Antimicrobial resistance in shigellosis, cholera and campylobacteriosis

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World Health Organization

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A BACKGROUND DOCUMENT FOR THE WHO GLOBAL STRATEGY FOR CONTAINMENT OF ANTIMICROBIAL RESISTANCE

Acknowledgement

Designed by minimum graphics
Printed in Switzerland

Contents

Summary of antimicrobial resistance in bacterial enteric pathogens

Important enteric pathogens are becoming increasingly resistant to the major antibiotics that are needed for optimal treatment of patients. The three bacterial pathogens chosen for this review (*Vibrio cholerae*, *Shigella* spp. and *Campylobacter jejuni*) are very different from one another. They cause quite different clinical syndromes; their ecology, epidemiology and modes of transmission are distinct; and they are widely separated genetically. The fact that three such different organisms are becoming increasingly antibiotic-resistant underlines the pervasiveness of the pressures that lead to the emergence and spread of resistance.

Shigella spp. show a pattern of steadily increasing resistance to antibiotics. Among the four species, *S. dysenteriae* 1 (Shiga's bacillus) is generally the first to develop resistance to a new antibiotic, but then the other *Shigella* species follow. Rarely does susceptibility reappear once resistant strains have become endemic in a region. In order to ensure appropriate treatment, continual surveillance is required to determine which antibiotics are still active. This strategy of "trying to keep one step ahead" implicates the continual development and testing of new antibiotics, which inevitably are more expensive. After extensive use of these new antibiotics, their prices do fall, but not to the level of the older, previously effective antibiotics. In this race between the development of new antibiotics by the pharmaceutical industry and the development of resistance in *Shigella*, it seems that the bacteria are winning, and we face the prospect of having no effective antibiotics for future epidemics of shigellosis. Expecting the pharmaceutical industry to develop a novel and cost-effective antibiotic every few years is unrealistic over the long term.

Vibrio cholerae, the agent that causes cholera, has a much different history of antibiotic resistance. For many years it was thought that cholera epidemics caused by antibiotic-resistant strains were unlikely to occur because the bacteria seemed to lack the ability to retain resistance plasmids. This

view was clearly incorrect, as resistant strains caused large epidemics in the United Republic of Tanzania and Bangladesh in the late 1970s. Since then, antibiotic resistance patterns have varied widely at different times and in different places, with multiply antibiotic-resistant strains commonly found during epidemics. Unlike *Shigella* spp., however, strains of *V. cholerae* frequently revert to antibiotic sensitivity. An example of reversion to resistance occurred with the new serotype, O139. Initially all strains of this new pathogen were resistant to trimethoprim, but now most strains are sensitive. The reversion to sensitivity is probably best explained by the ecology of the vibrio. Being primarily an environmental water organism and only secondarily a human pathogen, it must adapt to the conditions of its primary ecological niche, in which antibiotic resistance does not provide a major benefit to the bacteria.

Campylobacter jejuni has yet another ecological niche, being primarily adapted to animals, in particular to birds. The industrialization of poultry production has provided an environment in which resistant bacteria flourish, and these strains are then easily spread to humans. Especially worrisome is the routine use of fluoroquinolones for growth promotion in poultry. Generally, antibiotics used in animals should be different from those used for humans, but in this instance an antibiotic class with unique benefits for humans is, for economic reasons, being used in animals, with resulting loss of its effectiveness for treating human disease. This would seem to be a matter for government regulation. However, the difficulties are illustrated by the example of the United States of America, where different agencies regulate drugs for human and animal use. The loss of the fluoroquinolones as effective therapy for *C. jejuni* infections means that ciprofloxacin will no longer be efficacious in the syndromic treatment of dysentery. A question that is not yet answered is the extent to which the genetic determinants of fluoroquinolone resistance can be transferred from *C. jejuni* to other enteric and nonenteric bacteria. If this were to occur, the

spread of resistance originating in antibiotic-treated animals would represent an even more serious threat. Among the common bacterial infectious agents, *Neisseria gonorrheae* and *C. jejuni*, which were once generally sensitive to most antibiotics, are now becoming increasingly resistant to ciprofloxacin.

