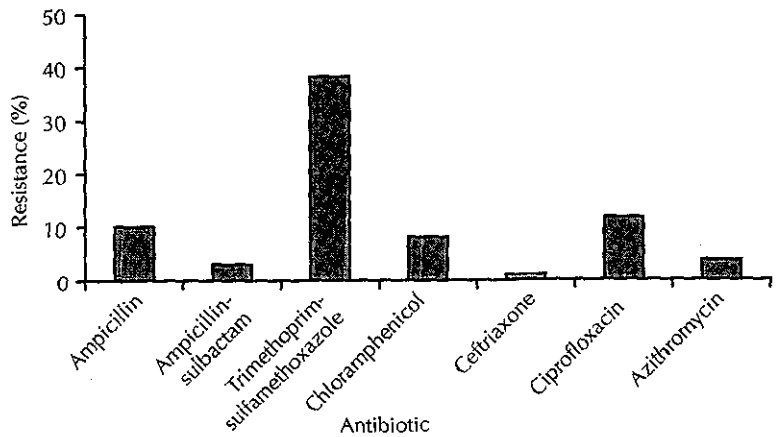


FIGURE 8. Resistance of 164 isolates of *Haemophilus influenzae* from the respiratory tract to various drugs. National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999



in the infectious processes in the hospital environment and in the community. The results presented show that in Ecuador the two most commonly isolated bacteria are *Escherichia coli* and *Staphylococcus aureus*, which together constitute 51.8% of the isolates reported in the period covered. These data agree with those of other national surveillance programs *(10)*.

Surveillance also helped to establish a consensus on the microbiological procedures to be used in all the participating centers. By using the WHONET software for data collection and analysis a centralized database has been created that makes it possible to detect quality control problems in the laboratories and then to correct and overcome them. In sum, the surveillance system has made it possible to identify the resistance profiles in each operating unit and issue recommendations on the best therapeutic options, and also to seek strategies to control the spread of resistance locally and nationally.

FIGURE 9. Sensitivity patterns presented by *Klebsiella pneumoniae* isolates from three hospital centers of the National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999

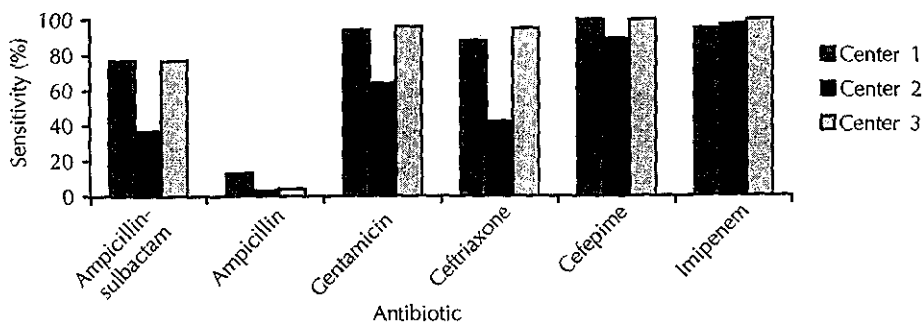
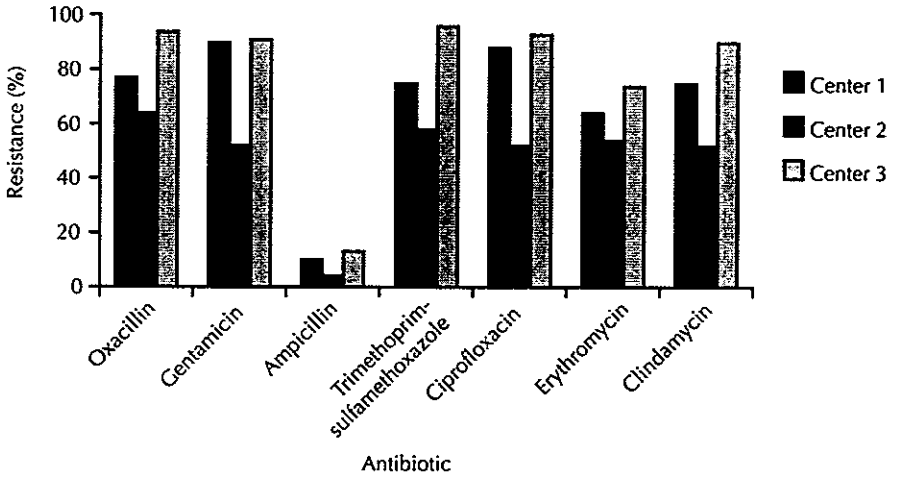


FIGURE 10. Sensitivity patterns presented by the *Staphylococcus aureus* isolates from three hospital centers of the National Network for Monitoring Bacterial Resistance in Ecuador, May to December 1999



It is imperative that this surveillance system be maintained, since the data obtained over time will serve to evaluate trends and variations in the infectious agents and their resistance profiles. This will in turn promote the development of national policies to help contain the increase in resistance.

ACKNOWLEDGMENTS

To Dr. Thomas O'Brien, Microbiology Laboratory, Brigham and Women's Hospital, Boston, Massachusetts, for the WHONET software; to John M. Stelling, WHO/EMC, for training in the use of the WHONET software; to Dr. Carlos Cornejo, Medical Director of Pfizer Laboratories, and Dr. Tito Vélez, Medical Director of Bristol Myers Squibb Laboratories, for their support; and to Mrs. Silvia García for entry of the network data.

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REPORT ON BACTERIAL RESISTANCE: PILOT STUDY OF SIX MEXICAN CENTERS[1]

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This article summarizes the findings of a pilot study on bacterial resistance carried out in six hospital centers located in various Mexican states. Information for 1996–1997 is given for the following species: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter *spp.*, Staphylococcus aureus, Streptococcus pneumoniae, *and* Haemophilus influenzae. *Isolates studied came from hospitals and communities.*

A dangerous rise in resistance to quinolones was noted among Gram-negative bacilli, especially E. coli *and* Klebsiella *spp. Resistance of* S. aureus *to oxacillin also poses cause for concern.*

INTRODUCTION

The increased use of antimicrobials initially promotes bacterial resistance at the local level, which subsequently can spread all over the world (*1–3*). Knowledge of available information on resistance to antimicrobials helps clinical physicians to make better decisions regarding empirical choice of medications (*4–6*). In Mexico, worldwide data on bacterial resistance were lacking; therefore, in 1996 an invitation to participate in the collection of data was extended to the leading hospital centers meeting accepted standards of quality control.

METHODS

During the first two years, six hospital centers gathered information on 17,269 clinical isolates including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* spp., *Staphylococcus aureus, Streptococcus pneumoniae,* and *Haemophilus influenzae.* These are all sentinel germs used in the surveillance of resistance to antimicrobial drugs in various regions of the world.

[1]Source: *Revista Panamericana de Infectología* 1999;(supl 1 mayo):S45–S47. Published by permission of the Asociación Panamericana de Infectología [Pan American Association of Infectious Diseases].

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The centers that sent information have internal and external quality controls. The bacteria were identified and their susceptibility to drugs established according to the recommendations set out by the National Committee for Clinical Laboratory Standards (NCCLS).

Data collection began in January, 1996. The present analysis includes data for 1966 and 1997.

RESULTS

Tables 1 to 6 show the total numbers of clinical isolates, which were divided into two groups: 8,371 obtained from hospitalized patients and 8,750 obtained from ambulatory patients. It was observed that in the isolates of *E. coli* obtained from the community (Table 1), more than 50% showed resistance to ampicillin and more than 20%, to ciprofloxacin. As regards strains of *K. pneumoniae*, there was more than 60% resistance to ampicillin in the total number of isolates. Resistance to ceftriaxone was 19% among the nosocomial isolates (Table 2).

With regard to nosocomial isolates of *P. aeruginosa* (Table 3), more than 30% were resistant to ceftazidime, over 40% were resistant to aminoglucosides, and above 30% to quinolones. Among the community isolates, over 30% showed resistance to *P. aeruginosa* and aminoglucosides, while resistance to ciprofloxacin exceeded 40%.

More than 35% of the strains of *Acinetobacter* spp. were resistant to ceftazidime, aminoglucosides, and quinolones (Table 4). Among strains of *Staphylococcus aureus*, resistance to oxacillin was higher than 15% in hospital isolates, and less than 10% in the community isolates (Table 5). Resistance to ciprofloxacin exceeded 10% in the hospital.

As for *Streptococcus pneumoniae* (Table 6), less than 10% resistance to penicillin was observed. It was noted that 30% of the strains of *Haemophilus influenzae* were producers of β-lactamase.

The resistance to ciprofloxacin observed among *E. coli* isolates was probably due to the administration of quinolones to ambulatory patients, according to data furnished by participants. Resistance to third generation cefalosporins among *Klebsiella* strains suggests that these are producers of wide-specter β-lactamase. Resistance notified in relation to

TABLE 1. Resistance (%) of *Escherichia coli* to different antimicrobials, according to community or nosocomial origin of isolates. Pilot study of six hospital centers, Mexico, 1996 and 1997

	Resistance (%)			
	Community		Nosocomial	
Antimicrobial agent	1996 (n = 3,437)	1997 (n = 2,192)	1996 (n = 1,565)	1997 (n = 2,317)
Ampicillin	63	63	43	68
Amoxicillin-clavulanate	7	9	17	24
Ampicillin-sulbactam	18	11	21	28
Cefalotin	49	32	29	35
Cefotaxime	5	1	2	5
Ceftriaxone	4	1	5	4
Ceftazidime	3	2	9	5
Imipenem	1	1	1	2
Gentamicin	7	11	18	18
Amikacin	2	2	21	6
Trimethoprim-sulfamethoxazole	55	55	44	61
Ciprofloxacin	21	25	15	24
Norfloxacin	17	28	14	19

TABLE 2. Resistance (%) of *Klebsiella pneumoniae* to different antimicrobials, according to community or nosocomial origin of isolates. Pilot study of six hospital centers, Mexico, 1996 and 1997

	Resistance (%)			
	Community		Nosocomial	
Antimicrobial agent	1996 (n = 267)	1997 (n = 211)	1996 (n = 433)	1997 (n = 587)
Ampicillin	80	78	77	85
Amoxicillin-clavulanate	12	14	30	40
Ampicillin-sulbactam	14	12	39	33
Cefalotin	17	23	48	46
Cefotaxime	13	11	25	20
Ceftriaxone	19	5	22	19
Ceftazidime	12	8	19	17
Imipenem	2	4	4	5
Gentamicin	13	14	32	24
Amikacin	6	3	30	24
Trimethoprim-sulfamethoxazole	27	49	42	52
Ciprofloxacin	6	29	9	5
Norfloxacin	5	30	5	6

TABLE 3. Resistance (%) of *Pseudomonas aeruginosa* to different antimicrobials, according to community or nosocomial origin of isolates. Pilot study of six hospital centers, Mexico, 1996 and 1997

	Resistance (%)			
	Community		Nosocomial	
Antimicrobial agent	1996 (n = 164)	1997 (n = 184)	1996 (n = 505)	1997 (n = 939)
Ceftazidime	25	30	42	31
Ticarcillin-clavulanate	5	34	10	34
Piperacillin	15	28	33	26
Cefoperazone	25	42	29	19
Imipenem	23	13	17	18
Gentamicin	41	36	51	43
Amikacin	33	32	41	37
Ciprofloxacin	43	40	26	35

TABLE 4. Resistance (%) of *Acinetobacter* spp. to different antimicrobials, according to community or nosocomial origin of isolates. Pilot study of six hospital centers, Mexico, 1996 and 1997

	Resistance (%)			
	Community		Nosocomial	
Antimicrobial agent	1996 (n = 79)	1997 (n = 32)	1996 (n = 160)	1997 (n = 231)
Amoxicillin-clavulanate	9	20	60	36
Cefotaxime	7	15	50	42
Ceftazidime	4	11	35	37
Gentamicin	19	13	54	33
Amikacin	4	19	45	35
Imipenem	4	9	4	2
Ciprofloxacin	11	13	35	32

TABLE 5. Resistance (%) of *Staphylococcus aureus* to different antimicrobials, according to community or nosocomial origin of isolates. Pilot study of six hospital centers, Mexico, 1996 and 1997

	Resistance (%)			
	Community		Nosocomial	
Antimicrobial agent	1996 (n = 499)	1997 (n = 391)	1996 (n = 508)	1997 (n = 1,126)
Oxacillin	4	10	16	22
Vancomycin	–	–	–	–
Gentamicin	11	6	10	15
Trimethoprim-sulfamethoxazole	3	13	9	14
Ciprofloxacin	8	12	10	25

TABLE 6. Number of *Streptococcus pneumoniae* strains and percentage resistant to penicillin according to type of infection. Pilot study of six hospital centers, Mexico, 1996 and 1997

	1996		1997	
Type of infection	Number	Percentage	Number	Percentage
Invasive, in adults	48	4	37	5
Colonizing, in adults	16	–	52	–
Invasive, in children	35	–	82	–
Colonizing, in children	119	–	286	1

isolates of *P. aeruginosa* and *Acinobacter* obtained from hospitalized patients shows the appearance of different resistance mechanisms in germs exposed to a varied number of antimicrobials. In this study, the *S. aureus* resistance to oxacillin that was notified did not represent an epidemiological problem.

Resistance to penicillin on the part of pneumococcus was low, but those results could be related to sample size. Production of β-lactamase by *Haemophilus influenzae* strains was not high.

At the present time, there are 13 centers participating in the program. All of them maintain strict quality controls and have external supervision and a center of reference.

CONCLUSIONS

A dangerous increase was observed in the resistance to quinolones among Gram-negative bacilli, *E. coli* and *Klebsiella* spp. in particular. Another cause of concern is the resistance shown by *Staphylococcus aureus* to oxacillin. On the other hand, the prevalence of resistance to penicillin remains low in pneumococcus isolated from well-defined infection.

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MONITORING OF ANTIBIOTIC RESISTANCE IN PARAGUAY

Wilma Basualdo[1] *and Antonio Arbo-Sosa*[2]

Bacterial resistance to antibiotics is a serious and growing problem in developing countries. Paraguay is no exception, as witnessed by the high levels of antibiotic resistance that have been observed among pathogens both in hospitals and the community. More than 70% of the Shigella *spp. strains isolated from diarrhea in children are already resistant to ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. With* H. influenzae *type b, 33% and 25% of the isolates studied were found to be resistant to ampicillin and chloramphenicol, respectively, and 11% were resistant to both. Of* Streptococcus pneumoniae *isolates from cases of invasive disease, 24% proved to be resistant to penicillin. The susceptibility patterns among hospital (nosocomial) pathogens are even more disturbing.* Acinetobacter *spp. were highly resistant to cefotaxime (86%), ceftazidime (70%), and ciprofloxacin (58%), and there was also 10% resistance to imipenem. Among the isolates of* Klebsiella pneumoniae, *60% were resistant to cefotaxime, 44% to ceftazidime, and 22% to ciprofloxacin. Finally, in the cases of* Pseudomonas aeruginosa, *56% were resistant to piperacillin, 48% to cefoperazone, 27% and 28% to ceftazidime and ciprofloxacin, respectively, and 15% to imipenem.*

INTRODUCTION

Although the increase in bacterial resistance to antibiotics is receiving considerable attention in first-world countries, the same is not true in Latin America, where bacterial resistance rates are high (*1–4*). The problem is more serious in the less developed countries, where infections, notably of the respiratory and gastrointestinal tract, are among the leading causes of morbidity and mortality, especially among children (*2, 3, 5, 6*). Several factors contribute to the high prevalence of infections in these latitudes: lack of minimum sanitary conditions such as safe drinking water and adequate excreta disposal, populations with little knowledge about hygiene and sanitation, and poor access to primary health care. The high frequency of infections and the concomitantly high use of antimicrobial drugs has set the stage for a triple phenomenon of excessive use, inappropriate use, and a shortage of antibiotics when they are really needed. Since a prescription is not usually required in order to

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obtain antibiotics, they are typically sold without any professional control. It should be noted that antibiotics are among the pharmaceutical products with the greatest impact on the environment. Their uncontrolled use in humans and animals has created a situation in which bacterial agents are subjected to selective pressure, which results in the constant appearance of germs newly resistant to the different antibiotics (2–6).

The present article examines such resistance in a number of the bacterial pathogens of clinical importance in Paraguay.

MATERIALS AND METHODS

A retrospective review was conducted of the information available for 1995–1998 at the Institute of Tropical Medicine, Ministry of Public Health and Social Welfare, on the in vitro sensitivity of germs found in the community (*Shigella* spp., *Salmonella* spp., *Haemophilus influenzae*, *Streptococcus pneumoniae*) and Gram-negative germs found in hospitals (*Pseudomonas aeruginosa*, *Acinetobacter* spp., *Klebsiella pneumoniae*).

RESULTS

Bacterial Resistance among Pathogens in the Community

Enteric Pathogens

The *Shigella* spp. are among the microorganisms on which antibiotics have exerted enormous selective pressure over time and which have thus been steadily acquiring resistance to antibiotics (*6–11*). Shigellosis is endemic in Latin America and causes between 8% and 12% of all episodes of diarrhea, including nearly 50% of the dysentery cases requiring hospitalization (*5, 12, 13*). In Paraguay this is one of the bacterial gastrointestinal infections with the greatest impact on child morbidity and mortality (*14, 15*).

The species *S. flexneri* and *S. sonnei* are predominant in Paraguay (*14, 15*). Susceptibility studies of Shigella isolates during the period 1995–1997 showed that less than 30% of the strains of Shigella were susceptible to ampicillin, trimethoprim-sulfamethoxazole, or chloramphenicol. On the other hand, resistance to nalidixic acid was only 2%, and no resistance to third-generation cephalosporins or fluoroquinolones could be confirmed (Figure 1) (*16*). However, another national hospital recently reported 4% resistance to third-generation cephalosporins (*17*).

Salmonella spp., except *S. typhi*, were isolated in 5% of the cases of dysenteric diarrhea in children under 2 years of age in Paraguay (*14*). In addition, there have been occasional epidemic outbreaks related to the consumption of contaminated food. The Institute of Tropical Medicine has observed a relatively constant sensitivity to antibiotics in the salmonellas isolated from cases of diarrhea in the community. Of the 40 isolates studied between 1995 and 1997, two (5%) were resistant to ampicillin and three (7%) were resistant to trimethoprim-sulfamethoxazole, while none showed resistance to chloramphenicol, cefotaxime, or ciprofloxacin.

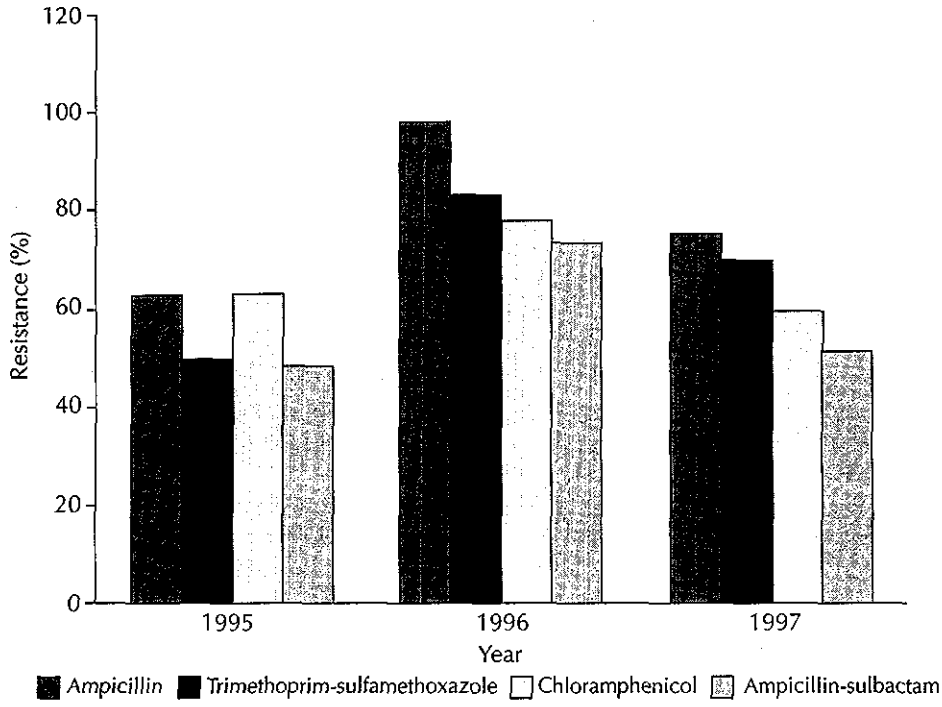
Invasive Pathogens

The profile of antimicrobial susceptibility in 52 isolates of *Haemophilus influenzae* type b (Hib) taken from children with bacterial meningitis (80%) and pneumonia (20%) showed that 32.7% of them were resistant to ampicillin and 25.0% to chloramphenicol, while 11.5% had combined resistance to both drugs. None of the isolates studied were resistant to third-generation cephalosporins (*18, 19*).

The principal etiologic agent of complicated pneumonias is *Streptococcus pneumoniae*, which also ranks second as the cause of acute bacterial meningitis in children under 5 years of age in Paraguay.

A study of antibiotic susceptibility in isolates of *S. pneumoniae* from patients with invasive pneumococcal disease, using the Kirby-

FIGURE 1. Resistance (%) of 205 isolates of *Shigella* sp. to antimicrobials in Paraguay, 1995–1997



Bauer method, showed that 23.6% (n=17) of these pathogens were resistant to penicillin. Application of the E-test to the 17 penicillin-resistant isolates to determine the minimum inhibitory concentration (MIC) yielded the following results: 13 had an MIC of ≥ 1 μg/mL (nonpenicillin-susceptible *S. pneumoniae*), while 4 had an MIC of 2 μg/mL (penicillin-resistant *S. pneumoniae*). Moreover, 1 of the 4 isolates resistant to penicillin was also resistant to cefotaxime, and 3 were resistant to chloramphenicol. None of these isolates was resistant to vancomycin. The frequency of reduced susceptibility to penicillin was analyzed vis-à-vis the age of the patients from which the *S. pneumoniae* samples were taken: of the 50 *S. pneumoniae* isolates from children with invasive disease, 12 were not susceptible to penicillin (MIC ≥ 0.1 μg/mL), and 3 of these were actually resistant to the drug (MIC 2 μg/mL); among the isolates taken from adults, one was nonpenicillin-susceptible (MIC ≥ 1 μg/mL) and one was resistant to penicillin (MIC 2 μg/mL) (P<0.05).

Resistance of Nosocomial Pathogens

Hospital, or nosocomial, infections are associated with high morbidity and mortality, higher hospital operating costs because of the need to use more expensive antibiotics and procedures, and prolonged hospital stays for infected patients. In Paraguay, more than 50% of the patients in intensive care units have had one or more episodes of nosocomial infection (*20*). These nosocomial infections have a highly varied etiology, but the predominant pathogens are Gram-negative bacteria, such as *Pseudomonas* spp., *Klebsiella* spp., and *Acinetobacter* spp.; Gram-positive cocci, such as *Staphylococcus aureus* (coagulase-negative and enterococcal); and fungi, such as *Candida*

TABLE 1. Percentage of in vitro resistance to nosocomial Gram-negative bacilli, Asunción, Paraguay

	Antibiotic							
Infectious agent	Pip	Cef	Cft	Cfz	Imp	Cip	Amk	Sul-cef
Pseudomonas aeruginosa (*n* = 100)	56	48	98	27	15	28	39	18
Klebsiella pneumoniae (*n* = 50)	76	76	60	44	0	22	38	25
Acinetobacter spp. (*n* = 50)	92	92	86	70	10	58	62	30

Pip = piperacillin; Cef = cefoperazone; Cft = cefotaxime; Cfz = ceftazidime; Imp = imipenem; Cip = ciprofloxacin; Amk = amikacin; Sul-cef = sulbactam-cefoperazone.

albicans. *Stenotrophomonas maltophilia* is also an emerging pathogen.

In studies of nosocomial Gram-negative bacilli from several intensive care units in the city of Asunción, a high degree of in vitro resistance to several antimicrobial agents was found (*21*). However, the drugs imipenem, ciprofloxacin and cefoperazone-sulbactam were active against more than 75% of the *K. pneumoniae* isolates, while imipenem and cefoperazone-sulbactam were also active (90% and 70%, respectively) against *Acinetobacter* spp., which were highly resistant to ciprofloxacin and other antibiotics. Finally, imipenem and cefoperazone-sulbactam were the most effective agents (85% and 82%, respectively) against *Pseudomonas* spp. (*22*) (Table 1).

This paper has reported on the susceptibility profiles of a number of different pathogens to the antimicrobial agents used most often in Paraguay. It shows high levels of antibiotic resistance in both the community and hospitals. One of the principal factors in this escalation of resistance is the inappropriate use of antimicrobial agents by prescribing physicians: 90% of antibiotic consumption takes place in the community, with inappropriate use increasing at alarming rates, in tandem with the vertiginous growth of bacterial resistance to these drugs (*1–4*). A recent study showed that 60% of the pediatric patients with respiratory infections seen as outpatients at a third-level center are given antibiotics (A. Arbo, personal communication). A related factor that seriously contributes to the problem is the population's unrestricted access to antibiotics, since in Paraguay, as in other countries, antibiotics are sold freely without prescription.

It is interesting to see that programs promoting the appropriate use of antibiotics have made the most progress in countries where health systems favor primary care.

Given the dramatic reality that must be addressed, a multidisciplinary effort involving primary care physicians, clinicians, pediatricians, clinical pharmacologists, pharmacists, microbiologists, and infectious disease specialists is the only possible or logical road to follow. Such a multifaceted and organized response will yield the best results in the programs we are promoting to improve the use of antimicrobial agents in our countries.

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STREPTOCOCCUS PNEUMONIAE RESISTANCE TO ANTIMICROBIALS IN URUGUAY: 12 YEARS OF MONITORING

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In Uruguay Streptococcus pneumoniae *is the bacterial agent most frequently isolated from invasive community-acquired infections.*

This study analyzes the findings of 12 years of monitoring of invasive Streptococcus pneumoniae *in children and adults in order to track the changes in resistance to nine antibiotics over time.*

Streptococcus pneumoniae *isolates from systemic infections in children under 5* (n = *402) and children over that age and adults* (n = *234) were sent to the Department of Public Health Laboratories to complete their characterization: typing by Quellung reaction and antibiogram using the disk diffusion method (oxacillin, trimethoprim-sulfamethoxazole, erythromycin, chloramphenicol, tetracycline, rifampicin, and vancomycin). For strains resistant to oxacillin, the minimum inhibitory concentration (MIC) for penicillin, cefotaxime, and ceftriaxone was determined.*

During the period 1987–1993, the percentage of resistance to β-lactam drugs did not exceed 5%. However, beginning in 1994, there was a marked increase in resistance to penicillin that was greater in young children (24.4%) than in older children and adults (13.5%). In subsequent years, resistance in the first group continued to rise (peaking at 44% in 1996), while in the second group, it reached only 14%. As of 1994, most nonsusceptible strains belonged to capsular serotype 14 and showed patterns of resistance with an MIC for penicillin of 1.0–2.0 mg/L; for cefotaxime and ceftriaxone, 0.5–2.0 mg/L; and resistance to trimethoprim-sulfamethoxazole, but susceptibility to all other antibiotics tested. Subsequent molecular studies revealed the presence of a clone imported to Uruguay from Southern Europe in the early 1990s. None of the 636 Streptococcus pneumoniae *isolates studied had diminished susceptibility to rifampicin or vancomycin.*

The results analyzed revealed a very pronounced, steady rise in resistance to β-lactam drugs in isolates recovered from infants and preschool children, which led investigators to

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question traditional therapeutic practices. In view of the prevailing situation, monitoring of the susceptibility to antibacterial drugs is fundamental for proper therapeutic management of pneumococcal infections in children and adults.

INTRODUCTION

In Uruguay, as in other regions, *Streptococcus pneumoniae* is the agent most frequently isolated from invasive community-acquired infections *(1)*. Bacteremic pneumonia and pneumococcal meningitis are frequent, with high morbidity and mortality in Uruguayan patients of all ages, but basically in children under 2 years of age and people over 65 *(2, 3)*.

The emergence of *Streptococcus pneumoniae* resistant to penicillin and other antibiotics is currently a serious problem in vast regions of the world *(4)*.

The first clinical isolation of penicillin-resistant *Streptococcus pneumoniae* was described in Papua New Guinea in 1967 *(5)*. After two years of sporadic findings, an outbreak of multiresistant *Streptococcus pneumoniae* was reported in South Africa *(6)*; *Streptococcus pneumoniae* resistance to penicillin and other β-lactam drugs subsequently spread to all parts of the globe.

The prevalence of nonsusceptible strains is correlated with antimicrobial use. In Europe, in countries such as the United Kingdom and Germany, where the use of antibiotics is strictly controlled and restricted, the percentages of resistance are low. However, in France and Spain, high frequencies of resistance coincide with high and unrestricted use of antimicrobials *(7)*.

The biological processes responsible for the emergence and spread of resistance to β-lactam drugs were, first, the alteration in successive passages of the genes that encode synthesis protein of the cell wall, with the consequent loss of the natural affinity for the drug and the subsequent geographical dissemination of resistant clones, which gave the problem a universal dimension *(8)*. Finally, the selective pressure exerted by the use of β-lactam drugs, which varied from region to region, either inhibited or facilitated the spread of resistance.

Of particular interest is the case of Iceland, where the prevalence of penicillin-resistant strains went from zero in 1988 to nearly 20% in 1993, after the importation of a multiresistant Spanish clone, serotype 6B. Since Iceland is a country with strict controls on the use of β-lactam drugs, this development was attributed to the selective pressure exerted by other families of antibiotics *(9)*.

Before the advent of antibiotics, mortality associated with pneumonia caused by *Streptococcus pneumoniae* was approximately 20%; with mortality from bacteremia at 50% and from meningitis at 80%–100% *(10)*. In the case of pneumococcal meningitides, the correlation between resistance to penicillin and third-generation cephalosporins and a deterioration in the course of disease has been repeatedly demonstrated *(11)*; in the case of pneumonias, however, the impact of the problem is still being studied. The U.S. Centers for Disease Control and Prevention (CDC) have called for continued surveillance, since some studies point to an increase in mortality among cases caused by resistant strains *(12)*. However, in Argentina and Uruguay, a retrospective analysis comparing 99 pneumococcias caused by nonsusceptible *Streptococcus pneumoniae* with 175 cases caused by susceptible strains treated with β-lactams revealed no significant differences in the course of the disease *(13)*.

Since 1987, Uruguay has conducted surveillance of invasive pneumococcal infections in children and adults, with special emphasis on determining the susceptibility of *Streptococcus pneumoniae*. This paper presents the results of 12 years of monitoring, which confirmed a steady increase in resistance to penicillin and cephalosporins in the isolates collected in this process.

MATERIALS AND METHODS

Collection of *Streptococcus pneumoniae* Isolates

Between 1987 and 1998, the Department of Public Health Laboratories (DLS) received 636 samples of *Streptococcus pneumoniae* isolated from invasive cases at public and private health facilities throughout the country. These isolates were analyzed in two separate groups: children up to 5 years of age ($n = 402$) and patients 6 years of age and older ($n = 234$), whom we will refer to as adults. Only specimens from normally sterile sites, such as blood, cerebrospinal fluid, and peritoneal and pleural exudate, were included. In children, the predominant clinical diagnosis was pneumonia (68%), followed by meningitis (20%) and other processes (12%), such as sepsis, bacteremias without apparent foci, peritonitis, abscesses, and cellulitis. Among adults, the same order of frequency obtained—that is, 44%, 31%, and 17%, respectively.

In 1994, surveillance was intensified with the country's participation in a multicenter study organized by the Pan American Health Organization's Regional System of Vaccines (SIREVA) *(14)*. The object of this study was to monitor the distribution of serotypes and antimicrobial resistance of the *Streptococcus pneumoniae* strains responsible for invasive disease in six Latin American countries.

Susceptibility to Antimicrobials

Identification of *Streptococcus pneumoniae* was confirmed by conventional methods *(15)*; the Quellung reactive technique was employed for serotyping, using 12 pools of sera and selected factors (Statens Seruminstitut, Denmark).

Susceptibility to oxacillin, erythromycin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, rifampicin, and vancomycin was determined by disk diffusion on Mueller-Hinton agar plates with 5% ovine blood incubated at 37 °C in 5% CO_2.

The MIC for penicillin, cefotaxime, and ceftriaxone was determined by E-test for all strains resistant to the oxacillin disk (≤ 19 mm). ATCC 49619 was utilized as the control strain. A subsample was also analyzed by microdilution in Mueller-Hinton broth and 5% lysed horse blood *(16)* to correlate this with the E-test. In the case of meningitides, the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) call for the determination of the MIC to be made without prior screening with a disk, which means that the E-test was done on the strains obtained from that source.

The cutoff points were the ones proposed by NCCLS *(16)*. The values for the E-test were estimated on the basis of the dilutions used in the reference method (for example, an MIC of 1.5 mg/L by E-test was read as 2.0 mg/L), as recommended by the manufacturer (AB Biodisk, Sweden). The MIC for trimethoprim-sulfamethoxazole was also studied in 120 isolates that included susceptible and resistant strains. The MIC for erythromycin and chloramphenicol was determined by microdilution only in isolates resistant to these drugs, after screening by disk diffusion.