Clinical consequences of resistance

The clinical consequences of antibiotic resistance vary among the three bacterial diarrhoea agents. For shigellosis, antibiotics are the primary treatment. Patients treated with an ineffective antibiotic may have more complications than if they had not been treated, because the antibiotic is likely to affect the normal intestinal flora, thus actually favouring the growth of the resistant shigella.

In treating cholera, antibiotics have been shown to reduce the duration of illness and the fluid loss, but they are not considered to be a "life-saving" treatment. If the hydration status is maintained with adequate rehydration fluids, patients will recover without antibiotics; however, the illness will persist about twice as long, lengthening the hospital stay and increasing the resources used (about double the amount of rehydration fluids). As long as skilled manpower and adequate supplies are available, case-fatality rates should remain stable, but cholera epidemics generally occur in areas where there is a shortage of services. Thus, in the "real world" where cholera epidemics occur, antibiotic resistance means higher costs, a greater need for supplies, and more deaths.

The consequences of antibiotic resistance in *C. jejuni* are more difficult to predict. Most cases are self-limited, and antibiotic treatment is needed only for severe cases. In developing countries, antibiotics are not normally recommended for the treatment of diarrhoea due to *C. jejuni*. However, some infections are severe, with high fever and bacteremia, and these cases do require antibiotic treatment.

Antibiotic resistance in vulnerable populations

Both shigellosis and *C. jejuni* infections are more severe among vulnerable populations, such as those who are malnourished and those with HIV/AIDS. Thus, one would expect a much greater proportion of adverse outcomes among these groups than among those who are healthy.

Factors involved in the emergence of antibiotic resistance

With all three organisms, the primary factor is the overuse of antibiotics; i.e. antibiotic pressure selects for resistant strains. Antibiotic pressure may be exerted directly (e.g. use of an antibiotic for shigellosis to which the bacteria are resistant, thus favouring the very bacteria one is attempting to eliminate). However, antibiotic pressure on an organism may occur indirectly, i.e. from using the antibiotics for entirely different reasons. Examples of inappropriate uses of antibiotics that exert selective pressure for resistance in various bacteria are: administering them to patients who have viral upper respiratory tract infections; feeding them to farm animals to enhance their growth.

Relationship between duration of treatment and development of antibiotic resistance

Several studies have found that patients frequently do not finish their prescribed course of antibiotic treatment. This is considered a factor in the development of antibiotic-resistant strains of some bacterial species, but there is no evidence for this in enteric pathogens. In enteric infections the strategy is shorter courses of treatment, providing sufficient antibiotic to treat the illness but no more. Giving an excessively long course of treatment (e.g. a ten-day course for shigellosis) simply adds to the antibiotic pressure, and favours the development of resistance. Some clinicians favour treatment schedules that completely eradicate the bacteria from the stool, on the theory that this will stop that patient from transmitting the organism to others. In fact, once clinically well the patient represents a very small risk to others. There are many more asymptomatic infected persons (or animals), who represent a much greater risk than a recovered patient with some residual bacteria in the stool.

Interventions

The interventions listed below are based on the following assumptions:

Firstly, the only way to reverse antibiotic resistance is to relieve the antibiotic pressure in the bacteria's environment (the human gut in the case of *Shigella* spp., the human gut and environmental water in the case of *V. cholerae*, and animal reservoirs in the case of *Campylobacter* spp.). While there are laboratory methods to "cure" bacteria of plasmids, these are not practical for public health

use. However, some bacteria seem to rid themselves of "genetic baggage" that is not useful to their survival and growth. Thus, antibiotic resistance genes are less likely to be maintained in the absence of antibiotic pressure.

Secondly, interventions that decrease overall rates of morbidity and mortality (by targeting the overall burden of disease) will also decrease the spread of resistant organisms.