Since 1994 the DLS has participated in an external quality control program coordinated by the National Streptococcus Center of the University of Alberta, Canada. As part of that quality control system, unknown strains are received regularly for characterization (for identification, serotyping, and susceptibility testing). Ten percent of the isolates in Uruguay are sent for confirmation of the results.

In this document, following the latest recommendations of the CDC, the term "not susceptible" refers to the sum of the intermediate and absolute resistance.

RESULTS

Between 1987 and 1993, 124 isolates of invasive *Streptococcus pneumoniae* obtained from patients of all ages were studied. For both children and adults, the isolation of strains not

susceptible to one or more antibiotics was an infrequent event, which occurred in no more than 5% of cases, except for resistance to trimethoprim-sulfamethoxazole, where the figure was 27%.

From 1994 on it was observed that strains in the pediatric population were steadily becoming more resistant to penicillin, reaching a maximum of 44% in 1996, with a decline in subsequent years (1997 and 1998). Among adults, in contrast, the percentage of resistance in the same period only fluctuated a little, never exceeding 14% (Figure 1).

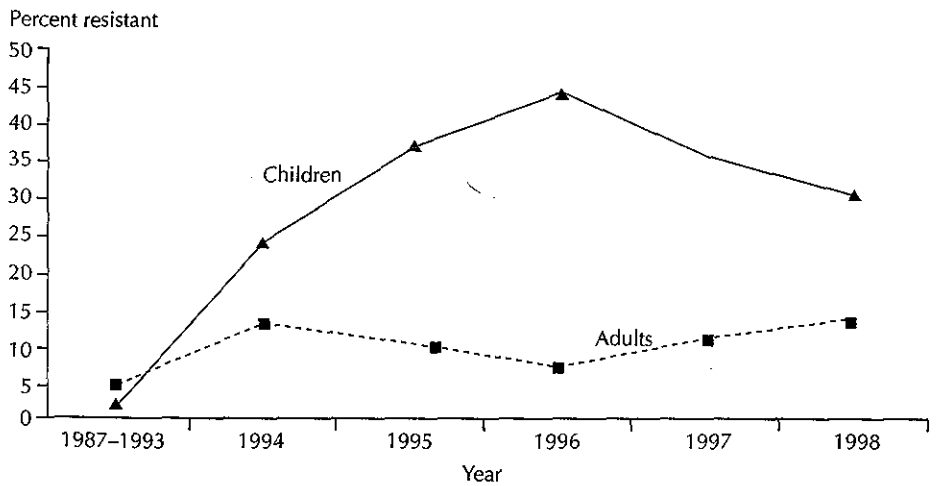
In children, the frequency of resistance varied with the disease in question, being higher in pneumonias than meningitides. In 1996, the percentage of resistant *Streptococcus pneumoniae* in pneumonia cases reached 53%, while for meningitis the highest level was 20%. Dividing the country into four regions—that is, western, central, eastern, and metropolitan area—the percentages of resistance for the strains isolated from children in the period 1994–1998 were 32%, 15%, 35%, and 36% for each region, respectively.

The increase in the global percentage of nonsusceptible strains (106/313 or 33.9%) was accompanied by a greater frequency of absolute resistance to penicillin, which in 1996 reached a peak of 24.3% (Table 1). Intermediate resistance to third-generation cephalosporins attained significant levels in the pediatric population, while full resistance to these drugs was low among children and insignificant among adults (Table 2). The highest MIC for penicillin was 4.0 mg/L and for cephalosporins, 2.0 mg/L.

The results presented in Tables 1 and 2 were obtained by E-test. However, a comparison between the two methods for determining the MIC (E-test and microdilution) for penicillin and ceftriaxone demonstrated that the E-test tends to systematically lower the readings. For example, in 16 isolates of *Streptococcus pneumoniae* with an MIC for penicillin of 2.0 mg/L by microdilution, the MICs by E-test were 1.0 mg/L, which implies a change in category. The results were similar with ceftriaxone.

The strains isolated from cerebrospinal fluid were for the most part susceptible to penicillin (Figure 2). Of the 22 fatal cases of pneumococcal meningitis in children, only two of the strains had an intermediate resistance to penicillin (0.12 and 1.0 mg/L) and one of them to ceftriaxone (1 mg/L). The 14 case fatalities recorded in the adult population were caused

FIGURE 1. *Streptococcus pneumoniae* from invasive infection in children and adults in Uruguay: percentage of strains not susceptible to penicillin, by year, 1987 a 1998*



* Through August.

TABLE 1. β-lactam-resistant *Streptococcus pneumoniae* isolated from invasive infections in children under 5 years of age, Uruguay, by year, 1994–1998*

	1994 (*n* = 78)				1995 (*n* = 81)				1996 (*n* = 70)				1997 (*n* = 61)				1998 (*n* = 23)			
	I		R		I		R		I		R		I		R		I		R	
Antibiotic	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Penicillin	13	16.7	6	7.7	22	27.1	8	9.9	14	20.0	17	24.3	12	19.7	7	11.5	1	4.3	6	26.1
Cefotaxime	10	12.8	1	1.3	9	11.1	2	2.5	16	22.9	4	5.7	7	11.5	3	4.9	6	26.1	–	–
Ceftriaxone	13	16.7	1	1.3	12	14.8	5	6.2	20	28.6	5	7.1	13	21.3	1	1.6	6	26.1	1	4.3

*Through August.

I = Intermediate resistance, MIC for penicillin 0.12–1.0 mg/L; MIC for cefotaxime and ceftriaxone ≥1.0 mg/L.

R = Resistant, MIC for penicillin, cefotaxime and ceftriaxone ≥2.0 mg/L.

TABLE 2. β-lactam-resistant *Streptococcus pneumoniae* isolated from invasive infections in adults, Uruguay, 1994–1998*

	Penicillin			Cefotaxime			Ceftriaxone		
	S	I	R	S	I	R	S	I	R
Number	177	10	12	189	10	–	187	10	2
Percentage	88.9	5.0	6.0	95.0	5.0	–	94.0	5.0	1.0
		11%						6%	

*Through August.
S = Susceptible, MIC for penicillin ≤0.06 mg/L and MIC for cefotaxime and ceftriaxone ≤0.50 mg/L.
I = Intermediate resistance, MIC for penicillin 0.12–1.0 mg/L and MIC for cefotaxime and ceftriaxone 1mg/L.
R = Resistant, MIC for penicillin, cefotaxime and ceftriaxone ≥2.0 mg/L.

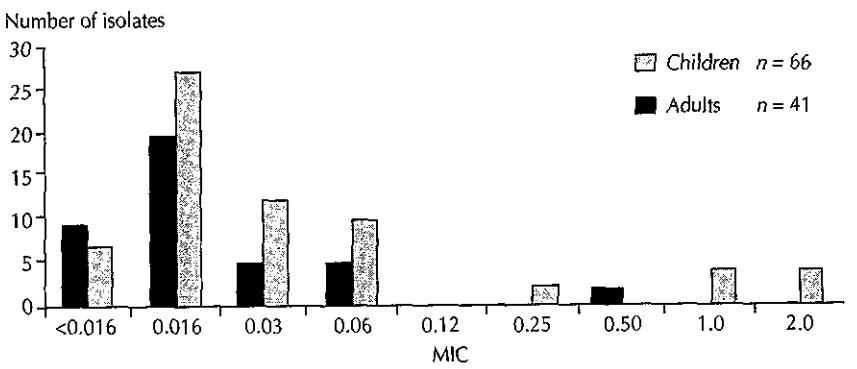
by *Streptococcus pneumoniae* susceptible to β-lactam drugs.

In 1992 the first isolation of serotype 14 not susceptible to penicillin or trimethoprim-sulfamethoxazole was recorded in Uruguay. From then on, most of the nonsusceptible isolates from children (90%) and adults (78%) exhibited the same phenotypical characteristics. The remaining serotypes associated with resistance were 6B, 6A, 19A, 23B, and 23F.

In the child population, resistance to trimethoprim-sulfamethoxazole reached its highest levels in the period 1994–1998 (45%), remaining at about 15% in adults (Table 3). When the MICs were determined, a good proportion of these strains showed high levels of resistance. Resistance to erythromycin went from 0.8% (1987–1993) to roughly 3% (Table 3). The MICs ranged from 4 mg/L to over 256 mg/L. These resistant strains belonged to 11 different serotypes in children (9V(3); 18C (2); and 1, 5, 14, 18B, 19A, and 23F) and to 8 in adults (9V (4); 6A, 11A, 18B, and 23B), and they usually exhibited resistance to another antibiotic (trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline).

Resistance to chloramphenicol in both populations remained low and stable during the 12 years of monitoring, and the MICs of the resistant strains were 2.0 or 4.0 mg/L. Strains resistant to rifampicin or vancomycin were not isolated, and only eight strains resistant to three or more antibiotic families were detected.

FIGURE 2. Minimum inhibitory concentration for penicillin in *Streptococcus pneumoniae* isolated in meningitis cases among children and adults, Uruguay, 1994 a 1998*



*Through August.

TABLE 3. ***Streptococcus pneumoniae*** **resistant to other antibiotic drugs, isolated from invasive infections in children and adults in Uruguay, by year, 1994–1998***

Antibiotic and population		1994		1995		1996		1997		1998*		Total	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
SxT†	C	34	44	36	44	33	47	27	44	10	43	140	45
	A	15	29	9	19	6	14	4	14	4	14	38	19
ER†	C	4	5	3	4	1	1	3	5	–	–	11	3.5
	A	1	2	1	2	–	–	1	4	2	7	5	2.5
TE†	C	4	5	6	7	6	9	–	–	1	4	17	5.4
	A	6	12	1	2	1	2	–	–	2	7	10	5.0
CL†	C	–	–	–	–	3	4	3	5	–	–	6	1.9
	A	1	2	–	–	–	–	–	–	–	–	1	0.5
Total strains	C	78		81		70		61		23		313	
	A	52		48		42		28		29		199	

* Through August.
† = Includes resistant strains only.
SxT = Trimethoprim-sulfamethoxazole. ER = Erythromycin. TE = Tetracycline. CL = Chloramphenicol.
C = children; A = adults.

DISCUSSION

Twelve years of monitoring the antimicrobial susceptibility of *Streptococcus pneumoniae* strains isolated exclusively from invasive processes made it possible to confirm a clear increase in resistance to penicillin, beginning in 1994—a phenomenon that, while coinciding with the start of SIREVA, was unrelated to that program or to changes in methodology.

While in the 1980s an increase in resistance to penicillin had been confirmed in only a limited number of countries, the 1990s were characterized by the universal spread of that resistance.

Uruguay was not exempt from that process, although the phenomenon exhibited special characteristics. Most of the strains that were not susceptible to penicillin were of a single capsular type, 14, and were also resistant to trimethoprim-sulfamethoxazole. Although it was the most frequent serotype in Uruguayan children *(17, 18)*, the absolute prevalence of this serotype among the resistant strains already suggested a clonal origin, which was later genetically confirmed *(19, 20)*.

The drastic increase in resistance to penicillin that occurred in Uruguay due to the importation of a single clone is comparable only to what occurred in Iceland *(9)*, since nonsusceptible strains in other countries are more genetically diverse.

In the late 1980s that clone, which was associated with serotype 14 and serogroup 9, had already spread in Spain, France, and Portugal *(21)*. A limited number of strains of the same clone were isolated in Brazil, Chile, Colombia, and Mexico, expressing serotype 14 or serogroup 9 *(22)*. By contrast, in Argentina *(23)* and, to a greater extent, Uruguay that clone predominated among the strains not susceptible to penicillin, although all belonged to serotype 14 and none to serogroup 9.

In 1992 surveillance at the national level made it possible to document the first isolation of that clone and its subsequent spread throughout the country; it was isolated more frequently in children from the metropolitan area with a clinical diagnosis of pneumonia. The lower percentages of resistance observed in the strains isolated from the adult population could be related to a lower frequency of that serotype, which may be attributed to greater acquired immunity.

Most of the strains not susceptible to penicillin had MICs by E-test around the cutoff point between intermediate and absolute resistance (1.0 or 2.0 mg/L). However, compar-

ing the results of the E-test with those of microdilution, on repeated occasions MICs of 1.0 mg/L by E-test and 2 mg/L by microdilution were verified. Although the difference with dilution is technically acceptable, E-test results may underestimate the percentages of absolute resistance. The same could be said for third-generation cephalosporins.

The introduction of the clone also led to an increase in global percentages and high levels of resistance to trimethoprim-sulfamethoxazole in children. In adults, the percentages of resistance to trimethoprim-sulfamethoxazole were lower, probably due to the lower prevalence of the clone. Nevertheless, doubts have been raised about the correlation between the in vitro results and the therapeutic efficacy of the drug (*24*).

In Iceland, the spread of another multiresistant clone (serotype 6B) was fostered by the administration of non-β-lactam antibiotics (*9*). In the case of Uruguay there is a possibility that the treatment of upper respiratory infections with trimethoprim-sulfamethoxazole may have exerted selective pressure, promoting colonization or infection with serotype 14 strains resistant both to that antibiotic and β-lactams. In addition, in Uruguay, the low levels of resistance found in invasive pneumococcal infections can be attributed to the very limited use of erythromycin and chloramphenicol; nevertheless, high levels of resistance to erythromycin (*25*) and chloramphenicol (*26*) have been reported in countries where these drugs are utilized on a regular basis.

The increase in *Streptococcus pneumoniae* resistance to β-lactams implies serious problems for therapeutic management of meningitides (*11*). Not so for pneumonias, however, since the low toxicity of these antibiotics makes it possible to increase the dosage to serum concentrations that exceed the MICs registered up to now (≤4 mg/L).

A recent CDC communication noted a possible increase in mortality in patients with pneumonia caused by resistant strains (*12*). However, research conducted in Argentina and Uruguay by Deeks et al. (*13*) found no significant differences in the outcomes of patients infected with susceptible and resistant *Streptococcus pneumoniae*. In that same work, an analysis of the risk factors for acquiring an invasive infection with nonsusceptible *Streptococcus pneumoniae* showed that only two of the parameters analyzed were significant: the administration of penicillin or ampicillin during the previous three months and receiving medical care in a private institution. Both parameters point in the same direction—that is, they link these risks with the overuse of antibiotics.

Hungary and Finland (*27*) have recently had success in reducing the frequency of resistance by controlling and restricting the use of antibiotics. These results confirm that the process can be reversed through a national antibiotic policy.

New experimental conjugate vaccines have proven to be immunogenic and safe in children (*28*). Since the proposed formulas include the serotypes associated with β-lactam resistance, the administration of these vaccines could reduce their prevalence. With the same objective, immunization of older persons with the 23-valent vaccine could be encouraged.

However, as long as conjugate vaccines are not available, the only possible measure is to attempt to control the steady rise in resistance through a campaign to educate health workers and the public alike to avoid the indiscriminate use of antibiotics resulting from self-medication and improper prescription.

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NOSOCOMIAL INFECTIONS IN URUGUAY: ANTIBIOTIC RESISTANCE OF THE PRINCIPAL MICROORGANISMS IDENTIFIED[1]

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In Uruguay the growing antibiotic resistance of pathogens in both the community and hospitals is creating serious public health problems. The Public Health Laboratories Department in the Ministry of Health is responsible for centralizing the information in this area, processing it in order to provide feedback at the operational levels, and standardizing quality assurance procedures. Accordingly, a series of studies was launched in 1996, with a view to making an initial diagnosis of the situation, with emphasis on nosocomial infections. For a period of seven months (1996–1997) all invasive processes (n=17) *representing 10 genera/species with different spectra of resistance and endemicity were monitored at three sentinel hospitals. Both* Staphylococcus aureus *and coagulase-negative* Staphylococcus *showed multiple resistance, 46% and 54% of isolates, respectively, being resistant to methicillin. All these cases, as well as the enterococci* (n=38)*, were susceptible to vancomycin. Variable resistance patterns were also observed in 103 invasive Gram-negative bacilli, all susceptible to imipenem. In addition, cases in the intensive care unit of a fourth hospital were monitored for six months in 1997 and again for six months in 1998. No differences were found at this latter institution, either in the frequency of the agents* (n=181) *or in their resistance. With the enterococci, 13% were resistant to ampicillin and 20% were highly resistant to gentamicin. Isolates of* Acinetobacter *spp. were obtained from both invasive infections and those of other origin.*

Apart from the risk to life and the treatment problems posed by the 352 nosocomial infections, the estimated cost of care alone is justification for improving microbiological surveillance to help to control the existing confirmed antimicrobial resistance and to prevent the appearance of other forms of resistance that have not yet been reported in Uruguay.

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INTRODUCTION

Antibacterial resistance has become one of the most serious public health problems worldwide (1) in recent years, and Uruguay has not been immune to this phenomenon. A number of different bacterial species are already known to have reduced susceptibility to antibiotics and are causing infections in both the community and hospitals alike. The treatment difficulties created by antibiotic resistance have not only led to higher morbidity and mortality, they have made it more difficult to manage patients and have significantly increased the cost of care.

It is already recognized that resistance is encoded in more than 100 genes, which can be transferred to bacteria of the same species or from one type to another (2). It is only a matter of time before new forms of resistance appear in different bacteria and spread to distant geographical areas. It is therefore essential to be constantly on the alert (3). A number of factors are implicated in the spread of resistance—for example, social and environmental change, the widespread use of antibiotics in agroindustry, the speed of communications, travel, and trade. The use and misuse of antibiotics in humans and animals are undoubtedly fundamental to the spread of resistance, since these practices exert selective pressure on bacterial populations and facilitate survival of the more resistant forms (4).

Recently, Greenwood (5) raised the fear that some of the multiresistant hospital pathogens, such as *Staphylococcus aureus*, might spread to the community and infect healthy individuals. An inverse case has already been documented: there have been outbreaks of *Streptococcus pneumoniae*, a typical community pathogen, in hospitals as well (6). In Uruguay there are exceptional cases that illustrate both risks. The situation created by antibacterial resistance is very complex; its study and control requires the coordinated effort of multidisciplinary teams. Clinical bacteriologists must have optimum skills in culture techniques and species identification, since finding the appropriate treatment for serious infections depends on being able to isolate the causative agent and determine its susceptibility to antibiotics. Although this is primarily a support task, once collected and analyzed, the data generated by clinical pathology provide valuable information for hospital infection committees. Moreover, this information should be compiled in national databases so that the ministries of health can offer guidelines to physicians on empirical treatments and establish policies on the use of antibiotics. Added to this challenge is the task of ensuring the comparability of results, including quality controls on the procedures for determining susceptibility to different drugs.

In the 1970s, hospital staphylococcal infections were replaced by Gram-negative bacilli infections. In the 1980s, new antibiotics designed to fight the infections caused by these germs offered hope that nosocomial infections could be fully brought under control. However, these infections have persisted, with the further involvement of multiresistant Gram-positive and Gram-negative bacteria. The factors contributing to this new stage in the history of the nosocomial infections include the changing biology of the bacteria as they become increasingly resistant, the extension of human life, technology advances, and immunosuppressive therapies.

The Public Health Laboratory Department (PHLD) in the General Bureau of Health, which acts as the Ministry of Health's national reference and communicable disease control laboratory in Uruguay, has not been indifferent to these problems. In 1996, it began to address the issue of nosocomial infections through actions aimed at:

- Strengthening the laboratories that provide support services;
- Having sentinel hospitals determine susceptibility profiles of the isolates mentioned above;
- Performing quality control with a view to standardizing techniques, identifying the

antibiotics to be tested, and setting up a uniform reporting system;
- Organizing and maintaining a collection of bacteria to be used in evaluating new drugs or techniques and conducting other related studies;
- Using molecular biology techniques such as pulsed-field gel electrophoresis or multilocus enzyme electrophoresis to genetically characterize the most important bacterial species;
- Demonstrating the feasibility and benefits of having a centralized microbiological system for monitoring resistance, with continuous up-to-date feedback to the operational levels.

MATERIALS AND METHODS

Definitions

- According to the World Health Organization *(7)*, nosocomial infections are infections that occur in patients who are currently hospitalized (48 h or more) or have recently been discharged (within the past month), the causative agents of which come from the endogenous flora of the patient or are endemic in the hospital.
- An invasive strain is one that is recovered from a normally sterile site.

Sentinel Hospitals

- The etiology of systemic infections reported in three hospitals was monitored for a period of seven months, from October 1996 to April 1997. Two of these hospitals, referred to here as Hospitals 1 and 2 (one for adults and one for children), are located in Montevideo, and Hospital 3 is a general hospital in the interior of the country *(8)*. All the isolates collected during this period were sent to the PHLD to confirm or complete their identification and determine their susceptibility to antimicrobial drugs. Information on 299 isolates (one per patient) was entered in the WHONET database. Of the cases in question, 57% were from nosocomial infections, and these are the ones that will be analyzed in the present paper.
- In 1997, a sentinel site was set up in a fourth hospital (Hospital 4), for adults, located in Montevideo, in order to include data from an intensive care unit (ICU) in the monitoring exercise and be able to compare the appearance of nosocomial infections from selected pathogens (*Staphylococcus aureus*, *Enterococcus* spp., *Acinetobacter* spp.) over time.
- As part of a multinational program (CEMNET) coordinated by Rockefeller University, isolates of methicillin-resistant *Staphylococcus* spp. were collected at Hospital 4 and another hospital for adults, also in Montevideo (Hospital 5), for the purpose of genetically characterizing them.

Techniques

Both the clinical bacteriology and the PHLD laboratories used conventional bacteriology techniques *(9)*. All the techniques for studying antibacterial susceptibility (disk diffusion and minimum inhibitory concentration (MIC) using microdilution or the E-test) were applied, following the recommendations of the U.S. National Committee for Clinical Laboratory Standards (NCCLS) *(10)*. Depending on the bacterial species, the antibiotype was tested against up to 16 drugs. In addition, in the case of *Staphylococcus* isolates, an oxacillin disk (1 μg) was included as a marker of resistance to methicillin, on Mueller-Hinton medium with 2% sodium chloride added. Readings were taken at 24 hours for *S. aureus* and 48 hours for coagulase-negative *Staphylococcus*. In the disk diffusion or E-test studies of *Enterococcus* spp. susceptibility to vancomycin, the results were read at 24 and 48 hours, with illumination to observe any occasional growth inside the halo.

For the genetic characterization of *Staphylococcus* spp., the first stage of training was completed abroad, after which training in

pulsed-field gel electrophoresis (PFGE) was continued in Uruguay. Chromosomal DNA extracts digested with Smal enzyme were separated using a CHEF-DRII apparatus *(11)*.

External Quality Controls

The PHLD cooperated with the World Health Organization (Prof. Vandepitte, Louvain University, Belgium) on an external quality control program for clinical bacteriology. In addition, under a PAHO/CIDA program, specialists from the University of Alberta in Canada assisted in setting up the microdilution technique and provided quality control for this aspect of the study.

Estimated Cost of Patient Care

The daily cost of patient care in a hospital ward (treatment, bed and meals, paraclinical services, and other costs) ranges from US$ 60 in a public hospital to $300 in a private institution. The cost of a day in intensive care, depending on the level of complexity and the institution involved, can be anywhere from US$ 400 to $1,200. At the University Hospital's ICU, the average cost is estimated at US$ 900.

Based on a study conducted in 338 hospitals in the United States, the additional days of hospitalization required as a result of nosocomial infection have been calculated at an average of 6.8 days for serious infections and 4 days for other infections *(12)*.

RESULTS

Hospitals 1, 2, and 3 recovered a total of 299 bacterial isolates from normally sterile sites. Of this total, 171 corresponded to nosocomial infections—106 patients from intensive care and the rest from hospital wards. Antibiotic susceptibility was tested for 10 predominant genera/species, each with at least six isolates. The breakdown by institution is shown in Table 1. The infectious agent isolated most frequently was *S. aureus*. Of the 39 isolates of this pathogen, 18 (46%) were resistant to methicillin, and 11 of these (61%) were susceptible only to vancomycin, while the 7 remaining were sensitive to rifampicin, amikacin, and chloramphenicol.

Only 13 isolates of coagulase-negative *Staphylococcus* were referred to the PHLD; 7 (54%) of these were resistant to methicillin, and the MIC for the rest of them was already borderline (4 mg/L).

Table 2 shows the behavior of Gram-negative bacilli vis-à-vis some of the antibiotics tested. Of the Gram-negative bacilli, *Escherichia coli* (n=29) was the pathogen isolated most frequently from systemic infections. These isolates were tested against 15 antibacterial drugs, and all of them (100%) proved to be susceptible to 7 of the antibiotics. In addition, all the isolates of *Enterobacter cloacae* (n=11), *Serratia marcescens* (n=11), *Klebsiella pneumoniae* (n=21), and *Klebsiella oxytoca* (n= 6) were resistant to ampicillin.

TABLE 1. Predominant genus/species of bacteria recovered from hospital invasive infections, in Hospitals 1, 2, and 3 during seven months of monitoring as part of study on nosocomial infections, Uruguay, October 1996 to April 1997*

	Staphylococcus aureus	Coagulase-negative *Staphylococcus*	*Enterococcus faecalis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Enterococcus cloacae*	*Serratia marcescens*	*Pseudomonas aeruginosa*	*Acinetobacter* sp.	Total
Hospital 1	36	11	6	19	14	4	7	8	4	25	134
Hospital 2	3	2	–	9	6	2	2	2	4	–	30
Hospital 3	–	–	–	1	1	–	2	1	2	–	7
Total	39	13	6	29	21	6	11	11	10	25	171

* Data modified from *(8)*.

The *Acinetobacter* sp. isolates (n = 25) came from only one of the three institutions, and the susceptibility varied. The following levels of resistance were found in these isolates: 52% to ceftazidime, 68% to amikacin, and 45% to ampicillin-sulbactam. Resistance to imipenem was not demonstrated by disk diffusion or MIC.

Six isolates were obtained from invasive *Enterococcus faecalis*, all of which (100%) were susceptible to ampicillin and vancomycin. No isolates of *E. faecium* were cultured.

Pseudomonas aeruginosa (n = 10) yielded a variety of susceptibility profiles. Only three of these isolates were multiresistant. However, two of them, which were sensitive to imipenem in the disk diffusion test (halos of 25 and 26 mm, respectively), had MICs of 8.0 mg/L, indicating reduced susceptibility to that drug.

Table 3 shows the isolates taken from intensive care patients in Hospital 4 over six consecutive months (March to August) in 1997 and again in 1998. A total of 181 cases of hospital infection were identified, corresponding to three of the most common pathogens. There was little difference between the two years in the reported frequency of the pathogens or in their levels of resistance: in 1997, 42 isolates of *S. aureus* were recovered and 45.2% of these were resistant to methicillin, while in 1998 there were 41 isolates, 36.6% of which showed resistance to this drug.

All the multiresistant isolates were sensitive to vancomycin. Of 32 enterococcal isolates, 31 corresponded to *E. faecalis*, and the remaining one, taken from peritoneal fluid, was identified as *E. faecium*. The latter was resistant to ampicillin, and 13% of the others were resistant to ampicillin, while 19% were resistant to gentamicin (MIC ≥ 500 mg/L). No β-lactamase production or resistance to vancomycin was detected. The numbers of *Acinetobacter* spp. (n = 66) isolates were similar in both years, but in 1997 the proportion of invasive strains was higher. The percentages of resistance were also similar in both years—namely, 30% for amikacin and 73% for ampicillin-sulbactam. None of the isolates was resistant to imipenem.

Between April and August 1997, Hospitals 4 and 5 recorded 167 isolates of *S. aureus*, 47 (28%) of which were resistant to methicillin. In addition, 27 isolates of coagulase-negative *Staphylococcus* (44%) were resistant to this same drug. The breakdown of this group was as follows: *S. epidermidis*, 13; *S. haemolyticus*, 7; *S. chromogenes*, 3; *S. hominis*, 2; and *S. capitis* and *S. cohnii*, 1 each.

Genetic analysis of the 1997 *S. aureus* isolates revealed a single pattern, B, with nine subtypes, B1–B9. Subtypes B1 and B2 were the most frequent, with 46.4% and 30.3%, respectively (*13*). Subtype B1 has a PFGE profile identical to that of strains from the clone found

TABLE 2. Resistance to seven antibiotics observed in Gram-negative bacilli isolated from invasive processes in hospitalized patients at Hospitals 1, 2, and 3, as part of a study on nosocomial infections, Uruguay, 1997 and 1998*

Organism	Ampicillin % R	Ampicillin-sulbactam % R	Ceftazidime % R	Imipenem % R	Ciprofloxacin % R	Gentamicin % R	Amikacin % R
Escherichia coli (n = 29)	79	28	–	–	7	–	–
Klebsiella pneumoniae (n = 21)	100	70	48	–	10	62	29
Klebsiella oxytoca (n = 6)	100	50	33	–	50	50	17
Enterococcus cloacae (n = 11)	100	91	91	–	–	27	–
Serratia marcescens (n = 11)	100	100	14	–	27	73	45
Acinetobacter spp. (n = 25)	N/P	46	52	–	50	36	32
Pseudomonas aeruginosa (n = 10)	N/P	N/P	10	0†	20	30	–

*Data modified from (*8*).
R = resistance (intermediate + absolute).
N/P = not processed.
†2 strains with intermediate resistance according to MIC.

TABLE 3. ***Staphylococcus aureus, Enterococcus* spp., and *Acinetobacter* spp. isolated in the ICU of Hospital 4, as part of a study of nosocomial infections, Uruguay, six consecutive months of monitoring in 1997 and 1998**

	1997			1998		
Organism	No.	Invasive	Noninvasive	No.	Invasive	Noninvasive
Staphylococcus aureus	42	9	33	41	5	36
Enterococcus spp.	19	4	15	13	3	10
Acinetobacter spp.	34	7	27	32	1	31

in Brazil in 1992–1994, which was later also described in Argentina *(14)*.

With the coagulase-negative *Staphylococcus* spp., the PFGE revealed great genetic diversity: there were 19 different profiles, 5 of which corresponded to the 13 isolates of *S. epidermidis* and the rest to the remaining species.

The estimated cost of providing hospital care for these infections was calculated by taking the average number of additional days of hospitalization required because of nosocomial infections and multiplying this figure by the lowest estimated daily cost of care in a hospital ward and the ICU. The results are shown in Table 4. Based on these calculations, the 352 microbiologically confirmed nosocomial infections in the four hospitals would cost a total of US$ 796,240. If the 287 ICU hospitalizations were calculated on the basis of the average cost of care at the University Hospital, the sum would be $1,756,440.

DISCUSSION

Epidemiological surveillance, by definition, is the ongoing systematic collection, analysis, and interpretation of data for utilization in the planning, implementation, and evaluation of interventions or programs *(15)*. The purpose of monitoring for antibacterial resistance, according to O'Brien, is to observe and explain the emergence and spread of the genes that express it *(16)*. This involves a highly intricate problem, which is virtually limitless, given the infinite number of bacterial populations.

The objectives of this study on nosocomial infections were very narrow. The first was to collect concrete data on specific pathogens and their behavior vis-à-vis antibacterial drugs in order to arrive at a diagnosis of the situation in the hospitals studied. Then, the exercise called for proposing some control measures and illustrating the potential benefits of monitoring antimicrobial resistance. Through this project it has been possible to recognize or confirm certain types of existing antimicrobial resistance and, at the same time, in some bacterial species, to confirm the persistence of susceptibility to drugs that in other geographical areas have lost their effectiveness due to resistance by the pathogen.

It was also determined that in Uruguay most of the species implicated in nosocomial infections have some loss of susceptibility to the antibiotics of choice and that they are often multiresistant. Nevertheless, reduced susceptibility to vancomycin has not yet been demonstrated in isolates of *Staphylococcus* spp. or *Enterococcus* spp. Fortunately, this antibiotic of last resort continues to be available for the treatment of patients infected with multiresistant Gram-positive pathogens. The appearance of isolates resistant to vancomycin would eliminate the last drug available for treating several infections, and this would definitely be considered a medical emergency requiring compulsory notification. The relatively privileged situation of Uruguay within the international context makes it essential to intensify surveillance in order to detect the appearance of vancomycin-resistant strains as soon as possible and implement measures to prevent their spread.

Of the patients with serious *S. aureus* infections, 37 had to be treated with vancomycin,

TABLE 4. Estimated cost of 352 nosocomial infections based on estimated costs of extended hospitalization at lowest daily rate, multiplied by the average number of additional days *(12)*

	Number of infections/cost in US$		
Institution	Ward	ICU	Total
Hospitals 1,2 and 3	65/15.600	106/288.320	171*/303.920
Hospital 4	–	181/492.320	181†/492.320
Total	65/15.600	287/780.640	352/796.240

*Invasive infections only.
†Hospitalized ICU patients.

an expensive and highly toxic drug. The outlook for the availability of other options in the future is not encouraging, since the pharmaceutical industry, for various reasons, has slowed its research for the development of new drugs.

A few years ago a development occurred that was a serious wake-up call: strains of *S. aureus*, an important pathogen both in the hospital environment and elsewhere, began to show reduced susceptibility to vancomycin, first in isolates recovered in Japan *(17)* and then in the United States *(18)*.

In our study, the percentage of coagulase-negative *Staphylococcus* recovered from systemic infections at Hospitals 1, 2, and 3 was relatively low, although in other studies their number has exceeded that of *S. aureus (19)*. The small number of confirmed coagulase-negative *Staphylococcus* may have been due to the problem of recognizing the isolates as invasive agents, since for years they were considered a habitual contaminant of hemocultures and other specimens. Its growing importance as a methicillin-resistant hospital pathogen is compounded by the fact that it provides a reservoir of resistant genes that are capable of being transferred to the same or other species.

In Hospitals 1, 2, and 3 the number of *Enterococcus* spp. isolates was very small, since only invasive strains were collected. However, at the ICU in Hospital 4, this microorganism was represented in greater numbers in both years, since other sites were included in addition to those that are normally sterile.

The prevalence of ampicillin- and vancomycin-resistant *E. faecalis* has been described in the United States since 1989 *(20)*. In Uruguay, 13% of the ICU isolates were resistant to ampicillin, but in samples obtained of other areas of the hospital, resistance was as high as 30%. The history of high-level resistance to gentamicin (19%) means that it is necessary to determine the MIC for all invasive strains or when the treatment of choice combines a β-lactam drug with an aminoglycoside. Prevention of the appearance of vancomycin resistance requires the rational and restricted use of all antibiotics, since any antibiotic that alters the fecal flora, even if it is ineffective against the enterococci (e.g., the cephalosporins and quinolones), favors the predominance of resistant forms *(21)*.

Both the Gram-positive and the Gram-negative bacteria identified in the four hospitals were repeatedly isolated as part of the endemic flora in those institutions. In Hospital 1, isolates from *Acinetobacter* spp. were repeatedly obtained from normally sterile sites, whereas at the ICU in Hospital 4 the isolates were recovered from various samples during the two six-month periods. This bacterium is usually associated with nosocomial infections, particularly in patients on ventilators. Sometimes it is difficult to differentiate between a mere colony and an infectious process, although the importance of its isolation must not be downplayed, because several authors have attributed a considerable increase in mortality to this pathogen *(22)*.

In Uruguay, patients are often transferred from one institution to another, and at the same time, health workers frequently hold more than one job. Both these factors would

facilitate the spread of *Acinetobacter* spp. and other agents proper to the hospital environment.

The molecular biology techniques available in the PHLD could be applied to the screening of *Acinetobacter* and *Enterococcus* infections as a way of adding to the body of information available on how these pathogens are transmitted and thus enabling the introduction of control and prevention measures. Also, at some future point this information might be useful in helping to assign responsibility in the event of legal problems *(23)*.

The cost of the 343 nosocomial infections evaluated in this paper measures only part of the economic impact, because the calculations were based on the cases for which a bacteriological diagnosis had been obtained, without taking into account those for which the cultures were not successful. Moreover, in Hospitals 1, 2, and 3 the estimate of the costs was based only on systemic infections and did not include other infectious processes. Also, in Hospital 4 the study corresponded to only three infectious agents, which had been selected because they were considered to be indicative of the situation. In any case, all four institutions were found to have resistance to the drugs of choice, multiple resistance, and a variety of endemic pathogens.

The information obtained in this study is barely a starting point toward the attainment of our objectives. This is the first time that a standard-setting reference institution such as the Public Health Laboratory Department has brought together properly validated information, analyzed it, and made it available at the operational level. The study demonstrated not only that it is feasible to organize a surveillance system, but the need to have such a system. In the current situation a surveillance system should be viewed as a high-yield investment, whose cost is very low compared to the costs involved in nosocomial infections. These arguments more than justify following the recommendations of the ad hoc commission of the Pan American Association of Infectious Diseases *(24)*, which highlight the need to increase human resources for drug surveillance and for efforts aimed at standardizing the tests, as well as the antibiotics to be tested and reported on.

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ANTIMICROBIAL RESISTANCE: A PERSPECTIVE FROM THE UNITED STATES

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Antimicrobial resistance is a problem of great concern in the United States and the world. For example, in some areas of the United States, up to 30% of infections with Streptococcus pneumoniae *are no longer susceptible to penicillin. In some intensive care units, 28% of bacteria that cause hospital infections are resistant to the preferred antibiotic treatment, and many strains of* Staphylococcus aureus *are now resistant to all antibiotics, except vancomycin.*

This section explores strategies to prevent the further spread of antimicrobial resistance of different infectious agents, and discusses a Public Health Action Plan to Combat Antimicrobial Resistance, including the surveillance system, data needs, and the translation of the latter into useful information for prevention and control. An appendix provides a summary of current CDC surveillance projects which focus on antimicrobial resistance.

Antimicrobial resistance is a serious problem in the United States and globally, involving bacteria, parasites, viruses, and fungi. In some areas of the United States, up to 30% of infections with *Streptococcus pneumoniae*, the most common cause of bacterial pneumonia, meningitis, and ear infections, are no longer susceptible to penicillin *(1, 2)*. Data from intensive care unit patients in the United States indicate that 28% of the bacteria that most frequently cause hospital-acquired infections are resistant to the preferred antibiotic for treatment *(3–5)*. One organism of particular concern is *Staphylococcus aureus*; many strains are now resistant to every available antibiotic except vancomycin. In 1996 a strain of *S. aureus* with decreased susceptibility to vancomycin was identified for the first time in a hospitalized child in Japan *(6)*. Similar strains have subsequently been reported in individual patients in the United States of America *(7)*, in Hong Kong, and in Europe *(8)*. In the United States, resistance has also been a problem for pathogens that cause tuberculosis *(9)*, gonorrhea *(10)*, AIDS *(11)*, salmonellosis *(12)*, and other common infections.

Strategies to prevent the development and spread of antimicrobial resistance differ for different pathogens. One common theme is that antimicrobial drug use exerts selective pressure favoring the emergence of resistance. For bacterial respiratory tract pathogens (e.g., *Streptococcus pneumoniae*) controlling outpatient antimicrobial use is critical; for some enteric pathogens (e.g., *Salmonella*) limiting an-

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timicrobial use in animals is important; and for pathogens that cause nosocomial infections, improving inpatient antimicrobial use and adhering to appropriate infection control practices are necessary. Addressing antimicrobial use and resistance is a priority in the United States Centers for Disease Control and Prevention (CDC) plan for confronting emerging infectious disease threats. The plan encompasses four goals: improving surveillance and response capacity, addressing applied research priorities, strengthening the nation's public health infrastructure, and enhancing prevention and control programs required to control emerging and reemerging infectious diseases (13). Additional information is available on CDC's antimicrobial resistance webpage at: *www.cdc.gov/ncidod/ar/*.

In 1999 an Interagency Task Force on Antimicrobial Resistance, comprising 10 federal agencies and departments and co-chaired by the Centers for Disease Control and Prevention, the Food and Drug Administration, and the National Institutes of Health was formed. The Task Force is developing a Public Health Action Plan to Combat Antimicrobial Resistance, with input from expert consultants and the public. The plan will address issues related to surveillance, prevention and control, research, and product development. Part 1 of the Plan, currently being developed, focuses on domestic issues—i.e., actions to address drug resistance within the United States. Subsequently, the Task Force will consider actions by United States federal agencies needed in collaboration with international partners to address drug-resistance issues on a global basis. When completed, the plan will serve as a blueprint for coordinated United States federal action to address antimicrobial resistance.

Goals of antimicrobial resistance surveillance include providing timely, relevant information to assist in therapeutic decisions, identifying priorities for new drug development, and targeting prevention and control measures. Necessary surveillance data include microbiologic data to quantitate levels of drug resistance, epidemiologic data on the patients with resistant infections to help identify risk factors, and clinical data to assess the severity and public health impact of resistant infections. Data are also needed to monitor trends in risk factors, notably antimicrobial drug use. Since resistance involves diverse pathogens requiring different prevention and control measures, CDC's strategy for national antimicrobial resistance surveillance involves multiple components. At present, these include projects for surveillance of resistance in nosocomial infections, foodborne pathogens (in collaboration with the Food and Drug Administration [FDA] and the U.S. Department of Agriculture [USDA]), gonococci, pneumococci, *Mycobacterium tuberculosis*, human immunodeficiency virus, and malaria, and, to a limited extent, outpatient antimicrobial drug prescribing (Appendix).

Surveillance systems require expansion and strengthening, both domestically and internationally. Several issues must be addressed, including which "bug-drug combinations" should receive particular attention, the amount of epidemiologic, clinical, and drug use data that should be collected, and optimal sampling strategies. For some pathogens, sentinel systems may suffice, but for pathogens in which resistance rates may vary considerably within a single geographic area (e.g., pneumococci), a population-based approach may be necessary. Of particular importance are standardization and quality control of clinical laboratory methodologies to ensure the timely and accurate identification of organisms and detection and reporting of resistance. In the United States and many other countries, there is variability in the capabilities and performance of clinical laboratories. Modern trends in health-care delivery in the United States may facilitate or hinder surveillance. Large databases acquired by networks of health-care organizations, including managed care organizations, and improvements in electronic data standardization and transmittal will be beneficial. On the other hand, in some cases, cost-containment pressures have led to fewer cultures being obtained and

less susceptibility testing, to the detriment of surveillance. Another issue to be addressed is how to coordinate or combine the several different surveillance systems currently in place, including systems in both the public and private sectors *(14)*.

Priorities for applied research include identifying the molecular basis of antimicrobial resistance and epidemiologic risk factors associated with its development and spread, developing new and improved diagnostic laboratory tests, and in collaboration with other agencies and private industry, assessing the role of new vaccines and orphan drugs in preventing and controlling the spread of resistant infections. As part of the implementation of the CDC Emerging Infections Plan, an extramural research program was recently established.

Perhaps the most daunting challenge is translating information from surveillance and research into practical public health prevention and control measures. A key element of the prevention strategy is ensuring that antimicrobial agents are used judiciously. The use of antimicrobial agents is determined by the policies and practices of numerous individuals and organizations. These include clinicians, patients, health-care institutions, managed care organizations, clinical laboratories, pharmacists, pharmaceutical companies, agricultural producers, public health agencies, regulatory agencies, and public agencies and private companies that pay for medical care. A public health approach to influencing these diverse groups encompasses several components, such as educational interventions to improve prescribing practices among clinicians and to promote more realistic expectations among patients. Clinical practice guidelines may be helpful if actively promoted and perceived by clinicians as a resource developed with their input. Adherence to practice guidelines may be evaluated by indicators of appropriate antimicrobial use in quality of care assessments. Cost-benefit analyses of judicious antimicrobial use are likely to be particularly important in affecting antimicrobial use. As a supplement to voluntary measures, more direct controls may also need to be considered, including reimbursement policies that do not support inappropriate antimicrobial use, as well as formulary controls and regulatory actions. Finally, public health systems at local, state, federal, and international levels, which have in many cases atrophied due to cost-cutting pressures in recent years, must be afforded sufficient resources to ensure appropriate surveillance and response and prevention and control activities.

Recent increases in antimicrobial resistance are cause for alarm, but not pessimism. Improving prescribing practices and decreasing the spread of antimicrobial resistance can be accomplished. The CDC, in partnership with State health departments, health-care delivery organizations, and professional societies, has begun to evaluate additional interventions to decrease antimicrobial use and, through surveillance, to measure the impact on antimicrobial resistance. The rapid spread of resistance, however, demands an immediate and aggressive response. All practicing clinicians can assist by examining their practices and identifying where improvement in diagnostic methods or communication with patients concerning the lack of benefit, potential patient side effects, and development of drug resistance may decrease unnecessary antimicrobial use. Professional societies, health-care delivery organizations, and the CDC can provide materials to assist in this process. By forming effective partnerships involving clinicians, public health officials, and patients, we can prolong the effectiveness of currently available antimicrobial drugs and reduce the threat of antimicrobial resistance for patients today and in future generations.

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APPENDIX: LIST OF CDC SURVEILLANCE PROJECTS WITH A MAJOR FOCUS ON ANTIMICROBIAL RESISTANCE

1. Pneumococcal disease

The Division of Bacterial and Mycotic Diseases has conducted surveillance for invasive pneumococcal disease, including drug resistant infections, since 1978. The first system was a voluntary sentinel surveillance system designed to assess serotype distribution and antimicrobial susceptibility. Over time, the system has involved 12–54 hospitals in 11–26 states. With this system, researchers at participating hospitals collect all pneumococcal isolates from sterile sites along with some demographic and clinical information. The isolates are sent to CDC for serotyping and susceptibility testing. In 1994, the Division began active, population-based surveillance for invasive pneumococcal disease. This system currently includes sites in eight states (total population of 16.5 million) and is administered, in part, through the Emerging Infections Program. Surveillance personnel in each site have regular contact with all microbiology laboratory directors or hospital infection control practitioners in their areas to detect patients with *Streptococcus pneumoniae* isolated from blood or other sterile sites. Clinical isolates are collected and undergo antimicrobial susceptibility testing at reference laboratories. Surveillance personnel complete a standardized report form for each case-patient. In addition to surveillance, the system provides an infrastructure for epidemiologic and microbiologic studies.

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2. Nosocomial infections

The Hospital Infection Program's (HIP) National Nosocomial Infections Surveillance (NNIS) system reports antimicrobial susceptibility profiles for nosocomial infections detected through active surveillance in approximately 260 voluntarily participating hospitals. In a subset of approximately 40 hospitals, additional data are collected from the microbiology laboratories and pharmacies in a special study (Project ICARE) conducted in collaboration with Emory University School of Public Health. Objectives of this project include more detailed examination of the relationship between antimicrobial use and antimicrobial resistance and early detection of new resistance mechanisms by establishing a sentinel network of microbiology laboratories in NNIS hospitals that can identify and send specific antimicrobial-resistant isolates to CDC. Both the NNIS system and Project ICARE provide hospitals with semi-annual reports containing data and methodology to make comparisons of nosocomial infection and antimicrobial usage patterns among hospitals. This allows hospital personnel to respond quickly to excessive infection, resistance, or usage rates in efforts to reduce antimicrobial resistance. [Contact person: Scott Fridkin, MD, Hospital Infections Program, National Center for Infectious Diseases, CDC, Atlanta GA 30333; phone: (404) 639-6436.]

In collaboration with several European investigators, CDC has also initiated an International Surveillance Program for Emerging Antimicrobial Resistance (INSPEAR) in

nosocomial infections. This project includes standardization of antimicrobial susceptibility testing and linkage of epidemiologic and laboratory data to assess the efficacy of surveillance and interventions in preventing the development and spread of antimicrobial resistance in hospitals. Thirty hospitals in France, Germany, Slovenia, Poland, Switzerland, United Kingdom, and the United States have joined INSPEAR to date and others are being recruited. [Contact person: Herve Richet, MD, Hospital Infections Program, National Center for Infectious Diseases, CDC, Atlanta GA 30333; phone: (404) 639-6413.]

3. Foodborne zoonotic pathogens

The National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996 by the U. S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and the U. S. Department of Agriculture (USDA) to monitor changes in antimicrobial susceptibilities of foodborne zoonotic pathogens in humans and animals. Goals include providing timely information to prolong the life span of approved drugs by promoting the prudent use of antibiotics, identifying areas for more detailed investigation, and guiding research in the area of antibiotic resistance. Antimicrobial susceptibilities to 15 antimicrobial agents are determined from randomly selected *Salmonella*, *Escherichia coli* O157:H7, and *Campylobacter* isolates from humans and animals. Human isolates are obtained from clinical specimens forwarded by clinical laboratories to the public health laboratories of 14 participating state and local health departments. Animal isolates are obtained from several sources including clinical specimens submitted to the National Veterinary Diagnostic Laboratory, surveys of healthy animals, and isolates collected at slaughter. [Contact person: Fred Angulo, DVM, PhD, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, CDC, Atlanta GA 30333; phone: (404) 639-2840.]

4. Gonorrhea

The Gonococcal Isolate Surveillance Project (GISP) was established in 1986 to monitor trends in antimicrobial resistance in *N. gonorrhoeae* in the United States and to establish a rational basis for the selection of gonococcal therapies. Data and isolates are collected from Sexually Transmitted Disease (STD) clinics in 26 cities. Data from this project have been used to revise the CDC's STD Treatment recommendations in 1989, 1993, and 1997. The specific objectives of GISP are (1) to monitor trends in antimicrobial susceptibilities in *N. gonorrhoeae* and (2) to characterize phenotypically antimicrobial-resistant isolates to describe the diversity of antimicrobial resistance in *N. gonorrhoeae*. [Contact person: Joan Knapp, PhD, Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, CDC, Atlanta GA 30333; phone: (404) 639-2840.]

5. *Helicobacter pylori*

Helicobacter pylori is a leading cause of peptic ulcer disease and chronic active gastritis. The *H. pylori* antimicrobial resistance monitoring project was established in January 1999 by CDC to determine the prevalence of *H. pylori* antimicrobial resistance in the United States and monitor changes in antimicrobial resistance. Goals of this project are to identify risk factors associated with the isolation of antimicrobial resistant *H. pylori* strains, determine the clinical outcome of patients infected with *H. pylori* resistant strains, and utilize data obtained from this project to help optimize future treatment strategies for *H. pylori* infected patients. Antimicrobial susceptibilities are determined from *H. pylori* isolates obtained from patients undergoing upper endoscopy at 10 centers in the United States. [Contact person: Cindy R. Friedman, MD, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, CDC, Atlanta GA 30333; phone: (404) 639-2206.]

6. Tuberculosis

The antimicrobial susceptibility profile of the isolate from every culture positive case of tuberculosis diagnosed in the United States is reported, along with epidemiologic and clinical data to State Health Departments, which in turn transit the information to CDC. [Contact person: Eugene McCray, MD, Division of Tuberculosis Elimination, National Center for HIV, STD, and TB Prevention, CDC, Atlanta GA 30333; phone: (404) 639-8117.]

7. Human immunodeficiency virus

During 1998, CDC began conducting antiretroviral drug susceptibility testing conducted on HIV strains obtained from groups of patients believed to have recently acquired infections and/or for whom recommendations for drug prophylaxis to prevent transmission have been made. These include adult seroconverters, adult patients beginning antiretroviral therapy, women and infants in perinatal transmission studies, and source-patients involved in health-care worker needlestick exposures. Contact person: Walid Heneine, PhD, Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, CDC, Atlanta GA 30333; phone: (404) 639-0218.]

8. Malaria

In the United States, surveillance for drug resistant malaria is passive; i.e., limited to reports of cases. In Africa, Peace Corps volunteers are followed prospectively to assess prophylaxis failure. In certain other countries, a sentinel system is in place to monitor treatment of ill patients to detect clinical failures which serve as a marker of resistance. [Contact person: Peter Bloland DVM, MPV Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta GA 30333; phone: (770) 488-7787.]

9. Antimicrobial drug use

CDC's National Ambulatory Medical Care Survey, conducted every 3 to 4 years, collects information on a sample of outpatient visits from a nationwide probability sample of office-based physicians. The survey provides data on patient diagnosis and medications prescribed, including antimicrobial drugs. A similar survey, the National Hospital Ambulatory Medical Care Survey, collects this information from hospital outpatient and emergency departments. [Contact person: Cheryl Nelson, MSPH, Division of Healthcare Statistics, National Center for Health Statistics, CDC, 6525 Belcrest Rd, Hyattsville, MD, 20782; phone: (301) 436-7132.]

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN BACTERIAL ENTEROPATHOGENS ISOLATED IN BRAZIL

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Bacterial resistance to antimicrobial drugs has been analyzed in Brazil since the 1950s. Cisalpino (*1*) described a progressive increase in the resistance of *Salmonella* strains isolated during the three-year period from 1947 to 1950. Later, Costa et al. (2) found 12% of *Shigella* spp. to be resistant to tetracycline and 9,8% of *Salmonella* spp. to be resistant to chloramphenicol in children treated in a University Hospital in Rio de Janeiro. Trabulsi et al. (*3*) detected multiresistance in nine *Salmonella* spp. strains isolated at hospitals in the state of São Paulo.

Studies carried out in the 1970s (*4–11*) revealed the progressive increase of simple or multiple resistance to different antimicrobial drugs in enteropathogens. Among these studies, the evaluations carried out by Costa and Hofer (*12*) and Hofer et al. (*13*) in *Salmonella typhi* strains isolated between 1923 and 1967 in different regions of Brazil revealed that resistance to chloramphenicol had increased since the introduction of this antibiotic into the country in 1953. Multiresistance to nine drugs in *S. typhimurium* (12%) was reported for strains isolated during the 1973 to 1980 period (*14*).

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In 1980, 94 laboratories from six Brazilian capitals participated in a project to evaluate the frequency of pathogens isolated from routine clinical material and their sensitivity to antimicrobial drugs. The data were collected and kept in a databank whose final analysis after 10 years (*15*) demonstrated a progressive increase of resistance and the need for clinicians, laboratories, and governmental organisms to be on the alert for this problem.

A directive published in 1993 by the Ministry of Health established that the Boards for the Control of Hospital Infections should also oversee the use of antibiotics in order to reduce bacterial resistance and, consequently, the number of nosocomial infections, especially among immunodepressed patients.

Parallel studies carried out in different regions of the country on strains isolated from human, food, and environmental sources showed simple or multiple resistance in enterobacteria from the indigenous microbiota, and in all *Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli*, as well as the transference capacity of these markers (*16–27*). Similar results were obtained by Solari et al. (22) in 240 strains of *Salmonella agona*, by Campos (*28*) in 658 salmonellas from different serovars, and by Reis (*29*) in an analysis of 948 isolates of *Salmonella enteritidis*. All strains used in

these studies were isolated from different sources and regions of the country.

Other interesting results refer to the detection of a high percentage of resistance in non-01 *Vibrio cholerae* strains isolated by Martins (30) from sewage waters in São Paulo and by Dias et al. (31) from a similar source in Rio de Janeiro, as well as in *V. parahaemolyticus* strains (97,2%) obtained from ocean fish. Asensi (32) reported resistance to nine antimicrobial drugs and the presence of conjugative plasmids in *Salmonella* spp. isolated in the period 1987–1992 from hospitalized patients in Rio de Janeiro.

With the reemergence of cholera in Brazil in 1991, the Ministry of Health reactivated the national reference laboratory at the Oswaldo Cruz Institute-FIOCRUZ through the Coordination of the Public Health Laboratories whose structure comprises a network of dependent laboratories acting all over the country. The municipal units send samples or isolates to the state units where the results are confirmed and forwarded together with their own strains to the macroregional units (Evandro Chagas Institute, Public Health Laboratory (LACEN) Dr. Milton Bezerra Sobral, Ezequiel Dias Foundation, Adolfo Lutz Institute, and the Federal District Health Institute). After additional tests, the macroregional units send the materials to the National Reference Center for final analysis.

Among the routine procedures performed at the National Reference Center are the surveillance of antimicrobial susceptibility as well as elaboration of half-yearly reports submitted to the Coordination of the Public Health Laboratories. Referenced analyses are carried out on all strains isolated and sent by the laboratories, thus allowing reliable characterization, particularly with respect to *V. cholerae* 01.

Since 1995, evaluations of antimicrobial susceptibility have been carried out according to the procedures established by the U.S. National Committe for Clinical Laboratory Standards (NCCLS). The Kirby & Bauer Method is applied to all strains of *Shigella* spp. and *Salmonella* spp. isolated from different sources and regions and, for *Aeromonas* spp. and non-01 *V. cholerae* isolates from human and animal sources. Also evaluated are the results of other strains received from public and private institutions and identified at this laboratory. As in the case of *V. cholerae* 01, the results obtained are reported half-yearly to the Ministry of Health.

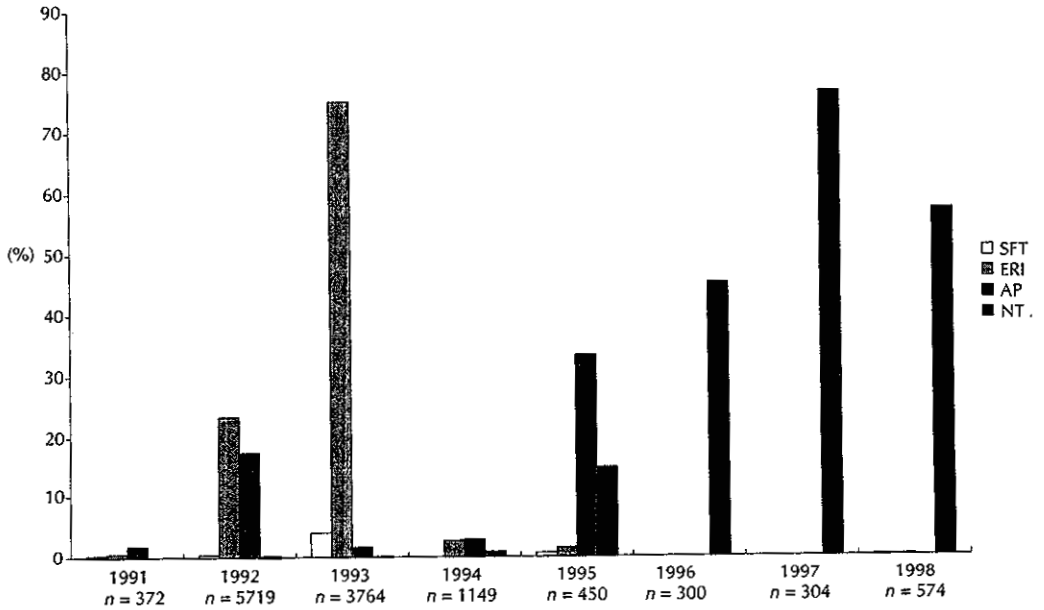
Erythromycin and/or ampicillin resistance were the prevalent profiles observed in *V. cholerae* 01 from 1991 to 1995 (Figure 1). Since 1996, the patterns of resistance have suddenly changed and all strains analyzed have been susceptible to erythromycin and ampicillin. However, high percentages of nitrofurantoin resistance have been detected.

Every year, about 7,000 strains of 50 to 60 different *Salmonella* serovars are isolated from humans, foods, the environment, animals, and foodstuffs or feed and identified. Routinely, the frequency and pattern of antimicrobial resistance are determined in 30% of the *Salmonella* strains isolated each year that are randomly selected from three different sources among all strains isolated from humans and food sources.

All *S. typhi* strains were susceptible to the antimicrobial drugs tested. The most important epidemiological agent identified was *S. enteritidis*, whose distribution is fairly wide in the food chain and whose resistance profile reflects lower selective pressure. Among all other *Salmonella* serovars, results indicated growth of the major antimicrobial resistance profiles observed year after year, particularly to multiple antibiotic drugs (Table 1). Those characteristics show that complex medical, socioeconomic, and political issues play a largely unmeasured but extensive role in the spread of antibiotic resistance.

In *Shigella* spp., 92.2% of strains showed resistance. A significant increase in their resistance to ampicillin, chloramphenicol, and trimethoprim-sulphametoxazole has become evident since 1996 (Table 2) and also in *Aeromonas* spp. (Table 3) isolated from humans and animals as well as from the aquatic environment and food sources. Data available on

FIGURE 1. Resistance profile in *V. cholerae* 01 isolated from different sources in Brazil, 1991–1998



SFT = trimethoprim-sulfamethoxazole; ERI = erythromycin; AP = ampicillin; NT = nitrofurantoin.

TABLE 1. Resistance profile among strains of *Salmonella* isolated from different sources and localities in Brazil, 1996–1998

Antimicrobial resistance profile	*S. agona*			*S. enteritidis*			*S. hadar*			*S. infantis*			*S. typhimurium*			*Total*		
	96	97	98	96	97	98	96	97	98	96	97	98	96	97	98	96	97	98
AP				1	2	6							1			2	2	6
CO															1			1
CRX	1			4	2	3										5	2	3
GN	1					25				1						2		25
SFT			2	1	1	1										1	1	3
TB					17	23											17	23
TT	3		6	7	11	6	22	18	10				1	4	7	33	33	29
CO-SFT	1	1	1	2	1											3	2	1
AP-CO													1			1		
AP-CF					1												1	
AP-CRX					1												1	
AP-SFT					1	1											1	1
AP-TT				1			2		3						1	3		4
CO-TT						2	1									1		2
CO-CFO								1									1	
CRX-CFO				1												1		
GN-TT				2		1										2		1
GN-TB			2	2		12										2		14
SFT-TT				1	1										1	1	1	1
TT-TB														1			1	
CIP-CO-CRX				1												1		
AP-CO-GN					1												1	
AP-CO-SFT														1			1	
AP-CF-CFO				1									1			2		
AP-CF-TT									1									1
AP-GN-CRX												1					1	
AP-SFT-TT				1				1								1	1	

TABLE 1. *(continued)*

Antimicrobial resistance profile	*S. agona*			*S. enteritidis*			*S. hadar*			*S. infantis*			*S. typhimurium*			*Total*		
	96	97	98	96	97	98	96	97	98	96	97	98	96	97	98	96	97	98
CF-CFO-CO					1												1	
CF-CFO-CRX				1	1											1	1	
CO-TT-SFT										1						1		
SFT-TT-TB															1			1
AP-CRX-CF-CFO				2												2		
AP-CRX-CO-GN											1						1	
AP-SFT-TT-TB					1												1	
AP-CO-SFT-TT				1												1		
AP-CRX-CO-SFT					2						4						6	
CF-CRO-IPM-NT																	4	
AP-CF-CRO-CRX-TB		4															4	
AP-CO-CF-GN-TT-TB	1															1		
AP-SFT-CO-CFO-CRX-TB	1															1		
AP-GN-CRX-CO-CF-TT-TB	3			3												6		
AP-CO-CF-CFO-CRX-GN-TB-CRO												1						1
AP-GN-CRX-CO-CF-CFO-TT-TB				1												1		
AP-SFT-GN-CRX-CO-CF-TT-TB	1															1		
AP-SFT-GN-CRX-CO-CF-CRO-TT-TB										6						6		
Total	12	5	11	33	44	80	25	20	14	8	6	1	4	6	11	82	85	117

AP = Ampicillin; CO = Chloramphenicol; CF = Cefalotin; CRX = Cefuroxime; CRO = Ceftriaxone; CFO = Cefoxitin; GN = Gentamicin; TT = Tetracycline; TB = Tobramycin; SFT = *Trimethoprim-sulfamethoxazole;* CIP = Ciprofloxacin; IPM = Imipenem

TABLE 2. Antimicrobial resistance pattern in *Shigella* spp. isolated from different sources and localities in Brazil, 1996–1998

Antimicrobial resistance profiles	Isolation year		
	1996	1997	1998
TT	1	–	1
SFT	–	–	11
AP-SFT	–	–	1
AP-ERI	1	–	–
TT-SFT	–	1	14
CO-TT	–	–	1
AP-CO-SFT	1	–	1
AP-TT-SFT	1	–	2
TT-SFT-ERI	–	1	–
AP-TT-CO	–	–	2
CO-TT-SFT	–	–	1
AP-CO-GN-SFT	–	–	1
AP-CO-TT-ERI	–	1	–
AP-CO-TT-SFT	2	1	34
AP-CO-GN-SFT	–	–	1
CO-TT-SFT-ERI	–	1	–
AP-CO-TT-SFT-ERI	4	8	–
AP-CO-NT-TT-SFT	–	–	1
AP-CO-TT-SFT-NT	–	–	1
Total resistant strains	10	13	72
Total analyzed strains	10	13	80
Total strains	46	50	80

AP = Ampicillin; CO = Chloramphenicol; CF = Cefalotin; CRX = Cefuroxime; CRO = Ceftriaxone; CFO = Cefoxitin; GN = Gentamicin; TT = Tetracycline; TB = Tobramycin; SFT = *Trimethoprim-sulfamethoxazole*; CIP = Ciprofloxacin; IPM = Imipenem

TABLE 3. Antimicrobial resistance profile in *Aeromonas* spp. isolated from different sources and localities in Brazil, 1996 to 1998

Antimicrobial resistance profile	Human			Food			Environment			Animal			Total		
	96	97	98	96	97	98	96	97	98	96	97	98	96	97	98
AP	14	26	30	–	–	10	15	–	–	–	–	18	29	26	48
SFT	4	1	1	–	–	–	2	–	–	–	–	–	6	1	1
NT	1	–	3	–	–	–	2	–	1	–	–	–	3	–	4
AP-SFT	2	4	7	–	–	–	9	–	–	–	–	–	11	4	7
AP-SFT-CO	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
AP-SFT-CO-NT	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1
Total strains	21	31	43	–	–	10	28	–	1	–	–	18	49	31	62

AP = Ampicillin; CO = Chloramphenicol; CRX = Cefuroxime; CRO = Ceftriaxone; CFO = Cefoxitin; GN = Gentamicin; TT = Tetracycline; TB = Tobramycin; NT = Nitrofurantoin; CIP = Ciprofloxacin; SFT = Trimethoprim-sulfamethoxazole; IPM = Imipenem.

the incidence of antibiotic resistance in Brazil probably represent only the tip of the iceberg and this means, therefore, that significant solutions are sorely needed.

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SECTION TWO

USE OF ANTIMICROBIAL AGENTS AND FACTORS LEADING TO THEIR CONSUMPTION

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THE USE OF ANTIBIOTICS TO PREVENT INFANT MORTALITY[1]

Yehuda Benguigui[2]

Antibiotics are essential for the treatment of many infectious diseases. They can keep such diseases from becoming worse and can prevent a probably fatal outcome. Since their introduction, these drugs have made it possible to save countless lives all over the world, helping to alter the trend in the rates of serious morbidity and mortality.

The use of antibiotics to treat children is even more important, because a large number of infectious diseases occur during the early years of life. Both acute respiratory infections (ARI) and diarrhea are included among the major infectious diseases due to their high incidence, but they are different in that diarrhea can be easily prevented with basic sanitation measures and good hygiene, and its treatment only occasionally requires antibiotics (for example, for the treatment of bloody diarrhea caused by Shigella). However, antibiotics are essential to prevent a worsening of the patient's condition and mortality from pneumonia, which is responsible for more than 80% of all deaths from ARI in children under 5 years of age in many developing countries.