Thirdly, there need to be programmes at local, national and regional levels. Resistance develops in specific habitats and patterns differ among geographical areas.

Finally, because antibiotic resistance genes move among bacterial species, programmes must aim at preventing resistance not just in certain specific pathogens, but in all of the gut flora, especially *Escherichia coli*.

Interventions aimed at antibiotic resistance in enteric bacteria include:

1. **Surveillance**. Patients must receive the best available treatment for acute illnesses. Determining the susceptibility of individual isolates is not cost-effective, nor would the results be available rapidly enough to be clinically useful. Therefore, a surveillance system is needed to determine the predominant patterns of resistance in a given locality.

   1.1 The surveillance system must use a representative sample. While random sampling is generally best, it is not as practical as systematic or periodic sampling. The patients included in the sample should be screened for recent antibiotic use; otherwise, the sample might include an excess of patients who failed antibiotic treatment, resulting in an overestimate of the proportion of antibiotic-resistant organisms.

   1.2 Susceptibility testing should include only those antibiotics that are clinically effective against the organism. Some antibiotics are not clinically useful, even though they may have *in vitro* activity. Testing for susceptibility to these antibiotics should be avoided because it may lead providers to prescribe inappropriate antibiotics.

   1.3 Antibiotic resistance patterns should be readily available both to policy-makers and providers. Policy-makers need the data to formulate drug policy for their programmes, to obtain the most appropriate antibiotics,

and to train the staff in their use. Individual providers need the information in order to give their patients the best possible treatment.

2. **Regulating and monitoring antibiotic use**. Programmes aimed at reducing inappropriate use of antibiotics require cooperation between government agencies and pharmaceutical companies.

   2.1 Government agencies should develop methods for monitoring antibiotic use in their country in order to measure the level of antibiotic pressure.

   2.2 The monitoring programme should include all antibiotics in use: for humans and animals; for therapeutic use as well as for animal feeds, on farms and in aquaculture.

   2.3 Government agencies should restrict the use of antibiotics to those that are appropriate and essential. Antibiotics used in animal feeds should be different from (and not closely related to) those needed for human therapeutic use.

   2.4 A single government agency should control all antibiotic use within a country, whether for human therapy or for agriculture.

   2.5 The choice of antibiotics for use by government health agencies should be based on data generated by the surveillance programme in order to ensure that the antibiotics available match the sensitivity patterns of the pathogens being treated.

   2.6 Promotional materials for antibiotics should be screened by and approved by the agency regulating antibiotics. Unwarranted claims and promotional strategies should be eliminated.

3. **Training health care providers in proper antibiotic use**.

   3.1 In countries where antibiotics are available only by prescription, providers need to be informed about their appropriate use for common illnesses, and also need training in counselling patients whose illness does not require an antibiotic. Frequently, patients "expect" (and providers feel pressured to prescribe) an antibiotic even when they have a viral infection that will not respond to antibiotics.

3.2 Readily available "standards of care" may help to reinforce good prescribing behaviour on the part of providers.

3.3 In countries where antibiotics are freely available, unqualified providers, as well as patients themselves, prescribe them. The use of antibiotics is thus difficult to control, and the choice of a particular drug may be determined more by the profit motive of the drug retailer than by guidelines for appropriate treatment. Even in these circumstances it may be possible to train providers in the appropriate use of antibiotics. This will require cooperation among drug companies, distributors, detail salespeople, and drug retailers. It will also require more careful preparation of package inserts and promotional materials for antibiotics.

4. **Standards of care in hospitals**.

Resistant bacteria often emerge in the hospital environment. Due to the number and proximity of very ill patients, who receive antibiotics for extended periods, antibiotic-resistant strains can emerge and spread to other patients. When the patients return home, they carry the antibiotic-resistant strains with them.

Training in the prevention of nosocomial infections is needed, as are adequate supplies and facilities for hand washing, laundry, and other basic hygienic measures.

5. **Interruption of transmission of infectious agents: hygiene and public education**. Interventions that interrupt transmission of shigellosis, cholera and *C. jejuni* infections will effectively stop the spread of both sensitive and resistant strains. Such interventions, which are not antibiotic-specific, are attractive because they are broad-spectrum, covering all bacterial enteric pathogens.

5.1 Water and sanitation. Most enteric organisms are spread by the faecal-oral route; hence, food and water should be from clean sources, or should be purified or cooked. Improvement in management of faecal waste (use of sanitary latrines, central sewers, etc.) is at least as important as pure water in preventing transmission of enteric bacteria, but programmes to implement this have not been adequately funded.

5.2 Poultry and seafood are especially likely to carry enteric pathogens and should be well cooked. Shellfish are particularly likely to be contaminated with vibrios during the warm season.

5.3 For enteric agents, hand washing is the most effective of the low-tech, low-capital interventions. Several studies have shown that an effective hand-washing programme (soap, education, and motivation) decreases the rates of cholera and shigellosis by about 30%. In spite of its demonstrated effectiveness in small, community-based studies, hand-washing campaigns have not been scaled up to the national level.

5.4 Leftover foods should be kept refrigerated if possible. If this is not possible, they should be recooked before being eaten.

5.5 Dishes or utensils that have been used to handle raw meat, especially poultry, should not come into contact with food that is ready to eat. It is very easy to contaminate food in this manner.

6. **Interruption of transmission of infectious agents using vaccines**. Immunity is independent of antibiotic-sensitivity patterns; thus, vaccination reduces the incidence of illness due to both sensitive and resistant strains.

6.1 An effective vaccine would be expected to reduce cholera rates in endemic areas. Preliminary evidence from studies of a killed oral vaccine suggests that it interrupts transmission of the vibrio organism and that communities may develop herd immunity.

6.2 Vaccines for *Shigella* are being developed but are not yet ready for wide-scale use. Because *Shigella* spp. are the most resistant of the enteric pathogens, and also the ones for which antibiotics are the only effective therapy, a vaccine would be of great value.

6.3 Vaccines for *Campylobacter* are being developed, but their future is not certain, since the burden of disease may not warrant large-scale vaccination.

7. **Short-course antibiotic regimens**.

7.1 When antibiotics are needed, the shortest effective course of treatment should be given. Additional studies of the newer antibiotics are required in order to determine optimal schedules.

7.2 Antibiotics should be packaged in a man-

ner that facilitates the correct dosing (i.e. packages should contain the correct number of pills for the course of treatment).

8. **Avoidance of antibiotic use for illnesses that do not require them**.

8.1 The only acute diarrhoeal illnesses for which antibiotics are indicated are cholera, shigellosis, severe campylobacteriosis, amoebiasis, giardiasis, severe travellers' diarrhoea, and *Cyclospora* infection. Antibiotics do not benefit patients who have viral gastroenteritis (e.g. rotavirus infection), and the use of antibiotics in these cases increases antibiotic pressure.

8.2 Upper respiratory tract infections without pneumonia do not require antibiotic treatment. Antibiotic use in these illnesses increases antibiotic pressure on enteric pathogens.

9. **Alternative treatments for enteric infections**.

9.1 Not all patients require antibiotics. Many enteric infections can be successfully treated without them.

9.2 Clear guidelines are needed to identify those patients who do not need antibiotic treatment.

9.3 Drugs other than antibiotics are being developed and may be useful in the future. For secretory diarrhoea, these may function by enhancing absorption or decreasing secretion, thus lessening purging. They include new oral rehydration solution (ORS) formulations (such as rice ORS), new antisecretory drugs, and short-chain fatty acids (which increase fluid absorption in the colon). These antisecretory drugs need further study before they can be widely used.

9.4 For shigellosis, drugs that decrease the excessive inflammatory response are being evaluated; these may have utility in decreasing the severity of the disease. They will likely have to be used in combination with an antibiotic, but they might make it possible to decrease the amount of antibiotic used.