On the other hand, antibiotics are frequently used unnecessarily and inappropriately, leading to increased bacterial resistance to said drugs. Bacterial resistance has led to frequent changes in treatment protocols. It has also created the need for ongoing investment in know-how and technology to develop new drugs to overcome antimicrobial resistance.

The strategy for Integrated Management of Childhood Illness (IMCI), developed by PAHO/WHO and UNICEF and suggested as a way of addressing health care for children seeking services at the primary health care level, is designed to prevent and control the most frequent illnesses in this population, among which infectious diseases are significant. The strategy seeks to rationalize the prescription of antibiotics and improve their use by health workers and the community alike. It is hoped that implementation of IMCI in all countries will lead to improvements in the use of this powerful tool for preventing serious morbidity and mortality and contribute to a reduction in infant mortality in most of the world's developing countries.

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INTRODUCTION

Each year some 500,000 children under the age of 5 years die in the Region of the Americas. Half of these deaths are due to a group of diseases that include acute respiratory infections (ARI), especially pneumonia, and diarrheal diseases. Prevention and control measures are currently available for both problems—for example, the timely and appropriate administration of antibiotics, especially in the treatment of pneumonia.

The use of antibiotics to treat diseases has helped to reduce serious morbidity and mortality among children. Unfortunately, this benefit has not been accessible to many children who need it in most developing countries. The lack of access to health services or health workers, frequent shortages of these drugs, and their inappropriate use are factors that, separately or in combination, have limited the impact of antibiotics on serious morbidity and mortality in children.

Acute respiratory infections are among the leading causes of mortality in children under the age of 5 years. Pneumonia is responsible for over 80% of deaths from such infections and can be treated with antibiotics. Nevertheless, the lack of timely access to antibiotics is a problem for a large part of the population, and it keeps children from benefiting from these drugs.

The inappropriate use of antibiotics to treat illness in children limits the availability of drugs for cases that truly require them. It also contributes to growing bacterial resistance to these drugs, and unnecessarily increases the costs of care, for both the health services and the family of the child involved.

Under these circumstances, monitoring bacterial resistance to antibiotics becomes twice as important. First, it allows us to continuously assess the impact of the inappropriate and excessive use of antibiotics, such as reduced effectiveness, and to determine the need for research and the development of new drugs. Secondly, the results of monitoring are used to issue recommendations on antibiotic prescription and treatment practices to guarantee the effectiveness of these drugs in treating illness in children.

Use of the results of monitoring to improve practices in the prescription and use of antibiotics is, in turn, essential for reducing mortality in children under 5 years of age due to the persistence of many infectious diseases.

MAGNITUDE AND CHARACTERISTICS OF MORTALITY FROM SOME CHILDHOOD ILLNESSES IN THE REGION OF THE AMERICAS

In this Region, approximately 40% of deaths (out of 500,000) in children under 5 are from a group of illnesses for which prevention measures, early diagnosis, and treatment are available.

Acute respiratory infections (ARI) and diarrhea are responsible for most of these deaths, and currently represent 27% of all mortality each year among children in this age group (*1, 2*). Other infectious diseases, such as malaria and vaccine-preventable diseases, cause nearly 4% of the deaths in this group, as do septicemia and meningitis.

Many of these deaths are associated with malnutrition, an extremely important risk factor in that it seriously affects the ability of the child's body to fight off disease and worsens the prognosis and evolution of clinical symptoms.

However, the distribution of these deaths is not even. An analysis of the situation by country reveals major contrasts. While in some countries this group of diseases is responsible for less than 5% of all deaths in children under 1 year of age, in others, the burden of these diseases is 10 times higher, and one out of every two infant deaths is due to one of these causes. The contrast seen in the burden of mortality from these causes among children aged 1 to 4 is even greater: While in some countries of the Americas the proportion of deaths from acute respiratory infections and diarrheal diseases is 8%, in others it is between 60% and 70%.

In both age groups, mortality from ARI and diarrheal diseases is closely related to the countries' infant mortality rates (IMRs), for which they are a major determinant. For example, in Canada and the United States, the two countries of the Americas with the lowest infant mortality rates, ARI and diarrheal diseases together cause 5% or less of infant deaths. However, in countries with infant mortality rates over 40 per 1,000 live births, ARI and diarrheal diseases are responsible for more than 30% of deaths among children under 1 year of age and more than 45% among children aged 1 to 4 years.

In those countries that fall between these two extremes, we find an intermediate situation, which points to the differences that still exist among developing countries. In countries with infant mortality rates of between 20 and 40 per 1,000 live births, these diseases represent between 20% and 40% of mortality among children aged 1 to 4 years, and less than 30% of mortality among children under 1 year of age. Nevertheless, among developing countries with infant mortality rates between 10 and 20 per 1,000 live births, ARI and diarrheal diseases account for less than 20% of deaths both in infants and children between the ages of 1 and 4 years.

These mortality figures are affected by underreporting, which is higher in countries where the infant mortality rates are also higher. For example, while the number of registered and estimated deaths from diarrhea and pneumonia and influenza is similar in countries with infant mortality rates below 20 per 1,000 live births, in countries where the IMR is over 40 per 1,000, the number of estimated deaths is more than twice the number of registered deaths. In countries where the IMR is between 20 and 40 per 1,000 live births, the difference between the number of registered and estimated deaths is greater than in the first group. It is estimated that the number of registered deaths is less than 50% of estimated mortality (Figure 1).

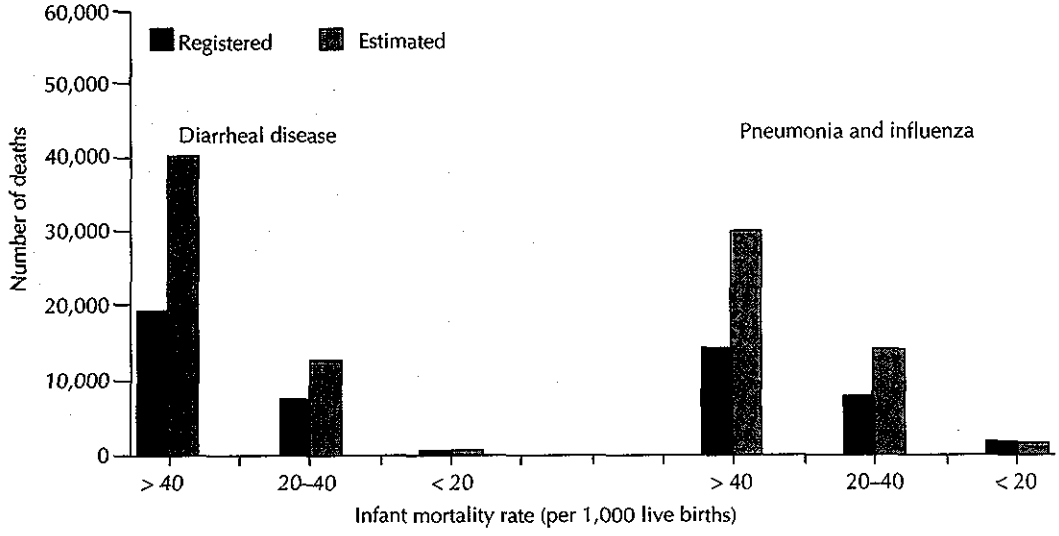
The fact that ARI and diarrheal diseases cause a lower percentage of mortality in some developing countries indicates, on the one hand, that it is possible to take steps to reduce deaths from those causes. On the other hand, it also points to the impact that reducing these diseases can have on infant mortality. Both aspects reinforce the need to support effective measures to control these problems as a way of contributing to a rapid reduction in infant mortality in the Americas. These actions, in turn, can help to improve equity in children's health conditions.

THE BURDEN OF MORBIDITY FROM SOME CHILDHOOD ILLNESSES IN CHILDREN UNDER 5 YEARS OF AGE

Various surveys in developing countries *(3–9)* reveal the frequency with which ARI and diarrhea, the two principal causes of mortality from childhood illness in children under 5 years of age, affect health. Some 17% to 24% of the families visited reported having had children under 5 years who had had a cough and difficult breathing in the two weeks prior to the survey. In addition, between 8% and 30% of families reported having had children who suffered diarrhea at some time during that period (Figure 2). In addition to being the two most frequent causes of disease among children in most developing countries, ARI and diarrhea contribute to a deterioration in their nutritional status, as a result of the difficulties that families encounter in feeding sick children. A cycle is thus created that increases the risk factors for a worsening of the condition and for mortality of the affected child *(10)*.

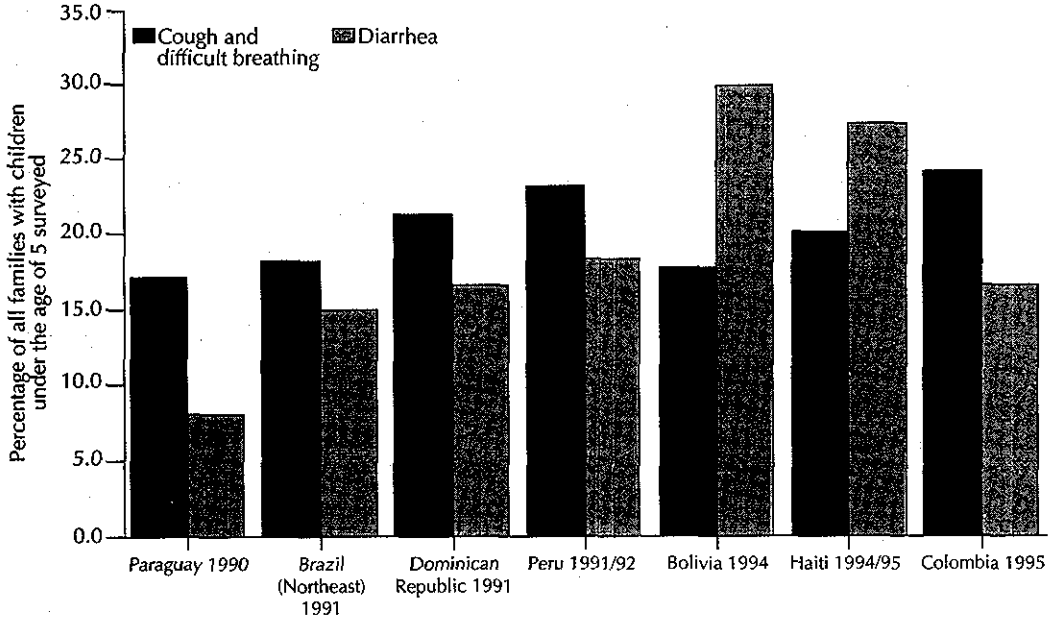
Another way to analyze the importance of ARI and diarrhea as causes of disease in children is by looking at the proportion of visits that these conditions represent in terms of total pediatric visits to the health services. In Peru, for example, visits for ARI and diarrhea account for approximately 90% of total care in the health services (Figure 3). Although this percentage is lower in other countries, the two causes represent approximately half of all visits to the health services for children at this

FIGURE 1. Registered and estimated number of deaths from pneumonia and influenza and from diarrhea in children aged 1 week to 11 months, in countries of the Americas, grouped by estimated infant mortality rate, around 1995



Source: Health Situation Analysis Program, Division of Health and Human Development; Communicable Diseases Program, Division of Disease Prevention and Control, PAHO/WHO.

FIGURE 2. Percentage of children who had an episode of cough and difficult breathing or diarrhea in the two weeks prior to the survey, selected countries of the Americas, 1990–1995



Source: *Demographic and Health Surveys.*

age. ARI and diarrhea are also responsible for a significant number of hospital admissions. For example, in Ecuador, these diseases represent some 50% to 60% of all hospitalizations of children under 5 years of age.

Another way to assess the importance of infectious diseases as causes of morbidity in the population is to examine the burden of disease that they represent for the population *(11)*, expressed in disability-adjusted life years (DALY) (Figure 4).

Acute respiratory infections and diarrhea are the two infectious diseases that cause the highest number of disability-adjusted life years, followed by AIDS, which ranks third.

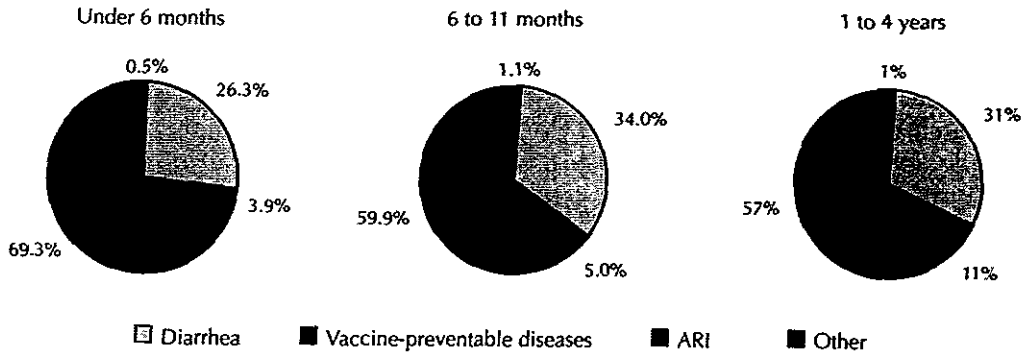
ACCESS TO CARE

ARI and diarrhea are responsible for a high percentage of outpatient visits and hospital admissions among children. This reveals the importance of providing adequate access to care in order to prevent more severe episodes and the death of many children. In developing countries of the Region, the population's access to health care varies, and there are sectors that have neither health services nor workers at a reasonable distance from their homes. Furthermore, even when care is available, many health services and workers do not have the capacity to respond to the diagnostic and treatment needs of the cases they handle.

Access to health care for children and the characteristics of the services to a large extent define the final outcome of episodes of ARI and diarrhea, because they determine patients' ability to receive timely, adequate treatment for their illness.

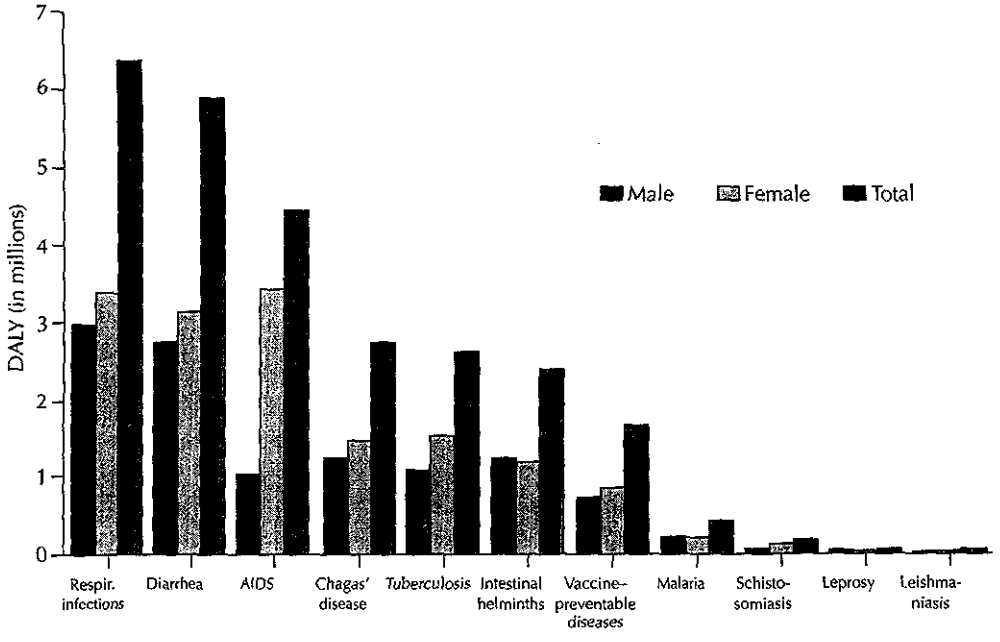
It is estimated that approximately one third of the population in the Americas does not have access to health services. This makes care difficult in terms of implementing preventive measures and providing early treatment of illnesses and health problems. Lack of access to services keeps many children from receiving care from health workers when they need it due to an episode of disease. These difficulties are directly related to a greater incidence of serious morbidity and mortality from avoidable causes. Both are seen more frequently in developing countries when compared to developed countries. These differences are also seen in comparisons among different regions within a single country, whether developing or industrialized.

FIGURE 3. Percentage distribution of consultations by children under 5 years of age, by cause, Peru, 1992



Source: Instituto Nacional de Estadísticas e Informática. *Compendio Estadístico 1993–1994*, Peru, July 1994.

FIGURE 4. Global burden of disease, in disability-adjusted life years (DALY), associated with communicable diseases, Latin America and the Caribbean, 1990



Deaths in the home can be considered the most serious event and are due to a set of circumstances that include factors related to the family caring for the child and the opportunities provided to seek care for the illness. The two aspects should be considered together, since the alarm that leads the family to seek care outside the home is affected by the difficulties the family must face when seeking care.

When there are no health services or health workers close to home, the family will delay the search for care. The family will do the same thing if the hours of operations are restricted, if waiting periods for service are long, if there is a history of a lack of drugs or bad outcomes from previous visits, if there are economic or cultural barriers, or if patients are treated badly.

The magnitude of mortality in the home has been studied in several countries. It has been observed that care-seeking behavior is affected not only by the distance between the home and the health services or health workers, but also by difficulties in obtaining timely and appropriate access to care when the family needs it. In an urban area of Argentina, deaths at home accounted for 32% of total deaths among children under 5 years of age, and in an urban area of Fortaleza, Brazil, the percentage of deaths at home was nearly 50%. In both studies, diarrhea and acute respiratory infections were the most frequent causes of death at home, according to an assessment based on the signs and symptoms described by the family (Figure 5). Many of these deaths could have been prevented if the children had had timely access to treatment.

Some community surveys (3–9) evaluated the attitude of parents and family members about seeking care outside the home for children with ARI. In general, less than half of the children studied with episodes of cough or

difficult breathing in the two weeks prior to the survey had been taken to the health services by their parents for assessment and treatment (Figure 6). This proportion was very low in some countries. In Haiti, for example, only 16% to 34% of the children with cough or difficult breathing were taken to the health services for consultation.

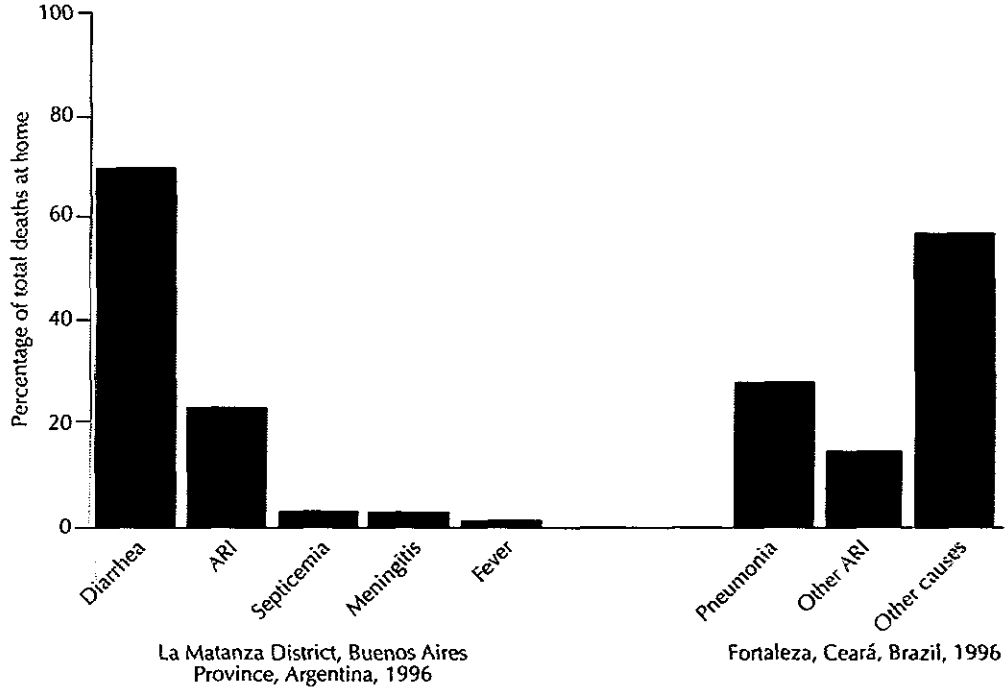
Not seeking care outside the home is not necessarily a poor decision on the part of the family, especially since the vast majority of episodes of cough in children under 5 years of age evolve favorably after a few days with only symptomatic treatment administered at home. However, some cases of ARI, especially those accompanied by difficult breathing, may be caused by pneumonia, and require timely treatment by health workers. If such care is not provided, the severity of the illness and mortality are greater.

USE OF ANTIBIOTICS TO TREAT CHILDHOOD ILLNESS IN THE HEALTH SERVICES AND COMMUNITY

Many children who could benefit from treatment with antibiotics do not receive these drugs due to limited access.

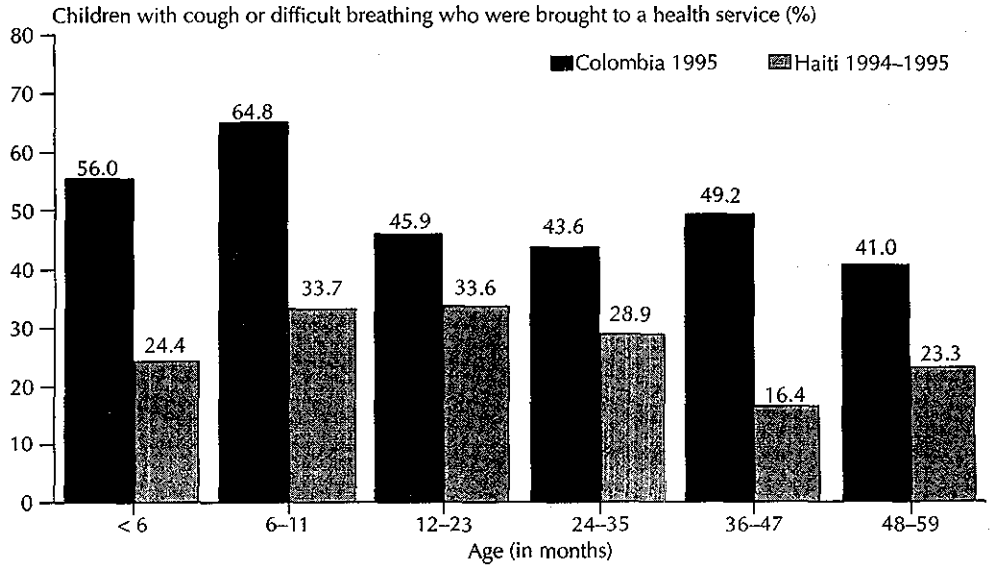
Most cases of ARI that come to the health services are treated with antibiotics, although these drugs may not be required for the favorable evolution or cure of the episode. Of all children consulting for ARI, some 40% to 60% customarily are treated with antibiotics, and this percentage exceeds 70% in many health services. Only a very small percentage of these antibiotics is used to treat pneumonia and otitis in children under the age of 5 years, and these are the two causes of ARI that most frequently require antibiotics, since streptococcal pharyngitis is rare under the age of 4 years. Thus, the

FIGURE 5. Principal causes of death at home in children under the age of 5 years. Studies conducted in Argentina and Brazil, 1996



Source: A. Barbato et al. (Buenos Aires, Argentina, 1996); L.C. Rey et al. (Fortaleza, Ceara, Brazil).

FIGURE 6. Percentage of children under the age of 5 years, with cough or difficult breathing who were brought to a health service for treatment. Results of surveys conducted in Colombia, 1995, and Haiti, 1994–1995



Source: National Demographic and Health Surveys. Institute for Resource Development/Macro International, Inc.

antibiotics used daily in the health services to treat ARI are for the most part not necessary to halt the progress of the disease, nor do they help to prevent a worsening of the condition.

Table 1 presents the magnitude of unnecessary investment in antibiotics for treating episodes of ARI among children in the United States in 1996. Approximately 60% of all antibiotics used to treat these illnesses, or almost 50 million prescriptions, are considered unnecessary for achieving a favorable result in the evolution of the illness.

The unnecessary use of antibiotics does not only affect the final cost of care. Given that no drug is entirely innocuous, the unnecessary administration of these antibiotics to children under 5 years of age, often repeatedly, exposes children to the potential adverse effects of these drugs and contributes to the development of bacterial resistance. In addition, particularly in developing countries, the unnecessary prescription of antibiotics creates a scarcity of drugs for the management of cases that do in fact need them, and puts the child population at risk of more serious illness and death.

The magnitude of the problem of unnecessary antibiotic prescription is accompanied by a failure on the parents' part to adhere to a prescribed treatment. This occurs in cases where use of the drug is justified and in those where it will not produce any benefit to the patient. Efficiency studies of health services in Latin America have shown that only one out of every four people visiting the health services understands, albeit with limitations, the health workers' treatment instructions. In these studies, moreover, only 8% of children correctly received the prescribed treatment at home. In the remaining cases (i.e., 92%) the treatment was administered incorrectly because of dosage or dilution errors, the antibiotic was diluted, the course of the treatment given was shorter than indicated, or the quantity of the antibiotic administered was incorrect *(12)*.

All these factors indicate that even when children have access to health services and health personnel prescribe the necessary and

TABLE 1. Unnecessary use of antibiotics to treat acute respiratory infections among children under 5 years of age, estimates for the United States of America, 1996

Type of infection	No. of antibiotic prescriptions per annum	Percentage unnecessary	Reason for error
Ear infection	23 million	30%	It is not necessary to administer antibiotics to a child who has a cold and secretion behind the eardrum but no earache, inflammation of the eardrum, or fever
Sore throat	13 million	50%	Antibiotics should not be used unless a test confirms that sore throat is due to streptococcal infection
Sinusitis	13 million	50%	In the absence of facial pain or inflammation, studies maintain that one should wait about 10 days to assess improvement in symptoms before prescribing antibiotics
Bronchitis	16 million	80%	Studies have not shown benefits from treatment with antibiotics, except in some specific infections or cases with serious chronic lung disease
Common cold	18 million	100%	Viral infections do not respond to antibiotics
Total	83 million	60%	

appropriate treatment for the illness, the drug is administered properly in less than 10% of the cases.

Parents do not follow the child's treatment indications fully. This is partially a result of the frequent use of antibiotics without proper explanation and instruction regarding the importance of responsible use of these drugs. Improper administration of antibiotics promotes bacterial resistance, as the drugs are often administered to the child in low dosages, which leads to the selection of resistant strains.

On the other hand, when antibiotics represent the essential treatment for remission of the disease, failure to follow the prescribed treatment in terms of dosage, length of treatment, or time between doses can contribute to longer or more serious episodes.

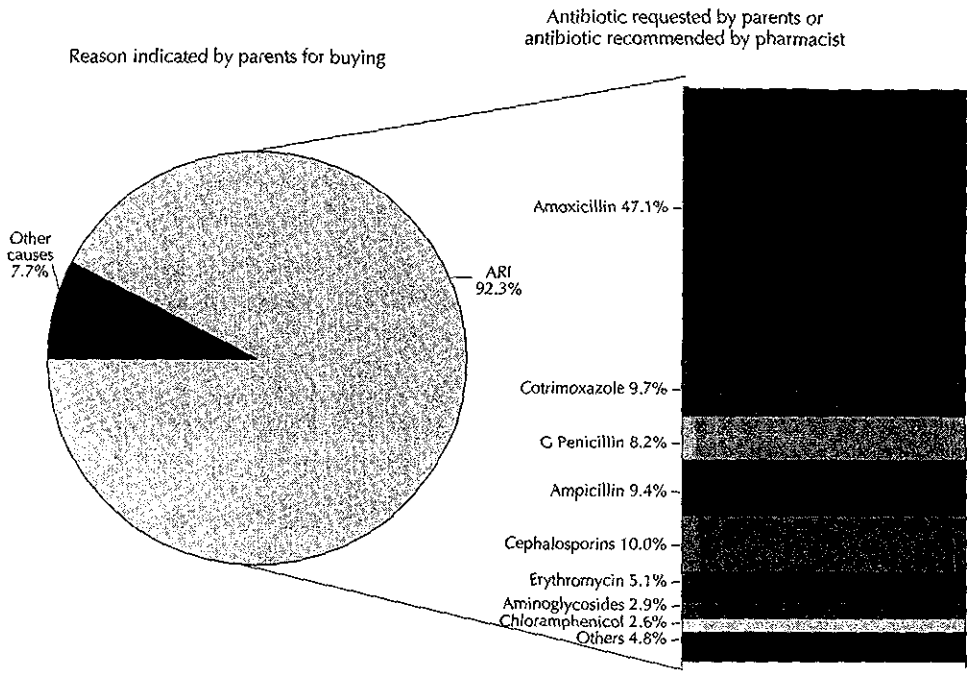
The above refers only to treatments administered in the health services. Nevertheless, in many cases parents themselves decide to treat the child with antibiotics, without any instruction from health workers. In this sense, self-medication is an additional factor contributing to the unnecessary and improper use of antibiotics.

Because of their high frequency and because antibiotics are the treatment that the health services customarily provide for them, ARI are the most frequent cause of parental decisions to administer antibiotics to children under 5 years of age without medical advice. A study of the family demand for antibiotics at pharmacies revealed that over 90% of the time these drugs are requested for the treatment of acute respiratory infections and less than 10% of the time for the treatment of another type of illness (Figure 7).

Although the same study showed that two out of three requests for antibiotics were for amoxicillin, ampicillin, or cotrimoxazole, the remaining third included a variety of antibiotics, even cephalosporins, erythromycin, aminoglycosides, and chloramphenicol.

Parents' requests for antibiotics from pharmacies were based on previous recommendations from the health services or information obtained from other people who had had "similar" experiences with episodes of childhood ARI. For this reason, the rational use of antibiotics in the health services is essential not only to limit the unnecessary administration of these drugs by health workers, but to instill a more responsible attitude in the community about indiscriminate self-medication with this type of drug.

FIGURE 7. Antibiotics requested in pharmacies for the treatment of acute respiratory infections (ARI) in children under 5 years of age, Santa Fe, Argentina, 1990



Source: Instituto Nacional Emilio Coni, Ministry of Health and Social Action, Argentina.

When antibiotics are used without instruction from health workers, their improper use can be even more frequent in terms of dosage, the time between doses, and the length of treatment, particularly because most places where drugs are sold are not run by health professionals. In addition, sometimes the antibiotic presentations available are not enough to complete the treatment, and this a priori prevents the child from receiving the dosage required for cure. Furthermore, some places where drugs are sold divide up antibiotics so that they can sell the number of tablets needed for only one or two days, although the correct length of treatment is seven to ten days.

There are several reasons for intensifying activities in the developing countries to ensure universal access to appropriate prevention and control measures, in both the health services and the home. These include the continued high mortality from preventable causes among children under 5 years of age, and the impact of access to early and appropriate treatment with antibiotics in terms of preventing mortality and reducing the severity of episodes of disease. If to these reasons we add the excessive and improper use of antibiotics for treating illness in children under the age of 5 years, thereby contributing to the development of bacterial resistance, it becomes necessary for interventions to include a strong component to improve the quality of care. As a result, the prescription and improper use of antibiotics should be reduced and emphasis placed on the monitoring of bacterial resistance to the antibiotics in use to guarantee the effectiveness of current treatments.

IMPROVING THE USE OF ANTIBIOTICS TO PREVENT MORTALITY

Greater access by the population to care for childhood illness and health problems and the use of standardized criteria to assess, classify, and treat episodes of illness in children are the two most effective lines of action for reducing mortality and serious morbidity in childhood. These activities, moreover, would help to improve the quality of care and reduce the excessive and unnecessary use of antibiotics in the health services and the community.

It is also essential to improve monitoring of the effectiveness of antibiotics in treating the most frequent childhood illnesses that require these types of drugs for their cure to ensure that proposed interventions actually help to reduce serious morbidity and mortality.

Access to adequate health care for children in the Americas

Since the late 1980s, the Pan American Health Organization/World Health Organization (PAHO/WHO) has lent its support for developing and implementing the interventions described above. This has been in the form of strategies for standard case management of diarrhea[3] and ARI,[4] which make it possible to increase access by the population to health workers and health services trained to appropriately assess, classify, and treat these childhood illnesses *(13)*. Given the high levels of excessive antibiotic use in the daily management of ARI in both health services and the home, the benefits of implementing these strategies go beyond reducing mortality and morbidity from these causes (severe morbidity in the case of ARI), because they help to reduce the inappropriate use of these drugs. Thus, adoption of these interventions and their implementation in the health services at the primary care level in the countries have given an ever-growing number of children access to adequate quality care for the illnesses that most frequently affect their health *(14)*.

In recent years, PAHO/WHO and the United Nations Children's Fund (UNICEF) have analyzed these and other strategies to control childhood illness and health problems and have developed a new intervention for children's care in the health services and at home and to improve the efficiency of care. The strategy for integrated management of childhood illness (IMCI) is the result of that joint effort and is presented as the single intervention that, when applied to the care of children seen in the health services, makes it possible to completely assess the health of the child, identify the most frequent illnesses and health problems, and provide full treatment for them. This care also includes the systematic application of disease prevention and health promotion measures to improve the treatment and care of children both at home and in the community *(15)*.

The IMCI strategy integrates all measures to control and combat the principal causes of death in children through prevention and treatment. It also helps to improve the capabilities of health workers, the operation of health systems, and family and community practices in caring for children *(16, 17)*. Implementation of IMCI can have a great impact on infant mortality, morbidity, and the severity of episodes of childhood illness. It also affects the quality of care and treatment that children receive in the health services and at home (Box 1).

By October 1998, as a result of country efforts to implement the IMCI strategy, almost 5,000 people had been trained to properly assess, classify, and treat children in 600 health services at the primary care level. This entire effort, which will be redoubled in upcoming years, is designed to increase the annual percentage reduction in mortality in infants and children aged 1 to 4 years. The strategy thus seeks to meet the targets of the World Summit

[3] Pan American Health Organization. *Diarrhea Treatment Manual*. PALTEX Series for Health Program Directors, No. 13; 1997.

[4] Pan American Health Organization. *Caring for Children with Acute Respiratory Infection*. PALTEX Series for Intermediate Technicians and Assistants, No. 21; 1992.

BOX 1. Objectives of the Strategy for Integrated Management of Childhood Illness

- To reduce mortality from prevalent childhood illnesses in children under 5 years of age, specifically deaths from diarrheal disease, acute respiratory infections, malnutrition, measles, and malaria
- To reduce the number and severity of cases of diarrheal disease, acute respiratory infections, and measles
- To improve the quality of care provided to children in the health services, reducing inappropriate use of diagnostic and treatment technologies
- To introduce aspects of child health promotion and disease prevention in the routine care provided by the health services
- To extend integrated care at the community level

for Children held in the year 2000, and to project the decline in infant mortality beyond that year, so as to reduce the number of deaths in children under 1 year of age by a total of 100,000 between 1999 and 2002 *(18)*.

Multicenter study on pneumococcal resistance to antibiotics

In recent years, PAHO/WHO has coordinated a joint system for monitoring pneumococcal infection with the Latin American and Caribbean countries to identify the prevalence of the various serotypes in the population of the different countries *(19)* and to evaluate bacterial resistance to antibiotics. The purpose of the system is to increase knowledge about the problem in the Region, since most of the current information on bacterial resistance comes from developed countries.

The multicenter study on pneumococcal resistance to antibiotics involves several countries in the Americas that are providing information on the serotypes of this pathogen—information that could potentially be used to develop a vaccine. Data are also being obtained on the sensitivity profile of pneumococci in order to evaluate the microorganism's resistance in the Region *(20)*.

Based on the results of this study, up-to-date information has been obtained on the resistance of *Streptococcus pneumoniae* to β-lactam antibiotics: 24% overall for isolates collected in Argentina, Brazil, Chile, Colombia, Mexico, and Uruguay, with variations among the countries *(21–24)*. Although these figures cannot be extrapolated to all cases of pneumonia from *S. pneumoniae*, because the samples were for the most part obtained from hospital cases, they do provide information on the circulation of strains resistant to first choice antibiotics in the treatment of pneumonia at the primary health care level based on the IMCI strategy.

Expanding these studies will provide more data on the sensitivity of bacteria to the various antimicrobials being used to treat pneumonia, as well as other invasive diseases such as septicemia and meningitis. This information will make it possible to continuously adapt treatment recommendations in the suggested strategy for reducing mortality and serious morbidity in children, and improving the quality of their care both in the health services and at home.

Monitoring bacterial resistance to antibiotics will also make it possible to underscore the need to improve criteria for antibiotic use in the health services and the community, in order to curb the development of bacterial resistance caused by the improper and excessive use of these drugs.

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RATIONAL USE OF DRUGS: REGIONAL POLICIES, REGULATIONS, AND STANDARDS[1]

Enrique Fefer[2]

The rational use of antibiotics is an issue that cannot effectively be addressed in isolation, since it is the result of numerous factors that affect the marketing, prescription, dispensing, and use of drugs in general. This paper summarizes the trends in the global pharmaceutical market that have particular implications for the health sector. It also describes some educational, administrative, and regulatory interventions that are within the reach of developing countries and that, when applied together, contribute to the rational use of drugs. Regulatory agencies control the entry of pharmaceutical products to the market. It is therefore essential that they have the necessary means to fulfill their mission to safeguard public health.

INTRODUCTION

We are living in an era where major advances in the field of medicine are a frequent occurrence. Certainly that is the impression people get from newspapers and television. The new drugs are aimed not only at preventing or treating disease but also at improving the quality of life. Some of them, the so-called lifestyle enhancers, such as Prozac and, of course, Viagra, are treated like Hollywood celebrities or Wall Street tycoons and are featured on the covers of popular magazines such as *Time* and *Business Week*.

The possibility of adverse reactions to these drugs is not as appealing a topic. Thus, an article on adverse reactions in hospitalized patients, published last May in the *Journal of the American Medical Association*, stating that drugs are the sixth leading cause of hospital deaths, went virtually unnoticed *(1)*. The case of Fen-Phen demonstrated that the enthusiasm generated by the positive, short-term effects of the drug led both physicians and patients to engage in practices beyond what is scientifically and, perhaps, ethically acceptable *(2)*.

All of this underscores the fact that promoting the proper use of antibiotics is no easy task. It is part of the larger problem of inappropriate drug use, which involves a lack of understanding about the risks associated with drug therapy. The special nature of antibiotics, true miracles of modern science, makes it even more difficult to alter the behavioral patterns that contribute to antimicrobial resistance.

Here, we will first review the global factors that affect the availability and use of pharmaceutical products; further on, we will outline a number of interventions and strategies that

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can lead to an improvement in their utilization. The strategies are generic—that is, they can be applied to antibiotics as well as other categories of drugs.

Let us begin with a pragmatic definition of the term "rational use." These two words summarize a complex process that, according to a publication by the World Health Organization, requires not only that the appropriate drug be prescribed but that it be available when it is needed at a price that people can afford; moreover, it should be taken in the proper dosage, at suitable intervals, for the appropriate length of time, and it must be effective, of acceptable quality, and safe (3).

There are many ways to stray from the path toward the achievement of this ideal situation: the long chain in the process from manufacturer to consumer offers many opportunities in this respect. The agencies responsible for regulating pharmaceuticals, when unsophisticated, permit the marketing of ineffective products or the advertisement of drugs for unauthorized uses; some pharmaceutical plants have no systems or procedures in place for quality assurance; health professionals have little access to objective information about the drugs they prescribe and dispense, and the information they have cannot compete with the sophisticated advertising strategies of the industry; pharmacies allow untrained auxiliary personnel to counsel clients about what products to buy, promoting drugs that yield the greatest profit. And finally, there is the user, the patient who cannot purchase enough tablets to complete the recommended course of therapy, or who, once he feels better, stops taking his antibiotics or hypertension medication.

This is nothing new; the *where we are* has been documented in numerous studies. We are responsible for this situation: governments, universities, professional schools, the pharmaceutical industry, health professionals, and consumers who regard drugs as an easy, immediate response to health problems whose cure requires well-considered individual and social action.

Given these circumstances, what can be done to promote better use of drugs? The necessary efforts will undoubtedly entail resources that are far beyond those provided by the international organizations. Thus, a basic strategy of the PAHO/WHO Program on Essential Drugs is to encourage all parties to participate in the achievement of this goal, without detriment to their legitimate interests.

It should also be recognized that decision-making at the national level is influenced and limited by what is happening in the world as a whole, given the international nature of the pharmaceutical industry and the profound political and economic changes stemming from globalization.

TRENDS IN THE PHARMACEUTICAL SECTOR AND NATIONAL IMPACT *(4–6)*

- The pharmaceutical market will continue to grow, due to well-known factors: the aging of the population, urbanization, attitudes and behavior, chronic diseases, emerging and reemerging infectious diseases, and, of course, the development of new products, thanks to greater scientific knowledge about processes at the cellular and molecular level.
- Paradoxically, in a growing market, the number of companies with the capacity to engage in research and development for the discovery of new drugs is shrinking. Mergers, acquisitions, and joint ventures have resulted in the creation of megacompanies with the resources to engage in ever more costly research and development and the capacity to market their products globally.
- Major companies recognize that they will have to develop new drugs if they are to prosper, since the patents on many of the products to which they have exclusive rights have expired or will do so within a few years. Expiration of the patents on a significant number of products, added to government interest in cost containment, has led to the rapid growth of the market for generic drugs. Until very recently, only

small national companies and a few multinational enterprises were interested in this type of activity. This picture has changed dramatically and all the major companies have entered this market, either with their own product lines or through acquisitions and partnerships with other companies.

- In addition, the introduction of patents and the extension of their protection as a result of the Uruguay Round of the General Agreement on Tariffs and Trade (GATT) will lead to higher prices for new pharmaceuticals.
- The opening of new markets, together with economic integration and the formation of free-trade blocs, has given heavy impetus to drug regulatory harmonization. Through the International Conferences on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Japan, the European Union, and the United States will draw up the international guidelines and criteria that will be imposed on Latin America, even though the countries of the Region have not participated in the debates.
- In our Hemisphere NAFTA, MERCOSUR, Central America, and the Andean Area are also moving forward in the formation of expanded pharmaceutical markets to facilitate product flows between the countries. Multinational corporations will no longer be required to maintain production plants in a country simply to comply with the protectionist laws that prevailed up to the 1980s. For example, Pfizer, Bayer, Squibb, and Lepetit no longer have plants in Chile. The opening to trade also threatens national companies, particularly small ones, which will have difficulty meeting international standards for quality and good manufacturing practices.
- The modernization of the State involves reducing its regulatory role and its bureaucracy, creating an environment that places economic considerations above health.

How do the aforementioned issues relate to the rational use of drugs in our societies and health institutions?

First, the new drugs are and will continue to be very expensive, and heavy pressures to use them will undoubtedly be brought to bear, since companies will have to recover their costs if they are to continue their research and development activities. Thus, it will be important to educate and inform physicians about the cost/benefit of alternative therapies, since "new" does not necessarily mean "better."

Second, the availability of similar products (generics) represents an opportunity to put the market on a healthy footing and permit competition, provided that a culture that encourages the use of generic drugs is fostered—a culture whose first step would be to require that the generic name appear clearly on the package, as well as the insert and all advertising. We must recall that the topic of generic drugs is always linked with quality and bioequivalence. There will be no significant market for these products as long as physicians, pharmacists, and the general public are not convinced that generics are quality products every bit as good as brand-name drugs.

Third, opening and integrating the markets will increase the number of circulating products. The proliferation of name brands will not contribute to good prescription practices, making the use of generic names even more urgent. It will also complicate the work of the agencies responsible for the monitoring and control of drug quality, which have had little success to date in adequately regulating the current market.

Furthermore, the expanded markets created by openness and economic integration may have a positive impact, since their operation requires regulatory consistency to facilitate the flow of goods between countries. This is forcing countries to review their criteria for the approval of manufacturers and products and, in the search for common criteria, they are resorting to internationally recognized standards. Thus, the Southern Cone has adopted the standards for good manufacturing practices (GMP) recommended by the World Health Organization (WHO), and the Andean Area intends to adapt the WHO Certification

Scheme on the Quality of Pharmaceutical Products Moving in International Commerce for its own use.

STRATEGIES FOR THE RATIONAL USE OF DRUGS

A number of strategies to improve the utilization of drugs and procure their rational use are within the reach of the countries of the Region. Those presented in this paper are described in the second edition of *Managing Drug Supply* (1997), published by Management Sciences for Health under the auspices of WHO, and refer to interventions in critical areas that affect the use of drugs *(7)*: the education of health professionals; the administration and management of drugs, and drug legislation and regulation.

Box 1 outlines possible interventions and steps that can be taken in these areas, some of which are discussed below.

Education

Knowledge of the basic principles of clinical pharmacology is necessary for understanding the benefits and limitations of new drugs and for a certain understanding of the clinical trials endorsing the indications for their use. There is much to be done in this area. In 1995 Hans Hogerzeil, a staff member of the WHO Program on Essential Drugs in Geneva, pointed out that prescription practices in university hospitals leave a great deal to be desired and cited studies that found that between 41% and 91% of prescriptions written for antibiotics were inappropriate *(8)*.

That same year, WHO published its *Guide to Good Prescribing*, which offers guidelines for good prescription practices and is geared to students beginning the clinical phase of their education *(9)*. The methodology used by the guide was developed at the University of Groningen in the Netherlands. Known as "problem-based teaching of drug therapy," its purpose is to teach prescribing as part of a rational, systematic process. In this approach, prescribing is not limited merely to writing prescriptions, but emphasizes the information that should be given to the patient, as well as monitoring to ensure that the medication prescribed performs as expected.

On the topic of education, also worth mentioning is the experience of the Costa Rican Social Security Fund, which offers a program for continuing education in therapeutics for its physicians and pharmacists. Guided by a colleague trained for this purpose, and utilizing specially prepared instructional materials, small groups actively examine a topic related to a specific therapy category, such as antibiotics. The methodology is dynamic and participatory, emphasizing the knowledge, skills, and attitudes required for the proper use of the drugs in question. With PAHO support, professionals from other Central American countries have received training to set up similar programs in their respective countries.

The schools of pharmacy in Latin America have recognized the shortcomings of their curricula and have embarked on the continuous review and updating of their educational programs to train professionals that can meet national needs and realities. The Pan American Health Organization, in collaboration with the American Association of Colleges of Pharmacy, has sponsored four Pan American conferences on education in pharmacy (1990, 1993, 1996, and 1999). The latter was held in 1999 in Santiago, coordinated by the University of Chile's School of Chemistry and Pharmacy.

Notwithstanding, the fact is that Latin American pharmacists are currently not fulfilling their role in promoting health: drugs such as antibiotics are frequently sold without prescription and without information to guide the user. The result is the unnecessary and inappropriate use of antibiotics, which is contributing to the development of antimicrobial resistance. The pharmaceutical profession should assume its responsibility in this field. This is a challenge for professional schools, which are attempting to raise the status of the

BOX 1. Strategies for the Rational Use of Drugs

Educational Interventions

Training of Health Personnel
- Training and continuing education
- Supervision
- Conferences, seminars, workshops

Printed Matter
- Scientific literature and bulletins
- Drug formularies and treatment guides

Interpersonal Contact
- Targeting
- Patient education
- Influence of opinion-makers

Management Interventions

Selection, Procurement, and Distribution
- Drug lists
- Utilization studies and feedback
- Pharmacy and therapy committees
- Cost information

Prescribing and Dispensing
- Standarization of selected prescriptions
- Treatment guides
- Prepackaging of selected treatments

Financing
- Setting prices
- Capitation

Legal Measures/Regulations

Drug lists
Restrictions on prescribing
Restrictions on dispensing
Registration of drugs

profession and establish a place for it in the health sector through genuine pharmaceutical care.

It is essential to recall that it is not enough to provide information if prescription practices are to be changed. Information by itself is not effective but must be part of a program consisting of education, monitoring, and supervision. The most effective intervention is the face-to-face, personal encounter, in which specific problems are discussed and possible solutions considered. The influence of well-respected professionals whose practices and recommendations are accepted by other professionals is also useful. The pharmaceutical industry utilizes both types of interventions and invests up to 25% of its sales revenues in activities for promotion and education. There is no scarcity of objective, up-to-date information on the appropriate use of drugs. What is needed is the distribution and application of the existing information.

In addition to pharmacology reference works in English (for example, Goodman and Gilman and *The Martindale*), pharmacy committees, universities, and health professionals

now have a valuable reference tool in Spanish, published with the collaboration of the Ministry of Health of Spain and the U.S. Pharmacopeia (USP): the Spanish version of the USP *Drug Information for the Health Professional*, which has been available since 1990. This publication fills a great void and has become the most complete source of pharmacological information available in Spanish. The Ministry of Health has also translated the volume on *Counseling the Patient*. Another important information source is the journal *Medicamentos y Terapéutica*, published by the Pan American Health and Education Foundation, an entity associated with PAHO. The publication contains Spanish translations of articles from *Medical Letter, Drugs and Therapeutics Bulletin*, and *Adverse Drug Reaction Bulletin*, all of them objective information sources of international repute.

PAHO's Program on Essential Drugs and Technologies takes advantage of the wide distribution of the Organization's official journal, the *Pan American Journal of Public Health*, to include a section on pharmacological information every four months. Covering topics related to pharmaceutical policy and regulatory decisions, this section also reviews the contents of relevant publications. In contrast, the current information centers in the Americas—some of them in universities, others in the ministries or schools of pharmacy—have generally not had much of an impact. It will take time to develop a culture that appreciates the value of this service.

Administration and Management

The best known tool for this purpose is the basic table or formulary that limits drug purchases to products that meet the needs of the health services. Almost every country in Latin America and the Caribbean has a national formulary for the public sector and even social security institutions. This management tool offers the opportunity to restrict prescription of certain antibiotics to specialists. It also constitutes a first and important step toward better use of resources and rational therapy, which, while clearly important, is not enough. Distribution of the formulary is very limited in many countries, to the extreme that some health workers are not even aware of its existence. For this reason, PAHO/WHO stresses the importance of accompanying the publication of the formulary with extensive publicity about its merits. The next step is to ensure that the list of products and the information are updated periodically; otherwise the formulary will lack credibility and become just one more document with limited impact. In the English-speaking Caribbean there are two excellent examples of how a formulary system should work. Barbados and the Organization of Eastern Caribbean States have established comprehensive drug supply services based on their respective formularies and on centralized purchasing determined by competitive bidding. The system has an active formulary committee, conducts drug utilization studies, exercises quality control over purchased products, provides training for technicians, and publishes information bulletins, all of which has contributed to the system's acceptance by health professionals and the public, in addition to politicians and government personnel responsible for approving and auditing the system's budget.

Drug utilization in a health facility or the health services should be monitored through utilization studies that will make it possible to detect inappropriate prescription practices—information that should translate into corrective action. The findings of such studies will document the current situation and permit impact assessments of interventions to improve drug utilization. The challenge, of course, is to translate the studies' conclusions into policies and programs that effectively modify the practices detected. Thus, it is important to establish pharmacy and therapeutics committees with the responsibility and authority to improve drug utilization in these institutions and, in the case of antibiotics, to develop policies for their appropriate use.

In addition to orienting health professionals, therapeutic guidelines or standard treatment protocols are management tools that indicate which drugs will be procured and how they should be utilized. Providing information on the cost of drugs can influence the selection of the products prescribed.

Legislation and Regulation

Regulatory entities act as gatekeepers to the national market and are therefore the first line of defense against ineffective or dangerous products—a first line of defense that some attempt to breach. Sanitary criteria are frequently ignored due to pressures from the economic sector, which means that the agency responsible for regulating pharmaceuticals should insist that its central mission and raison d'être is to *protect public health.* On this there should be no debate.

We are aware of the shortcomings of the regulatory agencies in Latin America and the Caribbean, whose problems range from the lack of skilled personnel for evaluating drug registry applications to the inability to pull hazardous or ineffective products off the market or to impose adverse impact sanctions. This situation will not change without a radical restructuring of the institutions responsible for controlling the pharmaceutical market, which requires a government willing to invest sufficient political capital to institute the necessary changes.

Below are some of the essential attributes of an effective regulatory agency that should be considered in any proposed restructuring *(6)*:

Institutional Visibility

An effective unit cannot be lost in the bureaucracy of the ministry; it should have presence and an identity.

Trained Staff

An ideal agency should have the administrative and financial flexibility to fulfill its mission and to hire a group of highly skilled staff to work full-time and devote themselves exclusively to the unit. Where personnel and expertise are limited, third-party contracting should be considered. Responsibility for evaluation, inspection, or quality control should be delegated only when clear procedures are in place and only to entities accredited for these functions.

Financing of Regulatory Actions

Financing has been a major constraint on the performance of regulatory agencies. Financing for these entities cannot be expected to come from the regular State budget. A viable alternative is for the regulatory agency to impose taxes or collect fees from the users of its services. This concept is widely accepted in the developed countries, where high fees are charged for evaluating registration applications, as well as for the inspection of manufacturing plants and the annual upkeep of the registry.

Transparency of the Processes

The procedures and criteria employed for the review and approval of registration applications should be public knowledge, as should the results of the process. Although this would appear to be obvious, in practice it is not the case in many countries, where political pressures and personal favors influence performance.

Application of International Standards

The capacity to evaluate pharmaceutical, toxicological, and clinical information varies from country to country. Due to the complexity of this task, duplication of efforts should be avoided—particularly now, when greater efficiency in the public sector is required. Thus, both industry and national authorities have recognized the benefits of standardizing regulatory requirements through the application of internationally accepted guidelines.

Interagency and International Cooperation

The importance of interagency and international cooperation in the efforts to curb the development of resistance to antibiotics is obvious, since infectious diseases recognize no boundaries. A strong national regulatory agency can and should play a prominent role, since once the marketing of a product is authorized, the agency must ensure that product information and advertising conform to internationally recommended ethical standards recommended in the international arena and that the sale of the product is properly controlled. The authorization can restrict the use of selected drugs to a certain level of care or medical specialty. In addition, the number of drugs dispensed by prescription can be limited.

It is important to recognize that even if the ministry of health has a well-organized and financed drug regulatory agency, there is another major front in the struggle against resistance to antibiotics, a front in which the ministry of health traditionally has little influence: the livestock sector. Efforts to rationalize the use of antibiotics in humans will have little impact if their use in animals is not controlled. For historical reasons, drugs for veterinary use are controlled by the ministries of agriculture, whose objective is to promote the livestock sector and ensure an abundant food supply. If antibiotics are required to accomplish this, the pressures to use them are obvious. Consequently, intervention policies in this field must extend beyond a country's health sector and borders. It is the responsibility of the ministry of health to take the lead in this area and to coordinate an intersectoral strategy that is both national and international in scope.

It may be necessary to develop new instruments like the *Codex Alimentarius* whose international application is compulsory. Let us recall that international agreements have subjected narcotics and psychotropic drugs to special surveillance to prevent their improper use. In the interests of public health, it may be necessary to adopt this practice for other drugs.

Users' Associations

Today, and certainly in the future, any discussion of the rational use of drugs must make mention of users' associations, whose influence in defining the social policy agenda is increasing and which can be valuable partners in initiatives to improve antibiotic use.

International Action for Health (AIS) and the International Organization of Consumer Associations have networks of collaborators throughout the Hemisphere who have documented improper prescription practices, particularly for the treatment of acute diarrheal diseases. In 1995 the AIS published the Spanish version of *Problem Drugs*. Several countries have national groups that are rather active, for example the Pro-Life Physician's Service of Peru and the Society for Drug Surveillance (Sobrevime) of Brazil. Pro-Life administers a network of community pharmacies and publishes an attractive journal called *Medicamentos y Salud Popular* [Drugs and Popular Health] that advocates putting a national pharmaceutical policy in place to promote the rational use of drugs and the use of traditional medicine. Sobrevime publishes an excellent bulletin with critical analyses of industrial and government activities, as well as the practices of health professionals.

CONCLUSIONS

It should be recalled that pharmaceutical products do not exist in a vacuum, since efforts to promote the rational use of drugs are affected both by international factors and the characteristic limitations of the developing countries. However, this is no justification for complacency. There are strategies and interventions within the reach of the countries of the Region that do not require high technology or enormous budgets. Political will, long-term institutional commitments, and the broad participation of all sectors involved will make it possible for our countries to begin to guarantee that antibiotics will be as miracu-

lous for future generations as they have been for ours.

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PROBLEMS AND DIFFICULTIES IN CONTROLLING ANTIBIOTIC USE

Barbara E. Murray[1]

Antibiotic resistance continues to increase in widely divergent areas, from intensive care units in highly industrialized countries to the community setting in lesser-developed ones. While the factors contributing to bacterial resistance, namely, antibiotic selective pressure combined with transmission of microorganisms, are well recognized, solutions to the problem remain elusive. This overview focuses on some of the problems inherent to developing strategies for controlling antibiotic resistance.

OVERVIEW OF PROBLEMS CREATED BY ANTIBIOTIC RESISTANCE

Antibiotic resistance can result in considerable cost to the patient and to the health care system *(1)*. The failure to choose an active antibiotic allows the continuation of the infectious process which may have serious consequences, for example, neurological complications from inadequately treated meningitis. Also, because resistance is more commonly seen with older and generally cheaper antimicrobial agents, the presence of resistance will often mandate the use of a newer, broader-spectrum and more costly agent, or the use of combinations of antimicrobials. For organisms such as vancomycin-resistant enterococci, the physician may be forced to use an antimicrobial agent of unproven efficacy, or combinations of different agents that have shown some activity in vitro or in animals but for which there may be little, if any, clinical data in humans *(2)*. Such alternatives may well be less efficacious than the therapy of choice would be for a susceptible organism. In addition, concern on the part of the prescriber that an organism causing a very serious infection may be resistant may cause the empiric choice of newer and more potent agents, or combinations of agents, for fear that waiting until an organism has been identified and its susceptibility determined will result in increased morbidity or mortality. Examples of the increased cost incurred include the widespread empiric use in the United States of America of the combination of vancomycin plus cefotaxime or ceftriaxone, instead of penicillin, for therapy of children with pneumococcal meningitis. Said recommendation arose from observations that a portion of pneumococci are now resistant to penicillin and cephalosporins *(3, 4)*. While this practice is appropriate for meningitis in areas with moderate rates of

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penicillin-resistant pneumococci, it unfortunately may be overextended to include empiric therapy for most cases of meningitis, including those of unknown cause, even when the likelihood of a bacterial process is rather low. This can result in unnecessary antibiotics for up to 48 hours pending culture results.

RESISTANCE BEGETS RESISTANCE

There is a strong association between resistance to newer antibiotics and the presence of resistance to multiple older antibiotics. This limits therapeutic choices and raises the possibility that the use of any of the antimicrobial agents to which a particular strain is resistant will select for and perpetuate all of the resistances of the strain. Methicillin-resistant *Staphylococcus aureus*, for example, not only express cross resistance to other β-lactams, but are typically resistant to multiple other antimicrobials such as ciprofloxacin, clindamycin, aminoglycosides, erythromycin, and others *(5)*. Studies comparing susceptibility of methicillin-susceptible and methicillin-resistant staphylococci show that resistances are much more common in the latter than in the former *(6)*. The same problem of multidrug resistance is seen in *Streptococcus pneumoniae;* that is, a penicillin-resistant pneumococcus is much more likely to be resistant to other potential therapeutic agents, such as erythromycin or trimethoprim-sulfamethoxazole, than a penicillin-susceptible organism *(7)*. Similarly, trimethoprim-sulfamethoxazole-resistant *Shigella* spp. and *Salmonella typhi* are often resistant to other therapies such as ampicillin, chloramphenicol, and tetracycline *(8–11)*. This has led to the use of nalidixic acid and, more recently, fluoroquinolones in some areas of the world, with the resulting emergence of nalidixic acid resistance in these same multidrug resistant organisms *(12)*. The presence of nalidixic acid resistance causes decreased susceptibility to newer fluoroquinolones, such as ciprofloxacin and ofloxacin, although these organisms may still have MICs ≤ 1–2 μg/mL *(9, 13–15)*. The economic impact of alternative therapy for typhoid fever, most often a parenteral cephalosporin, for strains resistant to the above mentioned orally administrable antimicrobials is quite large. Anecdotal reports from developing countries about finding fluoroquinolone capsules of lesser potency than stated poses a particular problem for fluoroquinolones. Resistance to these agents typically occurs by a series of mutational events, each of which adds incrementally to the resistance level of the organism. The easiest way to select single mutational steps, which may increase the MIC to only a small degree, is to use concentrations near the MIC. Thus, the use of a drug at doses lower than recommended would be an excellent way to select for fluoroquinolone resistance, as occurs when taking pills of lower potency.

FACTORS CONTRIBUTING TO RESISTANCE

Antibiotic Use

Some factors that contribute to resistance are not under human control. These include whether an organism intrinsically resistant to an antimicrobial is present in a person's flora at the time of treatment with antibiotics; whether a mutation causing resistance will occur during therapy, and whether resistance will be transferred from one microorganism to another. On the other hand, we do have control over antibiotic use, and it is the presence of antibiotics that gives the selective advantage to a resistant microorganism. The use of an antimicrobial allows a microorganism that is present in very low numbers to proliferate and increase thousands to millionfold and become the dominant organism of its species. Well-described examples include the marked increase in the number of trimethoprim-resistant *Escherichia coli* during the use of trimethoprim, and of vancomycin-resistant enterococci during the use of an oral glycopeptide *(16, 17)*.

Ease of Spread

Although the presence of an antibiotic is important in increasing the number of resistant organisms, the other very important factor contributing to the problem of antimicrobial resistance is the potential for spread of resistant microorganisms to others. Settings known to have high rates of transmission of infectious pathogens also tend to have high rates of antibiotic resistance, partly because these organisms are spread the same way (Figure 1). For example, day-care centers in both developed and developing countries are well known to have high transmission of enteric pathogens which have high rates of antibiotic resistance. Several studies have documented much greater resistance to antibiotics, including trimethoprim, in the flora of individuals who live in developing countries when compared to those in highly industrialized locations *(18, 19)*. Travelers to developing countries, including those not consuming antibiotics, have also been shown to acquire resistant organisms during their stay abroad *(20)*.

Relationship Between Antibiotic Use and Spread of Resistant Organisms

An inherent complicating factor that contributes to the increase in antibiotic resistance is that settings with the greatest potential for spread of antibiotic resistant organisms tend to be the ones with the heaviest use of antibiotics, which likely causes amplification of the resistance problem. Where there is overcrowding, poor sanitation, and/or failure to adhere to recommended sanitary procedures, spread of bacterial pathogens will be greater, leading to more disease and a greater need for antibiotics (Figure 1). The use of more antibiotics, in turn, creates more situations favorable for multiplication of resistant organisms while suppressing susceptible ones. Moreover, since the same conditions that spread the bacterial pathogens (e.g., *Shigella* spp.) also promote the spread of other enteric organisms (e.g., resistant fecal *E. coli*), the increased number of resistant organisms that are selected by antibiotic presence are then put back into the same milieu that favors the spread of bacteria. Examples of such settings include those where lack of clean water and irrigation with waters contaminated with feces, among others, create higher incidences of diarrheal diseases. Furthermore, intensive antibiotic use, including over-the-counter antibiotics, selects for resistance in the bacteria in normal flora which are then spread by the lack of clean water. In a similar manner, crowding, failure to wash hands, to disinfect equipment, and heavy antibiotic use contribute to outbreaks of antibiotic-resistant bacteria in intensive care units in tertiary referral hospitals.

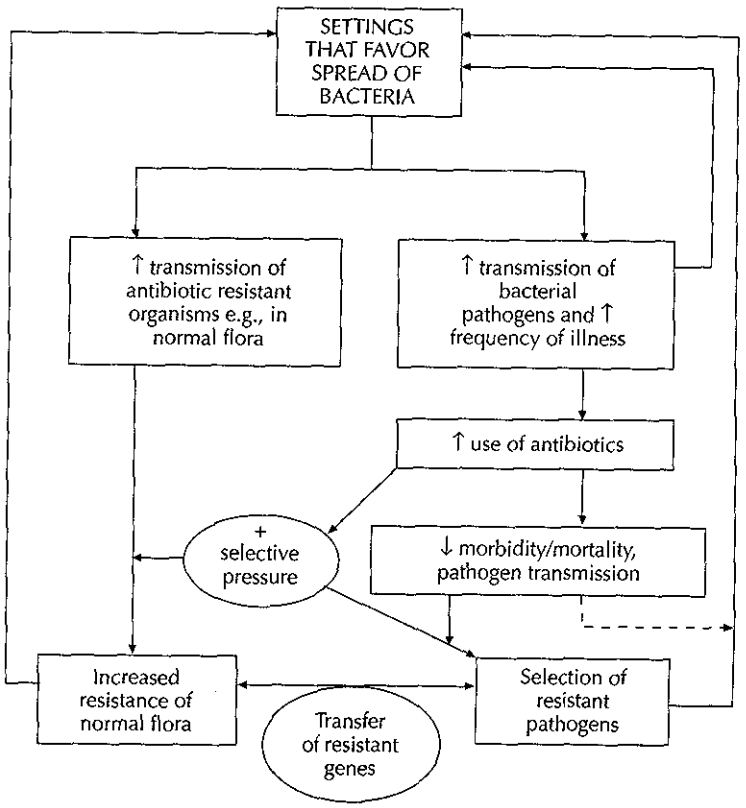
As mentioned above, day care centers in highly industrialized countries also have high rates of antibiotic resistance. These institutions, which may house large numbers of diaper-age children who are unable to comply with sanitary norms that might be practiced by older family members, are well known for outbreaks of various infectious diseases, including hepatitis A, *Haemophilus influenzae* type b, giardia, rotavirus, shigellosis, penicillin-resistant pneumococci, and trimethoprim resistant fecal *E. coli* *(21–23)*. In industrialized countries, the prevalence of resistance among fecal *E. coli* is much higher in larger day-care centers than in smaller ones, which in turn is higher than in children not enrolled in day-care centers *(24)*. Recent reports have indicated that day-care centers may also be a focus for emergence of methicillin-resistant *S. aureus* in the community *(25)*.

BARRIERS TO DECREASING OR CHANGING ANTIBIOTIC USE

There are a number of difficulties in achieving prudent use of antibiotics, some of which are listed below:

- Areas with high rates of disease have a great need for antibiotics, and it may be difficult to decrease use where there is a great need for use.

FIGURE 1. Interrelatedness of bacterial transmission, bacterial disease, antibiotic use, and antibiotic-resistant organisms



The dashed arrow indicates a diminution in numbers of pathogens if the pathogen is susceptible to the antibiotic used.