Research

Considerable research is needed if there is to be a reversal of the trend towards increasing antibiotic resistance in enteric pathogens. In principle, reversing antibiotic resistance is relatively simple: programmes can be implemented to reduce antibiotic pressure and to reduce overall transmission (by promoting personal hygiene, safe community water supplies, and the development and use of vaccines). In practice, reducing the amount of antibiotics used is difficult because of the economic interests of pharmaceutical companies, drug retailers and agriculture, as well as the desire of providers and patients for antibiotics. While an effective programme must aim to decrease the overall use of antibiotics, patients who require an antibiotic for treatment of an illness should not be denied the best available treatment.

1. **Surveillance methods**.

1.1 Simple and robust standard methods and computer databases are needed to monitor the agents that cause enteric infections and their antibiotic sensitivities. The research methodology includes the development of epidemiological and sampling strategies, as well as laboratory procedures. An unbiased sample should cover individuals who have significant enteric infections, but exclude those who have recently taken antibiotics. Since antibiotic resistance patterns may vary by geographical area, the sampling should use sentinel sites that reflect the various regions being studied.

1.2 Because patient history is frequently unreliable, assay of the patient's urine is probably the most reliable field test for recent antibiotic use.

1.3 The laboratory methods must be able to reliably detect *Shigella* spp and *Campylobacter* spp. These are the most difficult organisms to culture of the enteric bacteria. Thus, the laboratory should be close to the sentinel clinic/hospital.

1.4 Sample sizes should be sufficient to detect pathogens if they occur with a rate of 2 to 5% of all cases of diarrhoea. This will generally be about 500 stool specimens per quarter.

1.5 Sampling may be systematic (e.g. every 50 patients) or periodic (e.g. all diarrhoea

patients during a two-day period every two weeks).

1.6 Some patient information (e.g. age, sex, geographical location, evidence of malnutrition, type and duration of symptoms) must accompany the sample for correlation with the laboratory results.

2. **Methods for monitoring antibiotic pressure nationally and regionally**.

2.1 At the national level, pharmaceutical companies should be required to report the amount of each antibiotic sold, itemizing its intended use (human therapy, treatment of animals, nontherapeutic use in agriculture).

2.2 Before this can be accomplished, the regulatory environment needs to be understood. Research is needed into the policies both of the companies and the regulatory agencies, in order to understand their motivations and constraints and encourage mutual cooperation.

2.3 Methods are needed to quantitate, independently of data provided by the manufacturers, the amount of antibiotic that is being used in animal feed, veterinary medicine, aquaculture, and human treatment.

2.4 Potential indicators of antibiotic pressure need to be evaluated. These might include surveillance of antibiotic resistance in *E. coli* from normal stool specimens.

3. **Educating providers in order to change their prescribing patterns**.

The aims are understanding how best to: communicate to providers the information gained from surveillance; monitor prescribing and usage patterns to determine whether they have changed; decrease the demand for antibiotics by patients; decrease pressure from drug retailers.

4. **Educating policy-makers to help them implement rational drug policies**.

4.1 The aims are understanding how best to: transmit the information from surveillance so that it is useful in decision-making; determine whether, and to what extent, the surveillance data were used in the decision-making process.

4.2 Information is needed on antibiotic procurement procedures and policies to help

understand why, and by whom, antibiotics are purchased for use in national health programmes.

4.3 Similarly, information is needed on antibiotic procurement policies of hospitals in order to determine how to set and enforce standards of care for antibiotic use.

5. **Impact of various water and sanitation improvements on antibiotic resistance**.

5.1 Latrines (as well as modern sewage systems) are an effective way to limit transmission of enteric pathogens. To quantify their importance, the antibiotic-sensitivity patterns of faecal bacteria in areas with good and poor sanitation should be compared. This could be followed by an intervention study to determine whether improvement of latrines lowers the prevalence of antibiotic-resistant organisms.