- Ignorance as to the identity of the causal agent and, if bacterial, to its antimicrobial susceptibility, combined with fear of not starting a patient with severe bacterial infection on the proper antibiotic, causes greater use of antibiotics and use of different ones that may eventually be proven unnecessary.
- Lack of availability or delay in obtaining susceptibility results for documented pathogens.
- History of allergy which is not well documented but which alters drug usage patterns (e.g., a newer fluoroquinolone, a cephalosporin or vancomycin instead of penicillin).
- Patient expectation.
- Managed care and productivity issues. Treatment for certain infections, e.g., urinary tract infections, is increasingly being administered empirically with cultures taken only for failures. The necessity of seeing larger numbers of patients may not allow the physician sufficient time to explain to a patient the reasons for not giving an antimicrobial.
- Overestimations of local resistance problems, which may arise from extrapolation of surveillance data in tertiary care hospitals to the community and primary care hospitals.

- Recognition of new bacterial diseases (e.g., *Helicobacter pylori, Borrelia burgdorferi)* which leads to antibiotic treatment of a subset of the population which would previously not have been so treated.

CONFOUNDING ISSUES RELATING TO RESISTANCE

A fundamental problem relating to antibiotic resistance is the fact that we do not know how much resistance is due to appropriate use and how much is due to inappropriate use. Thus, even appropriate antibiotic use will likely be followed by emergence of resistance in some microorganism. For example, amoxicillin, however appropriately given for a pneumococcal otitis media, may nonetheless be followed by selection of ampicillin-resistant *E. coli* among the normal flora, which may also cause coselection for *E. coli* resistant to trimethoprim or other antibiotics. Because resistance to newer agents tends to be added to strains already multiresistant, it could be predicted that newer antibiotics would coselect for more resistances than older ones. In a study of normal fecal *E. coli* in Mexico in the early 1980s, strains resistant to only trimethoprim (the newest of the agents tested) were never encountered, while strains resistant to only ampicillin, sulfonamides, or other older drugs were common *(16)*. The ability of antibiotics to select for resistance among the normal flora as well as (or better than) in the infecting pathogen helps create an enormous reservoir from which resistance may be spread.

Other problems inherent to developing strategies to control resistance are that few, if any, data are available to indicate how much decrease in antibiotic use is needed to achieve an improvement in resistance rates. This is compounded by a paucity of information indicating how much of the resistance problem is due to antibiotic use and how much to transmission. Among penicillin-resistant pneumococci, the predominance of a few specific serotypes and the presence of some internationally spread clones *(26–28)* suggest that it is easier to spread a resistant clone once it has emerged than to recreate the resistance de novo. While the same may not be true for other types of resistance, such as mutational resistance to fluoroquinolones, the spread of resistant clones appears to be of major importance for certain antibiotics in some organisms. For these reasons, efforts to decrease antibiotic resistance in the hospital setting are clearly targeted to both decreasing transmission, as well as decreasing antibiotic use. While these goals may be achievable in sophisticated centers through continuing educational efforts, it is not clear to what extent reduction in bacterial transmission can be achieved in developing countries.

POSSIBLE SOLUTIONS TO THE ANTIBIOTIC RESISTANCE PROBLEMS

Efforts to achieve prudent antibiotic use must involve education of the decision-makers. In some settings, the decision-maker will be a physician. One approach to more prudent antibiotic use in hospitals in the United States has been to require approval by an infectious diseases consultant prior to the administration of the newest and/or most expensive antibiotics, or at least, approval for their continuation beyond a 24–48 hour period.

Information indicating what antibiotics are used and what they are used for will be needed in order to determine what adjustments in usage are desired, and to determine the types and targets of educational interventions. For example, hospitals that have determined that high usage of vancomycin relates to therapy of single blood cultures positive for coagulase-negative staphylococci, in cases of poorly documented allergies, or for routine surgical prophylaxis may achieve reduction in vancomycin prescription by focusing on these areas of inappropriate use. In other locations, pharmacists, other health care workers, or family members may be the decision-makers in regard to administering or request-

ing antibiotics, and education programs for these individuals will differ from those in tertiary referral hospitals.

IS THERE A PRICE TO PAY FOR DECREASING ANTIBIOTIC USE?

Antibiotics have been instrumental in decreasing the morbidity and mortality associated with a number of infectious diseases. Since diagnostics are imperfect, a reduction in widespread antibiotic use will likely incur some cost in terms of disease prevention or treatment. That is, we will occasionally be incorrect in not treating an illness that turns out to be bacterial and in using an agent to which an organism is resistant. Consensus guidelines may be useful in directing proper use, and will certainly be different depending on the infection and its possible sequelae (e.g., treatment of gonorrhea versus treatment of pneumococcal meningitis). While public health guidelines exist for empiric treatment of gonorrhea in a community when resistance reaches a certain level, there are few, if any, guidelines that suggest what level of resistance in a hospital justifies use of more broad spectrum or expensive agents for empiric therapy of nosocomial infections. Guidelines for antibiotic use would need to be based on disease symptomatology and severity, local disease prevalence and antibiotic resistance patterns, and would need to balance the risk of resistance (and potential adverse drug effects) with the risk of possible adverse outcomes with the strategies recommended.

ANCILLARY STRATEGIES

Ideal use of antibiotics requires rapid diagnostics to identify a bacterial etiology as well as its susceptibility at the time of presentation of the patient. Since such technology is at the least decades into the future, other efforts that may decrease resistance or at least slow its increase remain important. If bacterial disease can be prevented, then the need for antibacterials will decrease; in consequence, selective pressures exerted by antimicrobials will diminish and resistance problems should improve or slow in their increase. Improvement of general standards of living, including clean water, safe food, lack of crowding, and access to health care, as well as educational level, are of primary importance in this regard, but largely beyond our control. Other efforts to decrease disease rates may include targeted education toward improvement of water and food safety. Implementation of the highly effective vaccine for *H. influenzae* type b has reduced invasive disease caused by this organism dramatically in regions that can afford the distribution and administration of this vaccine. Conjugate vaccines based on the pneumococcal serotypes most commonly associated with penicillin resistance have been developed and appear quite efficacious, suggesting this strategy may decrease the problem of penicillin resistant pneumococci. While an increase in colonization by other serotypes may follow, if vaccines are successful against the more invasive and more resistant serotypes, they should at least temporarily be beneficial for the problem posed by penicillin-resistant pneumococci. However, administration of these vaccines to children in developing countries that may have the greatest need for them may not be achievable.

CONCLUSION

The problems of antibiotic resistance are unlikely to diminish. While some new antimicrobials are on the horizon for Gram-positive organisms, they are not likely to be inexpensive. Furthermore, probably the same cycle of resistance will follow if antibiotic use and transmission of bacteria remain high. Moreover, development of new affordable oral drugs for gonorrhea, dysentery and typhoid are further behind. This strongly suggests that efforts to prevent spread of bacteria and to decrease the selective pressure exerted upon

them remain paramount issues for decreasing resistance rates or, at least, slowing their increase.

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THE RISK OF LOSING THE TOOL FOR TUBERCULOSIS CONTROL: THE SITUATION IN ARGENTINA AS A BASIS FOR ANALYSIS

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Argentina is a country where the evolution of multidrug-resistant tuberculosis has been studied while validating known concepts. This resistance originated and spread among patients that received repeated treatments over a period of time in which the rate of cure was lower than that considered acceptable by international guidelines. Irregular or incomplete treatment led to the selection of multidrug-resistant strains in patients who were normally referred to infectious disease hospitals because of difficulties in the clinical management of these cases. Located in large cities where the prevalence of AIDS is greater, these institutions also have a high concentration of HIV-infected patients. There, the bacillus of multidrug-resistant tuberculosis found hosts where it could readily multiply and spread.

Multidrug resistance is still limited to groups of patients with specific medical histories, such as previous treatment or immunosuppression, and hospital stays in institutions which have reported nosocomial outbreaks. Nonetheless, this phenomenon serves as a timely warning for correcting poor practices, such as the inconsistent administration of drugs, as well as inappropriate decisions or conditions regarding patient hospitalization. If these changes are not made, chemotherapy will progressively lose its effectiveness. The result of extensive and in-depth research, this chemotherapy continues to be an essential tool for controlling tuberculosis—particularly given the lack of progress in developing new vaccines or antibiotics.

A great deal of effort and state-of-the-art technology have been devoted to overcoming the limitations of the BCG vaccine. However, neither the attenuated, recombinant, or mutated variations of the bacilli recently created by scientists to increase the expression of certain antigens, nor purified protein or DNA subunits, have resulted in significantly better immunogens. Without a vaccine to prevent the tuberculosis bacillus from becoming lodged in the lungs, the bacilli multiply and form a lesion, which then ulcerates, bursts, and spreads new bacilli. Thus, Koch's assertion in 1862 still holds true: the first step in controlling tuberculosis must be eliminating, as far as is humanly possible, the sources of infection. One of these sources, and certainly the most basic, is the sputum of the patients. Koch

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established that curing tuberculosis not only restores the patient's health but is essential in controlling the disease. Today, this continues to be the only effective action.

Using the most basic microscopy techniques, Koch observed that the bacillus had a cell wall with very unusual characteristics. His successors used electron microscopy and immunochemistry to map out the structure of this wall "shield," molecule by molecule. Thus they demonstrated why most broad-spectrum antibiotics cannot penetrate this microorganism and act on the metabolism or biosynthesis of the germ.

The discovery of streptomycin in the mid-twentieth century was followed by 20 years of intense research leading to a specific and effective treatment. The most active drugs were identified and selected when it was discovered that the bacillus had two weak points. The first was that RNA synthesis could be paralyzed by rifampicin, since it shows a high affinity for combining with the only RNA-polymerase of the bacillus. The second weakness of the bacillus is that isoniazid can prevent its cell wall from being synthesized. One of this drug's effects is the inhibition of the synthesis of mycolic acid precursors. It was also discovered that administering a single antibiotic was not sufficient, because the bacillus quickly became resistant to it. Thus, it was learned that multidrug therapy eliminated the selection of drug-resistant mutants and the drugs aimed at different targets on the bacillus had an additive action, preferring populations of microorganisms with different levels of reproduction and metabolic activity in the lesion. It was concluded that the way in which the drugs were combined and the quantities administered were relevant. Optimal treatments were designed that minimized the patient's infective period, relapses, and resistance to antibiotics. These remarkable experiences defined in minute detail the tool that promised to wipe out tuberculosis. And when this tool was not misused, its effectiveness remained unaltered—until today.

In Argentina, as in several other countries, a situation is coming to the fore that tests the veracity of this assertion. The National Program for Tuberculosis Control (PNCTB) has adopted an effective treatment regimen in which the patient is administered isoniazid, rifampicin, ethambutol, and pyrazinamide for 2 months, followed by 4 months of isoniazid and rifampicin. In the case of patients with HIV, the four months of the second stage are extended to seven months. This treatment's high degree of effectiveness is counteracted by resistance to isoniazid or rifampicin and can be nullified when there is resistance to both drugs—that is, multidrug resistance.

Surveillance of resistance to tuberculosis drugs has been in effect in Argentina since 1968 *(1)*. In 1994, the National Network of Tuberculosis Laboratories conducted the last national study. (See Table 1.) Acquired multidrug resistance among non-HIV patients increased from 0.9% during the period 1968–1978, to 7.8% in 1979–1986; and 10.4% in 1987–1992. In 1994, acquired multidrug resistance reached 19.1%. However, initial multidrug resistance was not detected during the period 1968–1978, and was 0.2% between 1987 and 1992, and 0.8% in 1994.

The vast majority of cases being detected do not have a history of prior treatment with tuberculosis drugs. Initial multidrug resistance, i.e. where a patient has been infected for the first time with a multidrug resistant strain, is a rarity. It was first documented in the 1990s, and has not surpassed 1%. Essentially, multidrug therapy as a means for controlling tuberculosis among patients who have not previously undergone this treatment continues to be effective in more than 99% of cases.

In Argentina, there are no exact figures on the number of diagnosed tuberculosis patients that undergo repeated treatments, but certainly they are a clear a minority when compared with previously untreated cases. However, the former have probably increased in the past 20 years, judging by the rate of cure recorded in Argentina. Between 1978 and 1994, the PNCTB conducted studies that showed the cure rate hovered around 60% *(2, 3)*. In general, the health system did not foresee the pos-

TABLE 1. National study of resistance to tuberculosis drugs. Initial and acquired resistance to tuberculosis drugs, by drug and HIV status, Argentina, 1994 *

	Initial resistance			
	HIV-negative or unknown		HIV-positive	
	Number	Percentage	Number	Percentage
Susceptible	478	91.2	51	62.2
Resistant	46	8.8	31	37.8
Resistant to				
Isoniazid	22	4.2	25	30.5
Rifampicin	4	0.8	27	32.9
Streptomycin	32	6.1	14	17.1
Ethambutol	5	1.0	14	17.1
Multidrug-resistant	4	0.8	24	29.3
	Acquired resistance			
	HIV-negative or unknown		HIV-positive	
	Number	Percentage	Number	Percentage
Susceptible	158	61.5	11	35.5
Resistant	99	38.5	20	64.5
Resistant to				
Isoniazid	77	30.0	17	54.8
Rifampicin	60	23.3	17	54.8
Streptomycin	62	24.1	10	32.3
Ethambutol	25	9.7	15	48.4
Multidrug-resistant	49	19.1	15	48.4

*Component of the World Health Organization's Global Project on Antituberculosis Drug Resistance Surveillance and the International Union against Tuberculosis and Lung Disease, 1994–1997 (*8, 9*).

Note: The sample studied consisted of 16% of the infected patients diagnosed in 17 provinces of Argentina and the Federal Capital.

sibility that patients would break off their treatment. Patients may have various reasons for abandoning treatment. For example, they may consider it unnecessary to maintain a prolonged drug regimen when they already feel well. In addition, sometimes the treatment may produce adverse side affects, and patients may have economic or social problems. The health system should have considered the possibility of patients abandoning their treatment, a situation it did not know how to prevent. Moreover, interrupting treatment meant that the health services had to prevent a public health problem. These factors were compounded by the occasional lack of drugs.

The matter was not given priority, and responsibility was spread out at several levels. On some occasions, there were bureaucratic delays in purchasing drugs, making it impossible to maintain an adequate supply and continuous distribution. It has been shown that insufficient dosages, incomplete drug regimens, and using suboptimal combinations of drugs lead to the selection of multidrug-resistant mutations of the bacillus, which then multiply and begin to occur naturally. If improper treatments continue, there is a selection of bacilli able to accumulate mutations, including multidrug-resistant ones. No other means have been discovered whereby the bacillus becomes resistant and later predominates in the lesion. Thus, acquired multidrug resistance is developed, reflecting poorly administered treatment. In Argentina, this was the situation among patients with a history of prior treatment. Multidrug resistance has steadily increased from very low levels in the mid-1970s to over 19%, showing a foreseeable curve as an emerging phenomenon.

The resistance of the bacillus to antibiotics does not spread naturally. It is not transmitted horizontally among strains, because it is

not coded in promiscuous mobile elements (plasmids, transposons, integrons) but in the chromosome. Furthermore, some evidence suggests that multidrug-resistant strains are less virulent than susceptible ones, as this is the behavior seen in animal models. This has a biological cause: the mutation that most frequently determines whether a strain becomes resistant to isoniazid affects the catalase-peroxidase enzyme of the bacillus. Without this enzyme activity, the microorganism is more vulnerable against super-oxygenated compounds produced by the host's macrophages to attack it. In this case, the host can win the battle with greater ease, decreasing the possibility of progression of the infection to disease and further spread of the bacilli.

However, in this decade, we have adopted a poor practice that fosters the spread of multidrug resistance. Since the clinical management of multidrug-resistant tuberculosis cases is very difficult, patients are often referred to specialized hospitals located in major urban centers where there is also a concentration of HIV-infected patients. In this environment, multidrug-resistant strains find a niche where they can reproduce and spread rapidly and freely. This niche is provided by the immunosuppressed host, which seems to foster the prevalence of certain strains. In 1994, the degree of multidrug resistance was measured for the first time in Argentina among patients with AIDS. The study was carried out during a peak period of nosocomial outbreaks of multidrug-resistant tuberculosis, which were later confirmed through excellent molecular epidemiology studies *(4–6)*. Under those circumstances, the proportion of patients with AIDS-associated tuberculosis included in the study (12.6%) was over fivefold the national estimate for that same time period *(7)*. The degree of multidrug resistance among these patients was extremely alarming. Based on this analysis, and in contrast with information disseminated in international documents *(8)*, the rates for the population with AIDS should have been analyzed independently and not averaged in with the rest of the population *(9)*.

Since this study, the PNCTB has given priority to the decentralization and supervision of the treatment in primary health services, placing special emphasis on geographical areas with greater numbers of patients and higher incidences of tuberculosis. A cohort study conducted in 1997 showed that 38.2% of the treatments administered had been supervised and that the rate of cure was 71.1% *(10)*. These results show progress, but are still far from the international objective to generalize directly observed treatment and achieve an 87% cure rate.

There still is no evidence of significant progress in controlling nosocomial transmission. Analysis of the registries at national reference laboratories and hospitals with the highest number of outbreaks in Argentina show that the number of multidrug-resistant cases remained relatively stable between 1995 and 1998 *(11)*. Care of multidrug resistance cases continues to be provided in specialized hospitals in cities with high population densities, and is strongly associated with AIDS. Most of these patients are infectious, and multidrug-resistant strains have already been transmitted to health workers in the hospitals where these patients receive care.

Judging by the distribution and profile of this multidrug resistance, nosocomial strains tend to prevail among patients with multidrug-resistant tuberculosis. Isolates recovered from patients and sent to reference laboratories in 1998 are being classified by the genetic fingerprinting method at the Pan American Institute for Food Protection and Zoonoses. This will allow the identification of the strain diversity currently circulating in the country, the prevalence of each strain, the length of the circulation period after their identification, and potential means of transmission, both within and outside of hospitals. Simultaneously, the National Network of Tuberculosis Laboratories launched a new national study that will make it possible to monitor trends of multidrug resistance rates and have a reliable indicator of the efficacy of control measures.

The following conclusions may be drawn from analyzing the information available on multidrug resistance in Argentina.

- Multidrug resistance is not a cause for concern among patients with no history of prior tuberculosis treatment, immunosuppression, or repeated hospitalization in specialized hospitals for infectious diseases.
- Multidrug resistance is the result of poorly administered treatment and it has increased because inadequate criteria and biosafety measures have been applied in hospitalizing patients. At present, multidrug resistance is confined to well-defined pockets of the population.

Once multidrug-resistant strains have been established in a susceptible community, they are very difficult to eliminate. There is no highly effective drug therapy to rotate antibiotics and halt the production of multidrug-resistant bacilli in patients, thereby eliminating the selection of these microogranisms. Furthermore, when it is not possible to enforce first-line drug regimens, it is difficult to ensure the continuous availability and proper administration of more complex, toxic, and expensive treatment regimes using drugs of restricted production. Under these circumstances, the multidrug resistance encoded on the chromosome is not transmitted horizontally, but vertically from one generation of bacilli to the next, becoming a stable and lasting characteristic of the species. The bacillus is capable of consistently retaining the information learned in its effort to win the battle against humankind.

Paradoxically, while we have not managed to discover new weak points in the bacillus, scientists have broken it down so that the complete sequence of its chromosome has been deciphered. With some exceptions, the new antibiotics being manufactured are actually old antibiotics with some modification in their structure or some new component. Once the bacillus has become resistant to an antibiotic, it need only evolve very little to become resistant to variations of the drug. Thus, if we do not heed the alarms and eliminate poor practices, we could succeed in destroying the precious tool that took so long to design. We could even put an end to the era of antibiotics.

The following institutions and individuals participated in the present study:

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Advisory Commission: Argentine Commission on Bacteriology of Tuberculosis (President, Gladis Amadio; Vice-president, Martha DiLonardo; Secretary, Lucía Barrera. Members: Omar Latini, Graciela Poggio, Isabel Terez, Carlos Fernández Pascua, Horacio Rouselle, Nilda Pascussi, Néstor Blazquez and Ana Etchart.)

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TRENDS IN ANTIMICROBIAL CONSUMPTION IN CHILE

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The purpose of this study was to determine the consumption patterns for antimicrobial drugs in Chile as part of a Ministry of Health project to develop a policy for the rational use of antibiotics. International Marketing System data on sales by private pharmacies countrywide in 1996 and 1997 were utilized. For each antimicrobial drug the information obtained was compared with data from 1988. The unit used to measure consumption was the number of defined daily doses (DDD) per 1,000 population per day. A large increase in consumption was detected between 1988 and 1997 for broad-spectrum oral penicillins, cephalosporins, fluoroquinolones, and macrolides. Only chloramphenicol consumption declined in the 10 years studied.

INTRODUCTION

Supposedly, there has been a notable increase in the consumption of antimicrobial drugs in Chile. The rising purchasing power of the population in the past 10 years, sales promotion by pharmaceutical laboratories, the business nature of pharmacies, and the lack of education and awareness of the problems generated by the overconsumption of these drugs are possible causal factors. However, the current uncontrolled sale of antibiotics in the country may also be a contributing factor.

Although the labels on antimicrobial drugs indicate "sale by prescription" and require the pharmacy to retain prescriptions for cephalosporins, aminoglycosides, and other drugs, in the absence of monitoring systems these requirements are not observed in practice in the majority of private pharmacies in Chile *(1)*. Thus, it is possible to purchase any antimicrobial drug with or without a medical prescription. There is the added risk of patients, on their own initiative, consuming improper dosages over inadequate periods of time, as these products have not been prescribed and are not accompanied by information or instructions from an authorized professional. The risk of creating antimicrobial resistance through indiscriminate mass consumption of antibiotics is real and has been demonstrated in closed studies and population studies *(2, 3)*.

The general objective of this study was to determine antimicrobial consumption patterns in the community in 1996 and 1997. The specific objectives proposed were as follows: to determine the trends in antimicrobial drug consumption in the past 10 years; to identify

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the groups of antimicrobials with the highest levels of consumption in the community; and to determine the approximate expenditure arising from community consumption of antimicrobials.

The first part of the study analyzed the information in the International Marketing System (IMS) corresponding to the sale of antimicrobials in the country's private pharmacies in 1996 and 1997. For comparison purposes, the unit used to measure consumption was the number of defined daily doses (DDD) *(4)* per 1,000 population per day *(3)*, which, as its name indicates, reflects consumption of the DDD specific to the antimicrobial *(5)* for every 1,000 inhabitants in a day *(6)*.

This unit of consumption carries some biases; for example, it utilizes the DDD of the specific drug as established for adults; it does not indicate whether consumption is by prescription, or provide information on the actual *use* of the drug after purchase. Nevertheless, it is the measure utilized most frequently in population studies worldwide, and makes it possible to obtain a homogeneous picture of consumption patterns *(7)*. This same consumption unit was utilized in a similar study conducted in Chile in 1989, against which the results obtained in this study were compared *(8)*.

MATERIALS AND METHODS

Population census data provided by the National Institute of Statistics (INE) indicated that the total population of the country was 12,748,207 in 1988; 14,418,864 in 1996; and 14,622,534 in 1997. Data on the sale of all antimicrobial drugs were grouped by generic name; the antibiotic groupings are shown in Table 1. With respect to consumption, it was assumed that antibiotics were sold to be consumed and that the quantities sold equaled those consumed.

Because there were multiple presentations and brand names for each antimicrobial under study, consumption was converted to grams, obtained by multiplying the dosage in grams by the number of tablets per container and then by the number of containers of each drug sold. In the case of liquid dosage forms (syrups and suspensions), the number of grams was calculated by dividing the number of milliliters per container by the number of milliliters per dose and then multiplying the result by the dose in grams and then by the amount sold.

To calculate the DDD per 1,000 population per day, the specific DDD for each antimicrobial as published by the Council of Nordic Medicine *(4)* was used. The DDD is an arbitrary measure based on the average daily maintenance dosage of a drug used for its principal indication for an adult in a given dosage form. The specific DDDs utilized are presented in Table 1.

The formula utilized for the unit of consumption used in the study was:

$$\text{DDD per 1,000 population per day} = \frac{(\text{no. of grams sold annually}) \times (\text{1,000 inhabitants})}{(\text{365 days}) \times (\text{specific DDD}) \times (\text{population})}$$

Thus, two DDD per 1,000 population per day is interpreted in the following way: on average, each day of the year under study, two out of every 1,000 inhabitants would be consuming the specific DDD of the antibiotic in question.

RESULTS

Table 2 shows the number of grams of each antimicrobial drug sold in 1996 and 1997. Consumption was highest for amoxicillin, with approximately 24 tons sold in 1996 and 27 tons in 1997. The table also shows the DDD values per 1,000 population per day for each drug listed, with amoxicillin reaching the highest number, 5.2, in 1997. This means that for every 1,000 population, on any given day more than five people, on average, were consuming the specific DDD for amoxicillin, which corresponds to 1 g. In Chile in 1997, on an average day, 12 out of every 1,000 people re-

TABLE 1. Groups of antimicrobials under study and their specific defined daily doses (DDD)

Group	Antibiotic	Specific DDD (grams)
Oral macrolides	Clarithromycin	0.5
	Azithromycin	0.3
	Erythromycin	1
	Lincomycin	1.8
	Roxithromycin	0.3
	Clindamycin	1.2
Penicillins, broad-spectrum, oral	Amoxicillin	1
	Ampicillin	2
	Amoxicillin-clavulanic acid	1
	Sultamicillin	1.5
	Sulbactam	1
Penicillins, med./narrow-spectrum, oral	Cloxacillin	2
	Flucloxacillin	2
	Phenoxymethylpenicillin	2
Penicillins, med./narrow-spectrum, parenteral	Benzathine benzylpenicillin	1.2 MUI*
	Sodium penicillin	3.6
Cephalosporins, oral	Cefadroxil	2
	Cefuroxime	1
	Cefixime	0.4
	Cefradine	2
	Ceftibutene	0.4
	Cefaclor	1.5
	Cefalexin	2
Fluoroquinolones	Ciprofloxacin	1
	Norfloxacin	0.8
	Fleroxacin	0.4
Trimethoprim assoc.	Cotrimoxazole	0.4
Tetracycline and associated	Tetracycline	1
	Minocycline	0.2
	Doxycycline	0.1
Chloramphenicol and associated	Chloramphenicol	3

Note: Because the objective of the study was to determine community consumption of antibiotics, parenteral dosage forms were excluded, except for sodium and benzathine penicillins and the dosage forms for topical use (for example, creams, ointments, and collyria).

* A specific DDD does not appear in the Anatomical Therapeutic Chemical Classification Index, thus 1.2 *MUI* was used.

ceived the DDD of some antibiotic, as indicated by the sum of DDDs per 1,000 population per day. For 1996 the sum was 10.5. Thus, the increase in antimicrobial consumption between 1996 and 1997 was 13.1%, while the increase in the population between those years was only 1.4% (see population data above).

Table 3 shows the percentages (grams) of antimicrobials consumed in liquid form, such as syrups and oral suspensions, used primarily on the child population. It also shows that consumption by children amounted to 50% of the amoxicillin plus a β-lactamase inhibitor, followed by cefadroxil.

TABLE 2. Annual amount in grams sold in 1996 and 1997 and DDD per 1,000 population per day for antimicrobials under study. Chile, 1996 and 1997

Antimicrobial drug	Grams sold		DDD consumed per 1,000 population per day	
	1996	1997	1996	1997
Clarithromycin	569,108	859,555	0.216	0.322
Azithromycin	320,748	537,075	0.203	0.335
Erythromycin	2,948,804	3,300,357	0.560	0.618
Lincomycin	862,905	820,188	0.091	0.085
Roxithromycin	81,549	62,856	0.052	0.039
Clindamycin	42,134	43,240	0.007	0.007
Amoxicillin	24,274,484	27,773,535	4.612	5.204
Ampicillin	6,105,441	6,546,963	0.580	0.613
Amoxicillin-clavulanic acid	1,877,418	2,207,017	0.357	0.414
Sultamicillin	106,089	98,201	0.013	0.012
Sulbactam	–	93,419	–	0.018
Cloxacillin	4,169,144	4,454,177	0.396	0.417
Flucloxacillin	1,430,689	1,765,354	0.136	0.165
Phenoxymethylpenicillin	1,699,713	2,127,526	0.161	0.199
Sodium penicillin	936,747	1,003,279	0.049	0.052
Benzathine benzylpenicillin	1,171,085	1,197,167	0.185	0.187
Cefadroxil	1,283,246	1,469,189	0.122	0.138
Cefuroxime	81,350	113,959	0.015	0.021
Cefixime	77,336	61,336	0.037	0.029
Cefradine	491,775	530,809	0.047	0.05
Ceftibutene	26,342	23,103	0.013	0.011
Cefaclor	8,546	44,397	0.001	0.006
Cefalexin	21,630	21,082	0.002	0.002
Ciprofloxacin	1,111,438	1,388,337	0.211	0.26
Norfloxacin	85,586	74,450	0.021	0.017
Fleroxacin	11,468	9,279	0.005	0.004
Trimethoprim	2,189,085	2,482,683	1.040	1.163
Tetracycline	3,131,264	3,352,864	0.595	0.628
Doxycycline	204,631	233,964	0.389	0.438
Minocycline	361,085	413,049	0.343	0.387
Chloramphenicol	1,255,326	1,271,500	0.080	0.079

Antimicrobial consumption can also be measured by the number of containers sold, as shown for each group of antimicrobials from 1988 to 1997 in Table 4. A substantial increase in sales can be observed, especially in the macrolides, broad-spectrum penicillins, cephalosporins, and fluoroquinolones. The only antimicrobial for which the number of containers sold decreased was chloramphenicol.

Table 5 shows that spending on antibiotics in private pharmacies in Chile increased almost by 300% between 1988 and 1996, and by 20% between 1996 and 1997, by which time it had reached nearly US$ 46 million. Figure 1 shows that macrolides and broad-spectrum penicillins account for the greatest proportion of the expenditure.

Table 6 compares the DDD values per 1,000 population per day for some antimicrobials in 1988 with those obtained in this study. Table 7 shows the percentage increase in 10 years; consumption of amoxicillin in association with clavulanic acid increased the most, and chloramphenicol was the only drug whose sales decreased.

CONCLUSIONS

Analysis of sales in private pharmacies in Chile reveals that the percentual increase in the consumption of most antibiotics studied is higher than the growth in the population, except in the case of chloramphenicol, whose

TABLE 3. Grams of antimicrobials sold in liquid oral dosage forms (syrups and suspensions) and percentage of the total grams sold, for antimicrobials having substantial sales of those dosage forms. Chile, 1996 and 1997

	1996		1997	
Antibiotic	Grams sold (oral/liquid)	Percentage of total	Grams sold (oral/liquid)	Percentage of total
Clarithromycin	205,228	36	241,526	28
Azithromycin	70,174	22	132,858	25
Erythromycin	1,304,639	44	1,362,300	41
Amoxicillin	8,717,766	36	775,736	35
Ampicillin	413,176	7	41,562	7
Amoxicillin-clavulanic acid	986,705	52	092,710	50
Cefadroxil	459,652	36	93,964	40
Flucloxacillin	376,009	26	11,556	23
Trimethoprim	534,874	24	43,219	22

TABLE 4. Number of containers of antimicrobials sold in private pharmacies in Chile, by year and by antimicrobial group, 1988 to 1997

Year	Macrolides	Penicillin, broad-spectrum	Penicillin med./narrow-spectrum	Cephalo-sporins	Trimetho-prim	Tetracycline	Chloram-phenicol	Fluoroqui-nolones
1988	765,800	3,199,000	3,488,600	...	1,409,800	836,500	392,600	...
1989	798,400	3,373,700	3,797,800	185,200	1,457,000	851,400	457,100	...
1990	989,235	3,633,271	3,379,425	214,084	1,478,396	870,288	398,428	...
1991	1,053,390	4,104,949	3,354,194	221,457	1,717,625	1,028,031	402,170	...
1992	1,118,807	4,368,913	3,240,031	247,261	1,578,412	914,297	278,566	...
1993	1,435,275	5,151,850	3,289,910	322,135	2,752,653	883,301	298,809	240,167
1994	1,400,078	5,090,712	3,250,074	367,539	1,543,922	844,178	276,392	269,452
1995	1,615,879	6,213,139	3,548,893	505,036	1,532,223	1,016,890	320,594	307,376
1996	1,818,824	6,858,590	3,676,448	537,154	1,550,226	979,917	341,639	335,728
1997	2,076,073	7,799,949	4,035,888	563,902	1,692,889	1,055,055	330,348	414,625

Source: International Marketing Service.
... Data not available.

TABLE 5. Sales (in US$) of antimicrobial drugs in private pharmacies, Chile, 1988, 1996, and 1997

Antibiotic group	1988	1996	1997
Macrolides	1,599,000	10,790,383	14,745,019
Broad-spectrum penicillins	3,062,000	11,606,151	13,869,812
Penicillins, medium- and narrow-spectrum	2,997,000	3,814,038	4,141,114
Cephalosporins	1,601,000	3,692,174	4,275,996
Fluoroquinolones	709,000	3,512,470	4,203,548
Trimethoprim	1,968,000	2,199,671	2,316,532
Tetracycline	772,000	1,682,546	2,014,394
Chloramphenicol	319,000	306,255	308,233
Total	13,027,000	37,603,688	45,874,648

Source: International Marketing Service.

consumption decreased between 1988 and 1997. This substantial increase in consumption reflects the shortcomings of the systems that control the utilization of antimicrobial drugs, whose indiscriminate use can cause these products to lose their effectiveness. This increased consumption also has an economic impact on the general population and on the health systems that must cofinance it.

When subjected to detailed analysis, a significant percentage of the consumption of macrolides, broad-spectrum penicillins, cephalosporins, and cotrimoxazole in 1996 and 1997 corresponded to liquid dosage forms, presumably for the child population. This heightens even further the perception of antimicrobial overuse, given that the specific DDD for each antibiotic is calculated for the adult population. Therefore, it may be inferred that consumption is greater than what is indicated by an analysis of the adult population alone.

TABLE 6. Comparison of the defined daily dose consumed per 1,000 population per day for some antimicrobials, Chile, 1988, 1996, and 1997

Antimicrobial agent	1988	1996	1997
Cloxacillin	0.39	0.396	0.417
Ampicillin	0.54	0.58	0.613
Amoxicillin	0.87	4.612	5.204
Amoxicillin-clavulanic acid	0.0025	0.357	0.414
Chloramphenicol	0.097	0.08	0.079
Norfloxacin	0.017	0.021	0.017
Ciprofloxacin	0.032	0.211	0.26
Cephalosporins	0.064	0.237	0.257
Trimethoprim	0.965	1.04	1.163
Fluoroquinolones	0.066	0.237	0.281

There has been an increase in the sale of drugs from the most recently developed groups of antimicrobials, (macrolides, penicillins combined with β-lactamase inhibitors, cephalosporins, and fluoroquinolones) that does not appear to be related to actual need.

FIGURE 1. Sales (in US$) of antimicrobials in private pharmacies, Chile, 1988, 1996, and 1997

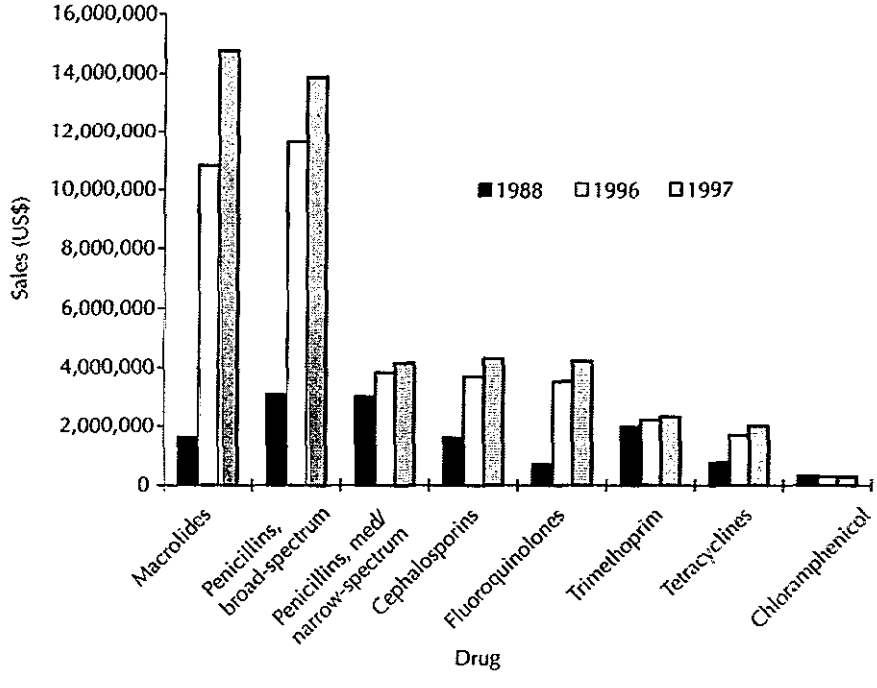

TABLE 7. Increase (%) in defined daily dose of some antimicrobials consumed per 1,000 population per day, Chile, 1988 and 1997

	Defined daily dose per 1,000 population per day		
Antimicrobial	1988	1997	% change
Cloxacillin	0.39	0.417	7
Ampicillin	0.54	0.613	14
Amoxicillin	0.87	5.204	498
Amoxicillin-clavulanic acid	0.0025	0.414	16,460
Chloramphenicol	0.097	0.079	–18
Cotrimoxazole	0.965	1.163	20
Cephalosporins	0.064	0.262	309
Fluoroquinolones	0.049	0.281	473

In Chile, this might reflect the impact of marketing activities and sales policies of both the pharmaceutical industry and private pharmacies, which target an uninformed consumer population.

In light of the data presented and the trends in antibiotic consumption over time, a policy governing the sale and control of antimicrobial agents in the community is urgently needed for their effective utilization.

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STUDY OF ANTIBIOTIC USE IN ARGENTINA AND URUGUAY[1]

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A comparative study of antibiotic use was conducted in the member countries of the Pan American Association of Infectious Diseases. The initial phase included a study of some of the oral antibiotics used most often on an outpatient basis in Argentina and Uruguay during 1997. The results were expressed in daily defined doses (DDD) consumed per 1,000 population per day. A large number of brand names were observed in both countries, even though more than 60% of the sales corresponded to one or two pharmaceutical laboratories. Antibiotic consumption in terms of DDD was greater in Argentina than in Uruguay. In both countries aminopenicillins were used more frequently than macrolides/azalides and quinolones. The ratio of aminopenicillins used alone to combinations with β-lactamase inhibitors was 6:1 in Argentina and 14:1 in Uruguay. Among the macrolides, clarithromycin and azithromycin tended to be used more frequently than older drugs of this group in both countries. This was especially true in Uruguay, where the use of erythromycin and roxithromycin was barely measurable in terms of DDD (0.009 and 0.004 per 1,000 population per day, respectively). The differences found are probably attributable to variations in the self-medication index, level of education of health professionals, and orientation of the pharmaceutical industry.

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INTRODUCTION

Drugs in general, and antibiotics in particular, have become such a familiar aspect of medical practice that, more than any other resource in the medical armamentarium, they run the risk of being used under uncontrolled conditions, and thus incorrectly *(1)*. It is clear that there is considerable pressure surrounding drugs, pressure that comes largely from the pharmaceutical industry and affects not only the professionals who prescribe and dispense them but the general population as consumers *(1)*.

According to data from the International Marketing Service (IMS), antibiotics are the second- or third-largest selling drugs in the world *(2)*. The effects of their improper use on the population—alteration of the normal flora, emergence of resistant pathogens, reduction in overall susceptibility to older first-line drugs in the treatment of common infections, and multiple adverse effects on the organism—are all widely known.

Aware that Latin America is no stranger to this universal situation, the member countries of the Antibiotics Commission of the Pan American Association of Infectious Diseases decided to undertake a study of antibiotic use. The initial objective was to understand and compare consumption of the oral forms of some of the drugs most frequently used in the community. This report presents the findings on the use in Argentina and Uruguay of some of the antibiotics selected for the first phase of the Pan American study.

MATERIALS AND METHODS

The data were obtained from IMS data on antibiotic sales for 1997 in Argentina and Uruguay. Inasmuch as the figures represented about 70% of all oral antibiotic consumption (capsules, tablets, and suspensions) in these countries per year, and included the corresponding dosage, it can be assumed that the findings are a very good reflection of the total consumption of these drugs. In order to allow for comparisons among countries, the results were expressed in daily defined doses (DDD) consumed per 1,000 population per day. The number of DDD consumed is the measure currently recommended by the World Health Organization *(3)*. This unit corresponds to the estimated average dosage used in the principal indication of the drug per day. Although it does not always correspond to the dosage typically prescribed, it comes close to that, and it serves as a good parameter for comparing consumption in different regions and at different times *(4–6)*. The formula for obtaining the DDD per 1,000 population per day at a given time and place is:

$$\frac{\text{Annual grams sold per 1,000 population}}{365 \text{ days} \times \text{DDD of antibiotic} \times \text{number of inhabitants}}$$

The DDDs established universally for the antibiotics included in the first phase of the study are as follows: ampicillin, 2 g; amoxicillin, 1 g; ampicillin + β-lactamase inhibitors, 1.5 g; amoxicillin + β-lactamase inhibitors, 1g; erythromycin, 2 g; clarithromycin, 0.5 g; azithromycin, 0.3 g; roxithromycin, 0.3 g; ciprofloxacin, 1 g; and norfloxacin, 0.8 g.

Argentina currently has an estimated population of 35,000,000 inhabitants and Uruguay, 3,173,700.

RESULTS

Table 1 lists, for the two countries, the total grams sold of each of the antibiotics, the number of brand names under which the antibiotic was sold, and the percentage of total consumption represented by each brand.

As it can be seen, there were large numbers of brand names in both countries, and considerably more in Argentina—particularly in the case of amoxicillin, the new azalides, and the fluoroquinolones. In the two countries, the largest volume of each antibiotic (more than

TABLE 1. Consumption of oral antibiotics and number of brand names under which they are sold in Argentina and Uruguay, 1997

Antibiotic	Grams sold		Number of brand names		Percentage sold	
	Argentina	Uruguay	Argentina	Uruguay	Argentina	Uruguay
Ampicillin	14,570,100	1,799,089	14	17	71 (1)	80 (1)
Amoxicillin	44,613,450	3,289,899	23	12	55 (2)	79 (1)
Ampicillin + β-lactamase inhibitors	260,475	95,860	3	4	92 (1)	68 (1)
Amoxicillin + β-lactamase inhibitors	8,469,900	206,639	4	3	66 (1)	100 (1)
Erythromycin	4,805,440	21,513	2	6	89 (1)	97 (1)
Clarithromycin	2,261,150	240,297	1	3	100 (1)	91 (1)
Roxithromycin	792,880	1,418	5	1	74 (1)	100 (1)
Azithromycin	2,162,019	108,536	10	4	69 (2)	63 (1)
Ciprofloxacin	3,193,950	22,315	11	7	64 (2)	72 (2)
Norfloxacin	6,736,800	15,995	10	4	71 (2)	79 (1)

60%) was sold by one or two pharmaceutical laboratories, regardless of how many brand names there were.

Table 2 shows DDD consumption per 1,000 population per day in the countries studied.

There was a clear preference for amoxicillin over ampicillin because of the posologic convenience offered by the former (it can be taken with food, smaller number of doses).

The consumption of ampicillins and simple amoxicillins vis-à-vis their respective combinations with β-lactamase inhibitors differed considerably in the two countries: whereas in Argentina the ratio was 6:1 (4.07 DDD per 1,000 population per day for ampicillin and amoxicillin versus for 0.67 ampicillin + inhibitors and amoxicillin + inhibitors), in Uruguay the ratio was 15.7:1 (3.62 and 0.23, respectively).

In Uruguay there was a marked difference in the use of macrolides/azalides. In terms of DDD, the consumption of erythromycin and roxithromycin was very low, and azithromycin was slightly ahead of clarithromycin. In Argentina, although erythromycin continues to be used, there was a clear trend toward the newer drugs in this group. The differences between the latter were not significant, although there was greater use of azithromycin than clarithromycin and roxithromycin.

DISCUSSION

There are several types of drug utilization studies *(1)*. In one of them, consumption is studied to determine the volume of a given drug sold at a particular place and time. This type of study is basically descriptive in nature, but valid conclusions can be drawn. The resulting information assumes greater importance if it can be used to evaluate strategies to modify existing situations through specific action. The work reported here falls within this type of consumption study.

As pointed out earlier, the comparison of consumption using defined daily dosages is

***TABLE 2. DDD per 1,000 population consumed* daily in Argentina and Uruguay, 1997**

Antibiotic	Argentina	Uruguay
Ampicillin	0.57	0.78
Amoxicillin	3.50	2.84
Ampicillin + β-lactamase inhibitors	0.01	0.05
Amoxicillin + β-lactamase inhibitors	0.66	0.18
Erythromycin	0.19	0.009
Clarithromycin	0.35	0.41
Roxithromycin	0.21	0.004
Azithromycin	0.56	0.31
Ciprofloxacin	0.25	0.02
Norfloxacin	0.66	0.02

an approach that largely avoids the type of biases that can emerge from an analysis expressed in unit type measurements (packages of drugs, ampoules), grams or cost. For example, in Argentina 2,261,150 grams of clarithromycin and 2,162,019 grams of azithromycin were consumed. An oversimplified analysis might suggest that these figures are comparable. However, the difference in the daily doses normally used for the two antibiotics would not be taken into account. In fact, the DDDs of azithromycin consumed were much higher than those of clarithromycin (0.56 and 0.35, respectively), despite the fact that absolute consumption was somewhat lower in terms of grams per year. Similar interpretation errors would surface if the total population of the particular country or countries were not taken into account.

When compared in terms of defined daily doses sold in the two countries, antibiotic consumption was substantially higher in Argentina than in Uruguay. Although there were no major differences in the use of older drugs such as ampicillin and amoxicillin, consumption of the new macrolides and azalides as a whole was 34% higher in Argentina (1.12 and 0.72 DDD in Argentina and Uruguay, respectively, per 1,000 population per day), and there was a significant difference in use of the fluoroquinolones ciprofloxacin + norfloxacin in Argentina and Uruguay (0.91 and 0.04 DDD per 1,000 population per day, respectively).

By the same token, there was a striking difference between the two countries in the ratio of ampicillins or amoxicillins with and without β-lactamase inhibitors: in Uruguay, for every 100 daily treatments using the drugs in their simple presentation, there were about 7 treatments combined with β-lactamase inhibitors, whereas in Argentina there were about 16 combined treatments.

In both countries there are a large number of different brand names for antibiotics. This situation points to the need for official regulatory agencies to strictly control the efficacy of all products.

The present study did not undertake a cost analysis, because of the difficulties in drawing comparisons among different countries, given the wide variety of brand names, dosage forms, prices, and stability of the drugs over time, not to mention socioeconomic factors—for example, the impact of treatment with a full course of antibiotics on the average monthly income of individuals or households.

Although this type of study cannot and does not purport to account for the differences in consumption between the countries, it is highly unlikely that they are due to variations in the incidence of diseases that require treatment with antibiotics. They are more likely to be due to such factors as self-medication and differences in the approaches taken by the pharmaceutical industry to influence decision-making by doctors. Self-medication is a daily fact in our countries *(7)*, fostered by widespread disregard for legislation on the requirement of prescriptions for the sale of antibiotics. This practice is condoned by the culture, particularly in regard to the management of febrile illness associated with respiratory symptoms, which for the most part are viral in origin *(8)*.

The level of medical education also varies, not only among countries, but even among professionals in the same institution. We believe that the problem of improper antibiotic use can be attributed in large measure to this factor. The education of health professionals and their continuing education should be designed, inter alia, to generate the correct and effective use of diagnostic and therapeutic resources. In reality, continuing education of health professionals is influenced to a greater or lesser degree by medical propaganda from commercial laboratories, which is geared more to a potential increase in sales of a given product than to important educational aspects.

Measuring the quality of antibiotic use is not a simple task. Furthermore, there are no definitions of acceptable quality in prescribing antibiotics, despite the many authors that suggest that these drugs are being used inappro-

priately in about 50% of the cases *(9)*. Other types of studies of antibiotic use, such as those that take into account the prescription versus indication and indication versus prescription of drugs in the context of the various diseases for which they are used, can contribute to the development of strategies for improving prescription practices, and hence, the utilization of antibiotics in our countries. The drugs evaluated in the present study are priority indications in outpatient care. It is essential to rationalize treatment in this context, not only for economic reasons but to reduce the development and spread of resistant organisms in the community *(10)*.

Finally, extending the present study to the rest of the member countries of the Antibiotics Commission of the Pan American Association of Infectious Diseases will be a valuable contribution to the compilation of information on new comparisons and to the search for a common strategy.

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ANTIMICROBIAL CONSUMPTION IN HOSPITALS: COSTS AND CONSEQUENCES OF USE AND ABUSE

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The increase in antibiotic consumption and antimicrobial resistance as a public health problem is analyzed, together with its economic impact on hospital care.

In 1992 the Infectious Disease Unit at the Hospital Español de Mexico instituted strict monitoring of all hospitalized patients to determine the incidence of nosocomial infections, evaluate hospital epidemiology and the use of antimicrobials, and determine and analyze the inappropriate prescription of antibiotics and the cost of their misuse and abuse.

Among the patients admitted to the Hospital Español de Mexico from 1992 to 1997, the percentage treated with antibiotics ranged from 52% to 77% per year; some 64% to 76% of the patients received antibiotics for perioperative prophylaxis. Roughly 30% of the time, antibiotics were prescribed for the treatment of a community or nosocomial infectious process. In addition, the improper use of antibiotics in prophylactic regimens primarily involved poor choice of the antibiotic (47% to 82%) and errors in the length of the treatment (9% to 36%).

During that same period an alarming increase was observed in the use of third-generation cephalosporins, fluoroquinolones, carbapenems, fluconazole, and vancomycin. It has been shown that the change in the use of antibiotics paralleled the changes in hospital microbiology and the increase in bacterial resistance. An increase in infections produced by Pseudomonas aeruginosa, *nonfermenting Gram-negative bacilli (mainly* Acinetobacter *spp. and* Citrobacter *spp.), and* Candida *spp. was observed, along with an alarming increase in the number of* Enterococcus *spp. infections.*

The data on antibiotic purchases in 1998 indicate that ciprofloxacin and two third-generation cephalosporins are the drugs that generate the greatest expenditures. Nearly 30% of antibiotic use in hospitals is inappropriate and can be avoided with restrictive policies.

INTRODUCTION

Two main problems related to the consumption of antibiotics are widely recognized: progressive bacterial resistance, to date unchecked; and the rising, often unnecessary, costs generated by the abuse of these drugs.

Knowledge about bacterial capacity for developing resistance is not new. Resistant strains began to appear at least 25 years ago. Initially such resistance was confined to a

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single antibiotic. For a little over a decade, however, it has not been unusual to isolate organisms resistant to 10 or more antimicrobial drugs *(1)*.

The abuse of antibiotics has three interdependent variants. On the one hand, patients expect to receive antibiotics for the most trivial infections, and not infrequently, for any type of fever. Physicians, for their part, are afraid of uncontrolled infections, and the false sense of security offered by antibiotics leads them to prescribe the drugs without first doing a thorough diagnostic workup. Furthermore, it should be noted that in many countries, particularly developing countries, antibiotics can be freely obtained without a prescription. In Mexico, quinolones, second- and third-generation cephalosporins, carbapenems, and monobactams can be obtained without a prescription. There is thus an urgent need to draft and implement restrictive legislation at the national and international levels.

With regard to hospital use of antibiotics, we must ask: Why is it necessary to restrict the use of antimicrobials in hospitals? We know that nearly 40% of antibiotic use is not for human consumption, and that more than 80% of these compounds are prescribed outside the hospital environment, particularly for respiratory infections that are mostly viral in origin *(1, 2)*. Why begin now to impose controls if we know that it will be impossible to return to the bacterial susceptibility that existed 20 or 30 years ago? The answers are simple. In addition to restricting the use of antibiotics in veterinary medicine and animal husbandry—a concern dealt with in another section of this publication—it is imperative to curb the abuse of antibiotics in current medical practice in order to: 1) prevent the further spread of bacterial resistance before our patients die of untreatable infections *(3, 4)*; and 2) contain increases in hospitalization costs before they climb so high as to impede the highly sophisticated and technically complex care provided by modern medicine. Another major reason for analyzing the costs of antibiotic use in hospitals and the benefits of a restrictive policy is that it is unquestionably easier to move from small-scale to large-scale in imposing controls. That is, it will be easier to control the 100 or 200 physicians who work in a public or private institution than to wait for agreements based on international consensus or compulsory national standards that could come too late *(4, 5)*.

BACTERIAL RESISTANCE: A WORLDWIDE PROBLEM

This topic is dealt with at greater length in other sections of this work. However, a brief review will serve as a foundation for the analysis in this chapter. As noted above, we have known for several decades that bacteria can become resistant to antibiotics *(1,5,6)*, but only for the last seven or eight years has this been considered a worldwide public health problem. Resistance has emerged gradually over a period of many years, and it is inexplicable that the medical community has not yet acted. We have known since the early 1980s about the emergence of methicillin-resistant *Staphylococcus aureus*, with the notable exception of the Netherlands *(6, 7)*. During the 1990s numerous reports have been published on serious infections caused by strains of *Escherichia coli*, *Neisseria gonorrhoeae*, *Salmonella typhi*, and *Streptococcus pneumoniae* that are resistant to penicillins and cephalosporins. There are reports of nosocomial epidemics of *Pseudomonas aeruginosa*, *Klebsiella* spp., and other multiresistant nonfermenting Gram-negative bacilli. In addition, there is mounting alarm about the emergence and spread of strains of vancomycin-resistant *Enterococcus* spp. *(8–14)*.

One reason the medical community has shown little concern about bacterial resistance is that since the 1980s the pharmaceutical industry has been developing new antibiotics that offer new therapeutic options. Why worry about bacterial resistance if other scientists have undertaken to develop new, more powerful, broader-spectrum drugs? There is no doubt, however, that the pharmaceutical

industry's ability to develop new antibiotic products has been, and will continue to be, outpaced by the defensive capabilities of bacteria. Today, just as new quinolones are reaching the market in countries such as Mexico, several authors have already reported significant patterns of resistance in different species of Gram-negative bacilli *(10, 12, 13)*. In 1998 we conducted a susceptibility study at the Hospital Español de Mexico, using disk diffusion technique, with two new quinolones (levofloxacin and trovafloxacin). We found resistance of more than 40% for *E. coli*, 42% for *P. aeruginosa*, 20% for *S. aureus*, and 22% for *Enterococcus* spp. (unpublished data).

Carelessness in implementing and maintaining strict hygiene, especially in the hospitals of countries with few economic resources, has led to the use of increasingly powerful and expensive antibiotics. Moreover, not only antibiotics, but heavy metals, disinfectants, and antiseptics have exerted selective pressure on bacteria in the hospital environment.

The cornerstone of any effort to reduce antibiotic use and thereby halt or slow the emergence of resistance and bring down costs must be the education of the medical community and the patient population. It is especially important to educate parents, who frequently demand that antibiotics be prescribed for their sick children to prevent complications *(15–17)*.

Since more than 75% of the prescriptions written for antibiotics are debatable *(1, 2, 15, 16)*, and the irrational increase in the use of quinolones and cephalosporins has had a major impact on the spread of bacterial resistance and the cost of hospital care, the answer may lie in addressing the poor academic training in infectious pathology provided to general physicians. In 1994, concerned about the study methods of medical residents and specialists at the Hospital Español de Mexico and about evidence of excessive antimicrobial use, we conducted an anonymous survey in order to weigh the possible introduction of a system to restrict antibiotic use (Table 1).

As Table 1 shows, 345 questionnaires were completed by the residents and specialists. Among the residents, a majority believe that they receive sufficient information in the classroom and less than 20% also study journals and books in the specialty. Although more than half the specialists said they obtain information from journals in the specialty, 100% acknowledged that many of their prescriptions are based on information provided free of charge by the pharmaceutical industry. A large majority of the residents said it would be useful to establish a system for the surveillance and control of antimicrobial use, but nearly half the specialists believe it to be unnecessary. More than 30% said they do not need a "policeman or guardian" who interferes in their practice of medicine. It should be noted that much of the information provided by the pharmaceutical industry is biased and some is erroneous.

A sponsored microbiology review aimed at introducing a new quinolone was published recently *(18)*. Among its many errors, the article puts the susceptibility of *P. aeruginosa* to vancomycin at 60%, erythromycin at 76%,

TABLE 1. Study methods of medical residents and specialists at the Hospital Español de Mexico. 1994 Survey

	Residents		Specialists	
	Number	Percentage	Number	Percentage
Number of questionnaires completed	175	50.7	170	49.27
Study method				
Hospital sessions	160	91.4	90	52.9
Individual instruction or personal communication	145	82.8	35	20.5
Specialized journals	32	18.3	95	55.8
Specialized books	22	12.6	34	20.0
Information from the pharmaceutical industry	170	97.1	170	100.0
Yes, agrees with a system to control antibiotics	140	80.0	90	52.9

clindamycin at 68%, and ampicillin at 100%. Is this the type of publication that the majority of our physicians use to obtain the information they need to prescribe antibiotics? Any effort made by governments, ministries of health, and microbiologists and infectious disease specialists will fall short in the absence of a minimum level of training and a commitment on the part of the pharmaceutical industry.

USE AND ABUSE OF ANTIBIOTICS IN HOSPITALS

Analysis of antibiotic consumption and costs in the Hospital Español de Mexico must begin with a description of the hospital setting. This is a tertiary-level university institution with 600 available beds. It serves two patient populations: private patients and beneficiaries of the Sociedad de Beneficencia Española de Mexico (Spanish Welfare Society of Mexico). For the latter group, medical care and the cost of hospitalization, surgery, and drugs are all free, which means that the cost of hospital care has a direct impact on the economics of the hospital. Given the special nature of this hospital, the conclusions to be drawn from an analysis of antibiotic use and associated costs cannot be generalized to other public or private hospitals.

Since 1992, the Infectious Disease Unit has strictly monitored all hospitalized patients. Each file is reviewed when the patient is discharged, and the data are entered into a database that is then analyzed using computer software specially designed for this purpose. The database captures multiple variables, among them the incidence of nosocomial infections, hospital epidemiology, consumption of antimicrobials, analysis and classification of antibiotic regimens that were prescribed incorrectly, and the costs generated by the misuse or abuse.

Table 2 shows the number of patients admitted in each year of this six-year period, and the number and percentage of patients who received an antibiotic. As indicated, in each year a significant percentage of patients received antibiotic treatment or prophylaxis, a figure that ranged from 52% to more than 77%. It is safe to say that no other group of drugs is prescribed for a greater number of patients.

The distribution of antibiotic regimens presented in Table 3 shows that a large number of patients received antibiotics for prophylactic reasons, for the most part perioperatively; while approximately 30% of the antibiotic prescriptions were to treat some community-acquired or nosocomial infection. On a fair number of occasions the patients received antibiotics for neither prophylactic nor therapeutic reasons.

Once the results are captured in the database, physicians of the Infectious Disease Unit analyze each use of an antibiotic and describe the administration of the drug as correct or incorrect; in the latter case, the reasons are specified. The error is classified as incorrect spectrum when the antibiotic, used empirically, is not active against the organism or organisms in question; as incorrect dosage when the dosage is larger or smaller than the one recommended for the age, weight, or metabolic status of the patient; and as incorrect interval or duration when the antibiotic is administered for a longer or shorter period than recommended or, in the case of surgical prophylaxis, when more than the standard three doses are given or when the drug is administered after the surgery. A toxicity error is noted when use of an antibiotic was unnecessary, and a cost error in cases where another therapeutic or prophylactic option was both acceptable and less expensive.

A therapy regimen is also described as incorrect when the physician, having received the results of the antibiogram that offer the option of another drug with a narrower spectrum or lower cost, does not change the drug. In some cases several errors are made in the selection or administration of an antibiotic; these regimens are described as incorrect for several reasons (Table 4).

As shown in Tables 4 and 5, the selection or administration of the drug is classified as in-

TABLE 2. Total number of patients admitted and percentage of patients who received antibiotics, per year, Hospital Español de Mexico, 1992 to 1997

	1992	1993	1994	1995	1996	1997
Total number of patients	16,355	14,073	12,131	13,747	13,102	14,095
Patients who received antibiotics	9,299	7,367	6,332	10,587	9,189	9,989
Percentage	56.85	52.34	52.19	77.01	70.13	70.89

TABLE 3. Distribution of antibiotic prescriptions by type of use, per year, Hospital Español de Mexico, 1992 to 1997

Type of use	1992	1993	1994	1995	1996	1997
Number of prescriptions	11,292	8,157	6,659	11,523	11,324	9,645
Percentage used in prophylactic regimens	68.2	76.2	54.8	66.4	65.1	64.2
Percentage used in therapy regimens	30.2	30.9	42.4	30.1	32.2	33.2
Undetermined use	1.46	1.8	2.6	3.4	2.7	2.6

correct in a significant percentage of cases. This has a direct impact on the selective pressure on hospital bacteria and on the cost of care, the latter resulting from the use of higher-priced antibiotics of a more recent generation.

It should be mentioned that in 1993 a campaign was launched to standardize the criteria for selecting prophylactic antibiotics in surgery, since there was no consensus on this subject. For example, it was found that for a single type of surgery, performed by a single surgeon, up to eight different regimens for preoperative prophylaxis were used.

In 1994 a guide was published and distributed to all surgeons, residents, and students *(19)*. Beginning in 1995 a marked reduction was seen in the consumption of broad-spectrum antibiotics, along with greater adherence to the suggested regimens. It cannot be denied, however, that the surgical teams feared infectious complications after using older drugs or those with a narrower spectrum, and this at times led to the administration of antimicrobials over longer periods than recommended, sometimes for up to seven days. For this reason, the proportion of errors associated with time of administration increased from less than 10% in 1992 to more than 36% in 1997. This situation is troubling, since not only was cost control not achieved but the time of antibiotic pressure was lengthened in comparison with 1993.

A new educational campaign is currently planned in order to reduce the number of doses administered, and there will be an effort to standardize criteria among the members of the different surgical teams on the same service, since it was observed that the incidence of surgical wound infections in clean surgery did not change when antibiotics with a narrower spectrum were utilized (0.93% before the change and 1.1% afterwards).

TABLE 4. Percentages of incorrectly used antibiotics in prophylactic regimens, by reason for incorrect use, Hospital Español de Mexico, per year, 1992 to 1997

Type of incorrect use	1992	1993	1994	1995	1996	1997
Incorrect use as a proportion of total prophylactic regimens (%)	35.6	24.1	20.1	21.6	19.4	16.1
Reason for incorrect use:						
Spectrum	81.8	68.4	64.2	70	52.1	46.7
Dose	0.3	0.45	1.32	2.2	2.1	3.2
Interval or time	9.2	27.1	31.2	24.6	31.1	36.2
Toxicity	2.9	2.5	2.2	0.9	0.7	0.7
Excessive cost	0.1	0.2	0.2	1.2	1.7	3.1
Several reasons	5.7	1.35	0.88	1.1	12.3	10.1

In analyzing the incorrect use of therapeutic regimens, what stands out is the dramatic reduction in errors derived from failure to consult the results of the antibiogram, as well as the significant reduction in antibiotic regimens with unnecessary toxicity. Unquestionably, improved technologies in hospital microbiology laboratories in recent years and better teaching, especially of medical students and residents, have had a positive impact.

On the other hand, a substantial proportion of incorrect prescriptions are linked to the use of excessively costly antibiotics; this percentage increased from less than 1% in 1992 to more than 15% in 1997. If the 1970s already saw an irrational or inappropriate use of antibiotics from 38% to 66% of the time *(20, 21)*, it is evident that after 25 years there have been no major changes.

Tables 6 and 7 show the evolution of antibiotic consumption in the Hospital Español de Mexico. There is a marked reduction in the use of first- and second-generation cephalosporins, cotrimoxazole, and first-generation quinolones. In addition, a drastic reduction in the use of natural penicillins is apparent. On the other hand, during this period there was an alarming increase in the use of third-generation cephalosporins (antipseudomonal), fluoroquinolones (especially ciprofloxacin), carbapenems, oral antimycotics such as fluconazole, and glycopeptides (mainly vancomycin). Table 7 shows a marked increase in the use of ciprofloxacin, ceftazidime, vancomycin, fluconazole, and amphotericin as individual drugs. Several years ago Castle et al. observed that, between 1977 and 1992, the only antibiotics for which no increases in consumption were recorded were penicillin, tetracyclines, and chloramphenicol *(22)*.

Many published studies have shown that the change in antibiotic use parallels the changes in hospital microbiology and the increase in bacterial resistance *(23–26)*. Table 8 shows a significant increase in infections produced by *P. aeruginosa*, other nonfermenting Gram-negative bacilli (mainly *Acinetobacter* spp. and *Citrobacter* spp.), and *Candida* spp. The number of infections produced by *Enterococcus* spp. has also risen alarmingly. Consequently, it is worth asking whether the emergence of multiresistant strains forced doctors to prescribe more broad-spectrum and antimycotic drugs, or if it was abuse of these antibiotics that led to the appearance of resistant bacteria. Various authors have correlated the increase in the use of ciprofloxacin and third-generation cephalosporins with a greater number of infections from resistant strains of *Pseudomonas* spp. and, particularly, *Enterococcus* spp. *(11, 24, 27)*. The results of our studies support these observations.

Although beyond the scope of this work, it is worth noting that despite the growing number of infections from *Enterococcus* spp., we still have not isolated vancomycin-resistant strains; but these undoubtedly will appear in a few years, if not sooner.