5.2 Hand washing is another effective way to limit transmission of enteric pathogens. Comparative and intervention studies (as above) are proposed to quantify its importance.

5.3 Pilot programmes are needed to determine how best to promote hand washing and the proper handling of food and utensils.

5.4 Computer models are needed to determine the impact of these interventions on the prevalence of resistance.

6. **Evaluation of the effect of vaccination on antibiotic resistance**.

6.1 During efficacy studies of cholera and *Shigella* vaccines, efforts should be made to quantitate their impact on the prevalence of antibiotic-resistant bacteria. The prevalence should be measured in terms of persons infected with antibiotic-resistant organisms (not the proportion of isolates that are resistant).

6.2 Computer models are needed to estimate the impact of vaccination on the prevalence of resistance.

7. **Short-course antibiotic regimens for common illnesses**.

7.1 Additional studies are needed to examine the effectiveness of short-course antibiotic treatment.

7.2 Pre-packaged standard regimens should be evaluated to determine whether they are cost-effective and if they improve compliance.

7.3 The effect of short-course regimens on the emergence of antibiotic-resistant faecal flora needs to be compared to that of traditional longer course therapy. Whether treatment is for enteric or respiratory illness, the emergence of antibiotic-resistant *E. coli* should be the parameter monitored.

7.4 The effect of different antibiotics on the emergence of antibiotic-resistant faecal flora needs to be determined. For example, there is little information about the comparative effects of sulfamethoxazole (SMZ) versus ciprofloxacin on the emergence of antibiotic resistance.

7.5 Especially important are studies to discern which patients with shigellosis require antibiotics and which will recover with no antibiotic. Simple clinical parameters are needed to minimize antibiotic use without compromising treatment results.

8. **Alternative approaches to prevention and treatment of enteric infections**.

Alternatives to antibiotics might include probiotics (such as lactobacilli and/or bifidobacteria) and prebiotics (such as inulin), certain amino acids such as L-histidine, as well as improved ORS formulations (e.g. rice ORS or ORS formulations that utilize short-chain fatty acids to improve fluid absorption), anti-secretory drugs and anti-inflammatory drugs. The potential advantage of these treatments is that, since they do not directly kill bacteria, their effect will likely be independent of antibiotic resistance. Additionally, some of these agents may potentiate the effect of antibiotics, making possible even shorter courses of treatment. Some of these alternatives might be used as treatment; others might be useful as prophylaxis during high-risk periods, e.g. for prevention of travellers' diarrhoea, or in family contacts of cholera or shigellosis patients.

9. **Development of rapid diagnostic tests to detect certain patterns of antibiotic resistance**.

9.1 Rapid and inexpensive tests would help guide therapy and rationalize drug treatment.

9.2 The ability to test frozen or otherwise preserved faecal samples would be useful in surveillance. The tests might use PCR, with primers for specific resistance genes.

9.3 Such tests would be useful in developing epidemiological methods to monitor the spread of particular resistance genes. Resistance determinants may be transmitted in a variety of ways (plasmids, phages, etc.) and among a variety of bacterial species. Detection of specific genes will help us understand their individual ecology.

10. **Understanding how antibiotic-resistance plasmids spread among enteric pathogens**.

10.1 Antibiotic resistance in normal faecal *E. coli* needs to be validated as a surrogate marker for resistance in pathogens.

10.2 The mechanism of transmission of resistance among different species needs to be established. For example, *C. jejuni* are now frequently resistant to ciprofloxacin, but the *Enterobacteriaceae* generally remain sensitive. If the resistance genes were to spread to *Shigella* a major treatment option will be lost.

10.3 Certain plasmids containing mutator genes have been found in shigellae. When transferred to other bacteria they enable the recipient to develop resistance more quickly by means of chromosomal mutations. Thus, plasmids can influence the development of chromosomal resistance. More work is needed to develop methods for recognizing mutator genes.