TABLE 5. Percentages of incorrectly used antibiotics in therapeutic regimens, by reason for incorrect use, *Hospital Español de Mexico, 1992 to 1997*

Type of incorrect use	1992	1993	1994	1995	1996	1997
Incorrect use as a proportion of total therapeutic regimens (%)	19.9	18.1	20.3	17.6	14.1	13.2
Reason for incorrect use:						
Spectrum	62.5	57.6	52.2	62.9	60.0	59.4
Dose or time	2.8	2.3	5.2	1.2	3.2	3.3
Not matched to antibiogram	9.8	3.3	1.4	1.2	1.9	1.2
Toxicity	10.7	17.7	29.1	22.2	19.4	19.6
Excessive cost	–	1.4	7.1	7.4	12.1	15.5
Several reasons	13.3	17.7	5.0	5.1	3.4	1.0

TABLE 6. Percentage of total consumption of antimicrobials, by groups of drugs, per year, Hospital Español de Mexico, 1992 to 1997

	1992	1993	1994	1995	1996	1997
First-generation cephalosporins	30.2	30.9	22.3	26.0	18.2	17.2
Broad-spectrum penicillins	15.3	14.6	12.8	13.1	9.2	7.4
Third-generation cephalosporins	11.3	10.9	12.0	13.3	16.0	17.2
Cotrimoxazole	7.6	6.4	6.8	6.1	5.0	3.7
Imidazoles	4.6	4.0	5.7	4.3	5.1	5.1
Aminoglycosides	4.7	4.2	3.9	1.3	2.2	3.3
Second-generation quinolones	3.2	4.0	6.2	6.3	7.6	9.4
Antipseudomonal aminoglycosides	3.5	3.7	5.8	3.4	4.2	4.7
Second-generation cephalosporins	3.8	3.8	3.3	1.6	2.2	1.7
Natural penicillins	3.9	3.3	2.4	1.2	0.9	0.9
First-generation quinolones	2.3	2.7	2.6	1.8	1.1	1.0
Carbapenems and monobactams	2.0	1.7	2.8	2.7	3.3	4.2
Semisynthetic penicillins	1.7	1.5	1.6	1.7	2.1	2.2
Macrolides	1.2	1.4	2.5	2.4	3.3	3.5
Antimycotics	0.7	1.3	2.9	3.0	3.4	4.1
Antivirals	0.7	1.0	2.4	1.6	2.4	2.8
Third-generation cephalosporins, antipseudomonal	0.7	0.9	2.0	3.1	4.5	5.1
Glycopeptides	...	0.2	0.3	2.2	3.1	4.4
Others	2.3	3.7	2.8	5.7	6.2	2.1

... No information

TABLE 7. Percentage of total consumption of antimicrobials, by drug, Hospital Español de Mexico, 1992 to 1997

Drug	1992	1993	1994	1995	1996	1997
Cefalotin	39.8	41.4	32.6	35.1	28.1	17.2
Ampicillin	15.4	13.0	9.9	9.3	7.1	4.7
Cefotaxime-ceftriaxone	13.5	12.3	14.6	16.2	18.1	19.9
Ciprofloxacin-ofloxacin	3.8	4.4	7.8	7.3	11.0	12.4
Cefuroxime	4.2	4.1	4.4	2.1	1.4	1.6
Gentamicin-tobramycin	4.3	4.0	3.2	4.1	3.2	4.0
Cotrimoxazole	3.5	2.9	3.3	0.9	1.2	1.1
Sodium penicillin	3.3	3.2	2.2	0.2	0.4	0.3
Norfloxacin	3.0	2.8	2.7	2.1	1.1	1.3
Amoxicillin-clavulanate	0.4	2.9	5.9	5.2	6.0	6.9
Dicloxacillin	1.8	1.6	1.8	2.2	3.1	3.0
Metronidazole	1.8	1.4	2.0	2.0	2.2	2.4
Amikacin	0.9	1.0	2.0	1.5	2.2	2.9
Ceftazidime-ceftixozime	0.6	0.7	1.9	2.0	4.0	4.7
Clindamycin	0.4	1.3	1.4	1.4	1.3	1.2
Erythromycin-clarithromycin	0.5	0.7	1.4	1.2	2.6	2.5
Chloramphenicol	0.7	0.4	0.4	0.2	0.4	0.3
Vancomycin	0.2	0.5	0.6	2.2	12.0	4.1
Fluconazole	0.2	0.3	0.7	0.8	1.3	3.7
Imipenem-meropenem	0.2	0.2	0.4	0.6	1.1	2.0
Ampicillin-sulbactam	0.2	0.1	0.1	0.2	0.2	0.3
Rifampicin	0.1	0.9	0.2	0.1	0.1	0.1
Tetracyclines	0.4	0.1	0.1	0.5	0.1	0.1
Piperacillin-tazobactam	0.1	0.1	0.1	0.6	1.0	2.2
Ticarcillin-clavulanic acid	0.0	0.0	0.1	0.2	0.6	0.0
Aztreonam	0.0	0.0	0.0	0.3	0.6	1.2
Amphotericin	0.0	0.0	0.0	0.1	0.5	1.0

TABLE 8. Infectious agents isolated from cases of nosocomial infections, as a percentage of total episodes of infection, Hospital Español de Mexico, 1992 to 1997

	1992	1993	1994	1995	1996	1997
Escherichia coli	26.1	22.2	17.6	7.1	8.4	8.2
Klebsiella spp.	15.4	9.9	8.2	6.6	5.6	5.8
Pseudomonas aeruginosa	12.4	8.7	13.4	19.7	16.6	17.6
Candida spp.	9.4	13.1	11.1	19.7	18.9	16.9
CNS (Staphylococcus epidermidis)	5.8	10.4	11.6	4.4	3.7	4.6
Staphylococcus aureus	9.2	5.6	6.5	3.3	4.1	4.2
Enterobacter spp.	5.7	7.6	10.5	4.9	5.6	5.5
Streptococcus spp.	5.6	4.2	1.2	6.6	4.7	4.8
Proteus spp.	4.1	4.7	1.2	1.6	2.2	1.8
Enterococcus spp.	3.1	2.6	1.2	18.0	18.7	19.1
Serratia spp.	0.4	2.4	11.1	1.1	2.4	3.4
*Nonfermenting GNB**	1.3	2.4	1.2	1.2	3.9	3.1
Staphylococcus viridans group	1.7	2.9	1.2	1.4	1.9	1.1
Clostridium spp.	–	–	–	–	–	–
Clostridium difficile	–	–	–	–	–	–
Bacteroid spp.	0.2	1.5	2.3	1.6	2.4	2.2

*GNB = Gram-negative bacilli.

In the United States of America, it has been calculated that the direct cost of antibiotic use plus the hidden costs of inappropriate use and complications from use is between $100 million and $30,000 million *(28)*. Table 9 shows the monthly expenditures for consumption of antibiotics in the Hospital Español de Mexico in 1998. As can be seen, the monthly cost of antibiotic purchases is over $103,000, almost $30,000 of which is spent on antibiotics prescribed incorrectly.

As the table shows, the three antibiotics that generate the largest share of costs are ciprofloxacin and two third-generation cephalosporins. These three drugs represent a very high percentage of total antimicrobials prescribed (see Table 7). The analysis of antibiotic regimens that were prescribed incorrectly showed, for example, that 33.5% of the prescriptions for ciprofloxacin are incorrect. There is clear abuse of that drug for the treatment of community-acquired diarrheal disorders, a high percentage of which are self-limited and require nothing more than dietary measures and correction of the hydroelectrolytic status of the patient. This quinolone is also frequently prescribed for treatment of community-acquired infections of the upper respiratory tract.

With respect to the third-generation cephalosporins, there are two main reasons for abuse: their use as a prophylactic in surgery, and the treatment of sepsis or bacteremias caused by enterobacteria susceptible to older cephalosporins or to broad-spectrum penicillins. Although the unit cost of cefalotin is low, the number of monthly doses is much higher than for other antibiotics. Incorrect administration is almost entirely due to the unnecessary prolongation of operative prophylaxis. It is noteworthy that two-thirds of fluconazole prescriptions are incorrect. One frequently sees treatment of patients whose sputum cultures were positive for *Candida albicans* in cases where the laboratory failed to exclude samples contaminated by saliva or those of patients in whom yeast was found in the urinary sediment. The most frequent incorrect uses of ceftazidime and piperacillin-tazobactam are in the treatment of Gram-negative bacillus infections (except for *P. aeruginosa)* that are susceptible to less costly narrow-spectrum drugs; they may also be given in suboptimal doses. Vancomycin is used incorrectly in a very high proportion of cases, either to treat infections from *Staphylococcus* spp. susceptible to methicillin or, worse still, as a prophylactic in surgery, most frequently orthopedic surgery.

TABLE 9. Hospital expenditures for antibiotic purchases, by drug, Hospital Español de Mexico, 1998 (in US$)

Antibiotic	Number of doses per month*	Monthly expenditure* (US$)	Percentage of incorrect prescriptions	Cost of incorrect prescriptions
Ciprofloxacin	1,100	17,530	33.5	6,223
Ceftriaxone	700	13,020	22.6	2,942
Cefotaxime	600	11,204	18.6	2,084
Vancomycin	250	8,547	19.8	1,692
Fluconazole	200	7,437	66.8	4,992
Cefalotin	1,700	6,889	22.5	1,550
Amikacin	395	6,382	55.8	3,551
Gentamicin-tobramycin	495	5,575	22.0	1,226
Erythromycin	200	5,136	7.3	374
Piperacillin-tazobactam	100	3,914	12.1	474
Ceftazidime	400	3,621	36.9	1,336
Metronidazole	700	2,520	33.9	854
Clindamycin	400	2,440	16.8	410
Imipenem	100	2,210	9.5	210
Ampicillin	575	1,575	16.8	264
Ofloxacin	120	1,376	45.8	630
Ampicillin-sulbactam	120	1,218	14.6	178
Amphotericin	20	954	3.5	33
Acyclovir	50	724	8.4	60
Clarithromycin	50	677	10.6	72
Aztreonam	20	490	2.5	12
Cefaloridin	76	124	12.9	15
Penicillin G	50	40	2.2	1
Total		103,603		29,376

*Figures for monthly doses and corresponding expenditures were obtained by courtesy of the Pharmacy Department.

As noted, the Hospital Español de Mexico has a special population of patients who are members of the Spanish Welfare Society. In order to track antibiotic abuse in this population and the associated costs, an observational study (*29*) was conducted from 1 May to 30 June 1997. Antibiotic prescriptions were analyzed on the ward for hospitalized members. Of 305 patients admitted, 177 received antibiotics (58%). Fifty-six of these (31.6%) received the drug as treatment for community-acquired or nosocomial infections and 119 (67.23%) as prophylaxis. The percentage distribution of drugs administered, by type, was 35% quinolones, 35% first-generation cephalosporins, and 24% third-generation cephalosporins. Antibiotics were changed or added in 71 patients and in the majority of these (43 patients) the drug chosen was ciprofloxacin.

Using the same system of classification described above, we find that 54% of the regimens were correct and 45% incorrect. The total cost of antimicrobials prescribed during the period was $62,814, of which more than $28,000 was spent on incorrect usage. The administration of unnecessary antibiotics cost $16,345.

A projection of annual expenditure based on the data obtained suggests that the hospital spends more than $98,000 per year on the unnecessary administration of antibiotics and more than $168,000 per year on incorrect or, at least debatable, prescriptions. These expenditures refer only to the drugs and do not take into account the equipment needed to administer them (syringes, infusion pumps, solutions), nor do they include labor costs.

Total data for the Hospital Español de Mexico show that the institution spends more than $1,500,000 per year on antibiotics and that more than $350,000 of that is for incorrect or unnecessary use. Although not all of the patients belong to the Spanish Welfare Society,

these costs must be covered by private insurance companies or by the patients or their families.

Various authors have calculated that nearly 30% of hospital antibiotic consumption is incorrect and can be avoided by means of restrictive policies *(27, 29)*. Our findings are similar, leading us to project savings of close to $500,000 per year from such measures.

All the data and figures analyzed in this work confirm once again that is urgent to establish policies and systems to restrict the use of antibiotics in hospitals. The threat of bacterial resistance is constant and progressive; in addition, there is a need to reduce the current cost of medical care *(29–31)*.

In sum, is it necessary to reduce antibiotic consumption? Yes. How? By reducing access. The bibliography includes many examples of modifications in the consumption of antibiotics in order to reduce costs and the generation of resistant strains *(32–43)*. There is agreement, then, on the need to reduce the use of antimicrobials—but how much, and which ones?

Nothing said here is meant to suggest that the most powerful or broadest-spectrum antibiotics, which are almost always the most expensive, should no longer be used; however, it is urgent to control the present abuse of these drugs. Antibiotics that definitely should be restricted, either because of their potential impact on the bacterial flora of hospital patients or because of their high price, include: quinolones of the second, third, and fourth generations; cephalosporins of the third and fourth generations, with or without antipseudomonal action; carbapenems; monobactams; antimycotics of the fluconazole type; rifampicin; vancomycin and teicoplanin; and perhaps some macrolides. The controls should be developed by specialized personnel, including specialists in infectious disease, microbiology, infection control, and pharmaceutics, together, of course, with the administrative authorities of each hospital.

The medical community still has time to put the brakes on the irrational use of these drugs. The words of Norman Simmons at the Pan European Conference on Antimicrobial Resistance held in Denmark are an accurate reflection of the views of many of us, and a wake-up call for others: "We have made a mistake; we should recognize it and apologize. Physicians had in their hands the marvelous gift of antibiotics, but they are destroying it through indiscriminate use. We do not need another committee, we know what to do: we simply have to use them less." *(44)*.

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ALLIANCE FOR THE PRUDENT USE OF ANTIBIOTICS:[1] BACTERIAL RESISTANCE TO ANTIBIOTICS IN LATIN AMERICA

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Bacterial resistance to antibiotics continues to be a serious problem all over the world, especially in Latin America, where many factors contribute to engender and develop it. Unfortunately, data on susceptibility to antibiotics are scarce and not all countries monitor resistance. Furthermore, lack of legislation in this field leads to the free distribution and sale of antibiotics without medical prescription. It is also common to find that health personnel, physicians in particular, have limited knowledge of the use of antibiotics and, therefore, prescribe these drugs without a sound clinical basis. In like manner, certain prophilactic prescription habits of antibiotics in surgery are inappropriate and do not conform to an evaluation of postoperatory infection risk. All of this is manifested in an indiscriminate use of antibiotics, which increasingly affects the frequency of resistance in bacterial strains.

This situation is well exemplified in the Region by the alarming rise of strains of bacteria with patterns of highly resistant susceptibility to the antibiotics used in the past. Strains of multiresistant *Mycobacterium tuberculosis* (MRMT) have emerged, causing very serious and often untreatable cases of tuberculosis. This problem is found in many countries. Moreover, in some Latin American countries, emergence and dissemination of MRMT strains have been observed among patients with and without VHI infection.

In Latin America, the sale, distribution, and prescription of antibiotics are greatly influenced by the pharmaceutical industry and this, together with patients' demands, has a substantial influence on the indiscriminate use of antimicrobial drugs. Nevertheless, it should be pointed out that this feature is not exclusive of the Region, since antibiotics occupy the second place worldwide among total sales of medications (*1*).

Surveillance of resistance to antibiotics in communities and hospitals is essential if health personnel are to select the proper antibiotic treatment. Surveillance data are also

[1]The Alliance for the Prudent Use of Antibiotics (APUA) is the only independent organization that aims exclusively at containing microbial resistance to antibiotics and promoting the prudent use of these medications throughout the world. Founded in 1981 and located in Boston, Massachussetts, the APUA maintains a net of chapters in 24 countries and has members in 100 countries. In order to achieve its mission, the APUA carries out a strategic plan for research and education in public health backed up by qualified personnel, a group of expert consultants, and an international scientific directing council.

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indispensable for health specialists in charge of proposing and applying control measures against resistant microorganisms. The World Health Organization (WHO) has undertaken surveillance of the susceptibility patterns of common bacteria and has created for that purpose a computerized system known as WHONET. Even so, there are also surveillance systems sponsored by universities or supported by the pharmaceutical industry. The information gleaned by these systems is more exact and complete, but unfortunately, it is not shared with physicians or other health personnel. The Alliance for the Prudent Use of Antibiotics (APUA) (2) is seeking to integrate these two public and private mechanisms, and to design a global surveillance system that will analyze not only the microbiology of communicable diseases, but also their epidemiology. Recently, the ARInfobank was launched (3), an electronic information bank on microbial resistance to antibiotics, which is sponsored by WHO and available at Internet address (http://who2.b3e.jussieu.fr/arinfobank/).

The rational and empirical use of antibiotics must be based on local profiles of susceptibility and resistance of the bacteria most commonly isolated in microbiological laboratories. At the same time, these laboratories must rely on quality-control systems. At present, a few international organizations are providing laboratories with training and resources to standardize their methods for determining the susceptibility of various bacteria to antibiotics.

In order to understand the factors affecting the prescription of antibiotics in medical practice, pertinent information must be obtained, analyzed, and presented systematically. Besides, it is important to know the opinion of health personnel, especially that of physicians, about antibiotics. The PAHO and the APUA have begun to study this subject in relation to the conduct surrounding prescriptions in various countries.

In general, the situation is more difficult in Latin America, since there are no regulatory measures for antibiotics, consumers can obtain these medications anywhere, and self-medication continues to be a great problem. However, a few countries like Chile, Costa Rica, Cuba, and Panama have passed laws regulating the sale of antibiotics. The next step is to ensure compliance to these laws. Unfortunately, in other countries private industry exerts a great deal of control over governmental authorities, and the latter´s indifference toward this matter leads to overlook the observance of control measures on the use of antibiotics.

The appearance of resistant pathogens is a critical public health problem. Diseases like malaria, cholera, tuberculosis, diarrhea, and pneumonia caused by resistant strains have become leading causes of morbidity and mortality, while antibiotics continue to lose their efficacy. This has weighty consequences due to the fact that, besides excessive morbidity and mortality, the costs of treating hospitalized patients have drastically increased, while the manufacture of new antibiotics has decreased.

The new antibiotics, above all the fluoroquinolones, are producing high degrees of resistance, especially in nosocomial infections (4) and in institutions offering specialized nursing care to the elderly (5). At the same time, more and more invasive procedures are performed and no guidelines are followed for empirical administration based on microbiological reports of resistance in the hospitals involved.

The study of resistance to vancomycin by the *Staphylococcus aureus* species reveals that this constitutes another growing problem in Latin America. Over 60% resistance was notified in a study carried out by Brazilian researchers (6). Likewise, vancomycin-resistant enterococci have been detected in the Region and many hospitalized patients are colonized by this highly dangerous germ.

On the other hand, the improper use of antibiotics triggers important changes in the epidemiology of pathogens. This is the case of *Streptococcus pneumoniae*, as reported by several authors (7, 8). At the present time, penicillin-resistant strains of *S. pneumoniae* with a minimum inhibitory concentration of ≤0,1 μg/mL are widely disseminated.

In a special issue of the *Revista Panamericana de Infectología* on bacterial resistance in the Americas, research workers from various countries of the Region describe very important findings. Several of the articles germane to the same topic have been included in the present book.

After analyzing available information, it appears to be of the essence to have in place a surveillance program on bacterial resistance, to report and divulge among physicians the local susceptibility and resistance patterns, to develop and publish empirical recommendations on the commonest infections, to emphasize quality control in microbiological laboratories, and to standardize the tests for microbial susceptibility. Perhaps the most important issue is to educate the public on antibiotics and to keep these medications from being dispensed without prescription by authorized personnel. For all of us this is a challenge and a commitment to the people of the Region.

In an effort to restrict unjustified use of antibiotics, the APUA has established nine of its organizational chapters in the following Latin American countries: Argentina, Colombia, Cuba, Ecuador, Guatemala, Mexico, the Dominican Republic, Uruguay, and Venezuela. Other countries are now going through the process of establishing their own chapters, after which the APUA will collaborate with initiatives aimed at increasing public knowledge on bacterial resistance to antibiotics and the correct use of these drugs. Thus, with support from mutual and international collaboration, the Alliance seeks to develop each country's capacity to influence the policies regulating the use of antibiotics.

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VETERINARY USE OF ANTIMICROBIALS IN ANIMAL AGRICULTURE IN NORTH AMERICA, LATIN AMERICA, AND THE CARIBBEAN

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INTRODUCTION

Use of antimicrobials for therapeutic and growth promotion purposes is common in modern production agriculture. However, this use is not without controversy. Scientific debate about possible public health risks posed by the use of antimicrobials in food-producing animals has been going on for over 30 years. There is general agreement within the scientific community that the development of resistant human pathogenic bacteria results not only from the direct use of antimicrobial agents in humans, but also from acquisition of infections by resistant organisms or resistance factors from animal and environmental sources. There is concern that use of antimicrobials in food animals may contribute to the development or enhancement of antibiotic-resistant zoonotic organisms. These, in turn, may contaminate food products at the time of slaughter and subsequently be transmitted to humans, thus posing a risk to human health.

Recent meetings of the World Health Organization (WHO) have focused on the use of antimicrobials in food-producing animals. A WHO expert meeting held in Berlin in 1997 sought to define potential medical problems that could come from antimicrobial use in livestock, and to recommend the next actions the WHO should take in response to these risks. Recommendations from the meeting included the need for further assessment of the risk and defining what constitutes resistance. There was also a recommendation against the use of antimicrobials for growth promotion that are also used in human medicine, or that can cause cross-resistance to human medicines. Another recommendation indicated that more research for replacing growth-promoting antimicrobials should be carried out. Monitoring for resistance in food animals and foods of animal origin was supported, as well as managing risk on the farm through the prudent use of antimicrobials.

A 1998 WHO expert meeting in Geneva specifically addressed the use of quinolones in food-producing animals. The experts agreed that the use of antimicrobials will cause the development of resistance, and that there is a potential that resistant strains of *Salmonella*, *Escherichia coli*, and *Campylobacter* may be transferred to humans through the food supply. However, the experts also agreed that antimicrobial drugs, including quinolones in certain instances, are needed to treat sick animals, but that more research is needed to find out what uses limit the rise of antimicrobial resistance of public health concern. More research on the effects of animal use of quinolones on human illness was also recommended.

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This paper seeks to examine the use, distribution, and control of antimicrobials in animal agriculture throughout the Americas (Table 1). Additionally, guidance currently available to veterinary practitioners concerning the prudent use of antimicrobials in animal agriculture will be addressed. Programs to monitor the development of antimicrobial drug resistance in food-borne pathogens will also be examined.

The information presented here is based on a limited sample, with information provided by Argentina, Canada, Colombia, Mexico, and the United States of America. Because of the importance of collecting accurate information in order to provide a basis for formulating recommendations to address public health concerns, it is suggested that a more comprehensive survey be undertaken and information gathered from all countries to provide a more complete picture of the situation in the Americas.

DRUG DISTRIBUTION

In Argentina, antibiotics, anticoccidials and growth promotants are marketed by veterinary clinics and organizations staffed with veterinarians that provide technical assistance.

TABLE 1. Antimicrobials approved* for use in two or more of the countries surveyed

Drug	Argentina	Canada	Colombia	Mexico	United States
Amikacin		X			X
Amoxicillin	X	X	X	X	X
Ampicillin	X	X	X	X	X
Apramycin	X	X			
Bacitracin	X	X			X
Carbadox	X	X			X
Cefacetrile	X		X		
Cefadroxil		X			X
Cefoperazone	X		X		
Ceftiofur	X	X		X	X
Cefapirine	X	X		X	X
Chloramphenicol	X	X†			X†
Clavulanic acid	X				X
Clindamycin		X			X
Cloxacillin	X	X	X	X	X
Danofloxacin			X	X	
Dicloxacillin	X			X	
Difloxacin	X			X	
Dihydrostreptomycin	X		X	X	
Doxycycline	X	X		X	
Enrofloxacin	X	X	X	X	X
Erythromycin	X		X	X	X
Florfenicol	X	X		X	X
Flumequine	X			X	
Furazolidone	X	X		X	
Gentamycin	X	X	X	X	X
Kanamycin	X			X	X
Lincomycin	X	X	X	X	X
Monensin	X	X			X
Nalidixic acid	X			X	
Neomycin	X	X	X	X	X
Nitrofurantoin	X	X			
Nitrofurazone	X	X		X	

(continued)

TABLE 1. ***(continued)***

Drug	Argentina	Canada	Colombia	Mexico	United States
Norfloxacin			X	X	
Novobiocin	X	X			X
Ormetoprim		X			X
Oxolinic acid	X			X	
Oxytetracycline	X	X	X	X	X
Penicillin	X	X	X	X	X
Pirilimycin	X	X			X
Polymycin B	X	X			
Salinomycin	X	X			
Sarafloxacin				X	X
Spectinomycin	X	X	X	X	X
Spiramycin	X		X	X	
Streptomycin	X	X	X	X	X
Sulfachloropyridazine	X		X	X	X
Sulfadiazine	X	X	X	X	
Sulfadimethoxine	X	X	X	X	X
Sulfadimidine	X		X		
Sulfadoxine	X	X	X		
Sulfaguanidine	X	X	X		
Sulfamerazine	X	X		X	
Sulfamethazine	X	X		X	X
Sulfamonomethoxine	X		X		
Sulfanilamide	X			X	
Sulfapyrazole			X	X	
Sulfaquinoxaline	X	X	X		X
Sulfathiazole	X	X	X	X	
Sulfisoxazole	X	X			
Tetracycline	X	X		X	X
Tiamulin	X	X	X	X	X
Tylosin	X		X	X	X
Virginiamycin	X	X			X

*No distinction was made between drugs approved for food animals or nonfood animals.
†Chloramphenicol is not approved for use in food animals in Canada or the United States.

These clinics and organizations are authorized to operate and are inspected by the government regulatory authority. The distribution of prescription animal drugs in Canada is mostly through veterinarians.

In Colombia, approximately 75% of antimicrobials are distributed through agricultural warehouses. Between 18% and 20% are marketed through cattle funds and agricultural cooperatives, and 5%–7% are marketed directly to the end consumer (poultry and hog farmers and cattle ranchers). Government regulations which address the distribution, marketing and sale of products for livestock now require a written prescription from a licensed veterinarian for the sale of antibiotics.

In Mexico, veterinary drugs are sold by distributors and pharmacies registered with the Secretary of Agriculture, Livestock, and Rural Development (SAGAR). Sales of veterinary drugs are limited to finished products which have been registered and authorized for sale.

Most prescription antimicrobials in the United States are sold by veterinarians. Prescription drugs are available only on the order of a licensed veterinarian. There are various sources of distribution for over-the-counter/nonprescription antimicrobials, including veterinarians, distributors, feed suppliers, cooperatives, and farm supply stores.

AVAILABILITY: PRESCRIPTION OR OVER-THE-COUNTER (OTC/NONPRESCRIPTION)

In Argentina, antibiotics, anticoccidials, and growth promotants are available to the user by prescription only. Canada, on the other hand, has many OTC antimicrobial products for use in animals. Most of the newer approvals are prescription. Any new approval for an antimicrobial to be used in animals that is also used in human medicine will be prescription only.

Recent legislation in Colombia requires a written prescription from a licensed veterinarian for the sale of antimicrobials.

The United States has many OTC antimicrobial products available for use in food animals, both for therapeutic purposes and for growth promotion. However, since 1992, all new therapeutic antimicrobials have been approved as prescription only. On the other hand, to date, antimicrobials for use in animals in Mexico do not require a prescription.

OFF-LABEL OR EXTRA-LABEL USE POLICIES FOR ANTIMICROBIAL DRUGS

Off-label or extra-label use refers to the use of a drug product in a manner other than what is indicated on the product label. It may refer to use of a different dose level or route of administration than indicated on the label for a particular species, or may refer to use of a drug in a species not indicated on the label. Because pharmacokinetics data and other important information are generally limited for off-label uses of drugs, the veterinarian bears an important responsibility to ensure the safe use and adequate withdrawal times for drugs used in food-producing animals.

Regulations in Argentina do not allow extra-label use of antimicrobial products. The product must be used following the indications on the labeling, which were submitted, reviewed and approved by the government regulatory authority. Extra-label use is legal for veterinarians in Canada. There are no restrictions or prohibitions against extra-label use of approved antimicrobial drugs.

In the United States, extra-label use is only permitted by a licensed veterinarian in a valid veterinarian-client-patient relationship. Additionally, there is a list of drugs for which extra-label use in food animals is strictly prohibited. This list includes the quinolones and glycopeptides, because of concerns related to the development of antimicrobial resistance.

DRUG USE DATA COLLECTION

Collection of detailed information on the use of antimicrobials in food-producing animals can be a valuable tool to allow more direct correlation between loss of susceptibility or development of resistance to a particular antimicrobial agent, and the marketing of the individual drug product. Sales information collected by geographical area, on a calendar year basis, is particularly helpful in analyzing use trends. As monitoring programs are developed, collection of this type of data becomes increasingly important.

In Argentina, the Servicio Nacional de Salud Animal (SENASA) maintains a data base that records import and export of raw ingredients and finished products, including antibiotics, anticoccidials, and growth promotants. Drug marketing information is collected in Colombia. The Canadian Bureau of Veterinary Drugs does not collect drug sales or usage data.

In the United States, drug-use data are collected as part of the Drug Experience Report which is submitted after the first six months of marketing, and yearly thereafter for each approved drug (whether prescription or over the counter). Manufacturers submit a listing of the quantity of the drug marketed. In Mexico, drug-marketing data are collected in a database with information relating to veterinary drugs registered in the country, which also includes information relevant to the use of these products.

PRUDENT USE GUIDELINES

In order to minimize the development of antimicrobial resistance and ensure the continued efficacy and availability of antimicrobial products for use in food-producing animals, it is critical that prudent use of antimicrobials be promoted. Key elements of such activities may include developing prudent use principles, therapeutically-based antimicrobial use guidelines, recommendations on appropriate measures to reduce disease transmission, and educational programs for the prescribers and users of these drugs.

In the United States, a committee chaired by the American Veterinary Medical Association has been established with broad representation from the veterinary community, which is drafting prudent therapeutic use guidelines. The Center for Veterinary Medicine has representation on this committee and strongly supports these efforts. Health Canada is currently forming a similar committee to look at prudent use recommendations.

In Argentina, regulations for the prudent use of antimicrobials apply to all formulations and routes of administration, including those used as growth promoters. Currently, there is a very active discussion in the government regulatory authority concerning the impact on human medicine of the use of antimicrobial growth promotants in animal feeds. Regarding the use of antibiotics in animals that may impact on bacterial susceptibility to treatments of humans, Argentina uses strict standards during the registration review, and strives to approve antibiotics based not only on safety and effectiveness considerations, but also on the impact that the residues may have on antimicrobial resistance. Labeling for growth promotants (limited use in poultry, swine, and beef cattle) provides pre-slaughter withdrawal times and milk discard periods. Slaughter houses and processing plants have adopted Hazard Analysis Critical Control Point (HACCP) monitoring standards for *Salmonella* spp., *Escherichia coli*, enterobacteria, *Campylobacter* and *Listeria* spp. using ELISA methods, microculture, and disks, to characterize the sensitivity of antibiotics used in animal production. A draft regulation has been prepared to prohibit the marketing and use of avoparcin.

MONITORING PROGRAMS

Concerns about the enhancement and/or development of organisms resistant to available antimicrobials from food-animal use has led to efforts to monitor the development of resistance in pathogens of public health concern. Officials in Canada and Argentina are currently considering the establishment of monitoring programs.

In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) was established in January 1996 as a collaborative effort among the U.S. Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and the United States Department of Agriculture (USDA). The NARMS was started (and has recently been expanded) because of the human health concerns related to the use of antimicrobials in food animals. This program monitors changes in susceptibilities to 17 antimicrobials of zoonotic enteric organisms from both animal and human sources. The food animal specimens are gathered from healthy farm animals, animal clinical specimens, and from carcasses of food animals at slaughter.

The objectives of the NARMS include: to provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in *Salmonella* and other enteric organisms, to facilitate the identification of resistance in humans and animals as it arises, and to provide timely information to veterinarians and physicians. The ultimate goal of these activities is to prolong the lifespan of approved drugs by promoting prudent and judicious use of antimicrobials in food animals and taking appropriate public health action. The system is set up as two nearly identical parts: a veterinary arm and a human

arm. Recently, the monitoring system has been expanded to include *Campylobacter* and *E. coli* isolate testing and reporting on the veterinary side. Human *Campylobacter* isolates had been included in the monitoring system beginning in 1997, and new sites and sources of isolates have been added.

Epidemiologic research has been initiated to characterize and reduce the incidence of foodborne disease associated with emerging and drug-resistant pathogens and includes a field study, several farm-based efforts, and molecular genetic research. FDA, USDA and local health authorities are currently in the field studying risk factors associated with an outbreak of *Salmonella typhimurium* DT104 on a farm. Information from this field study will be used to educate farmers to prevent future outbreaks and spread of this multiresistant organism among animals and to man. Also with industry cooperation, on-farm poultry studies have been initiated to examine management, production, and drug use practices that influence the development of resistant zoonotic pathogens. It is hoped that this information will be helpful to formulate appropriate prudent use recommendations by the veterinary industry.

Plans for the future in the United States include further augmentation of the existing monitoring system, as well as support for the development of an international veterinary resistance database, in order to facilitate international response to the emergence of resistance and spread throughout the world. Additionally, veterinary and producer drug prescribing surveys to assess the impact of antimicrobial use patterns on resistance emergence and prevalence are being planned.

RECOMMENDATIONS

- The creation of a standardized veterinary database in the Americas can be extremely useful to facilitate response to resistance emergence and spread throughout the Region. Sharing of information from resistance monitoring activities will be an important step in encouraging the prudent use of antimicrobials in animal agriculture, and in addressing the problem of antimicrobial resistance.
- Because of the importance of collecting accurate information to provide a basis for formulating recommendations to address public health concerns, we recommend that a more comprehensive survey be prepared and information gathered from all PAHO member countries to give a more complete picture of antimicrobial use in animal production in the Americas.
- Collection of standardized drug marketing data is encouraged, since this kind of information is extremely valuable for use in conjunction with resistance monitoring programs.
- In order to minimize the development of antimicrobial resistance and ensure the continued efficacy and availability of antimicrobial products for use in food-producing animals, it is critical that prudent use of antimicrobials by veterinarians and producers be promoted by all PAHO member countries.
- Countries may also want to consider whether changes should be instituted in current controls and/or regulations on antimicrobial drug distribution, availability and extra-label use policies.

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