In summary, antibiotic-resistant enteric bacteria represent a major problem, which is becoming increasingly complex. Great effort will be required, both in basic and applied research, but there is much that can be done in the meantime. Programmes to control resistance in enteric pathogens should focus on public health approaches that reduce the number of infections by targeting transmission.

Review of *Vibrio cholerae*

Introduction

Cholera, caused by infection with the toxigenic bacteria *V. cholerae* O1 or O139, continues to cause severe outbreaks of dehydrating diarrhoea in much of the developing world. Historically, cholera has been one of the major pandemic "plague-type" diseases, capable of spreading and devastating large populations in epidemics, as well as occurring during regular seasons in endemic areas. Its traditional home is the Ganges delta area of India and Bangladesh, but over the last two centuries cholera has spread in waves throughout the world. Since its spread to Latin America in 1991, nearly all developing countries have been threatened by it. Although cholera is a reportable disease, its global incidence is not known because cases are not reported from those countries in Asia where it is most common, and because of underreporting in African and Latin American countries where it occurs more sporadically. Based on sample reporting, the United States Institute of Medicine in 1986 estimated the global disease burden from cholera at about 6 million cases annually, with over 600 000 hospitalizations and 120 000 deaths. This was before cholera spread to Latin America. However, the World Health Organization (WHO) received reports of only 293 121 cases in 1998, with 10 586 deaths; most of the reports came from Africa (WHO, 1999). In recent years, global climatic change (e.g. the effects of *El Niño* and Hurricane Mitch) is thought to have contributed to increasing rates of cholera in some regions.

Case-fatality rates should be close to zero because treatment is simple and inexpensive. However, many cases occur in areas lacking adequate treatment, so that fatality rates are commonly about 3% and are often greater than 10%. The highest fatality rates occur in refugee or displaced populations and in remote areas. Global death rates are difficult to estimate, but during peak years (e.g. the Goma epidemic in 1994) have likely exceeded the 120 000 estimate of the Institute of Medicine.

Microbiology

Cholera is caused by strains of *V. cholerae* O1 or O139. *V. cholerae* has many serotypes, but only toxigenic strains (which produce cholera toxin, or CT) belonging to these two serotypes have caused epidemic diarrhoea. *V. cholerae* belongs to the Vibrionaceae family of bacteria, which are normal inhabitants of fresh and salt water; thus, an understanding of cholera requires an understanding of the bacteria's role in the environment as well as in the human host. Other species of *Vibrio* cause diarrhoea or systemic illness, and some may even produce cholera toxin, but they do not cause epidemic diarrhoea.

Patients with cholera excrete large numbers of the bacteria in their faeces. The bacteria are easily cultured using special media for their isolation (TCBS or TTGA), and their presence can also be detected using rapid immunoassay methods at the bedside (SMART Test or coagglutination tests). Specimens for culture should be placed in transport medium (e.g. Cary Blair) if they cannot be cultured immediately; they are then stable for several days and can be sent to a regional laboratory. After isolation, standard tests, including agglutination with specific O1 or O139 antiserum, are available to confirm identity. *V. cholerae* is a motile, curved, Gram-negative rod. If trained technicians and the proper microscope are available, motility is helpful for rapid diagnosis, since the bacteria can be readily visualized.

Serogroup O1 has been subdivided into 3 serotypes (Ogawa, Inaba, and Hikojima) based on differences in factors A, B, and C of the O antigen, and also into two biotypes (classical and El Tor). An individual strain of O1 *V. cholerae* will thus have both a serotype and a biotype designation (for example a strain might be serotype Ogawa, biotype El Tor). All recent isolates of serotype O1 belong to the El Tor biotype, but there is frequent switching between Ogawa and Inaba serotypes in various locations. Hikojima strains are very rare and are not important from a public health standpoint. The clinical illnesses caused by Ogawa and Inaba strains are indistinguishable.

In 1992, a new serogroup (O139) of epidemic cholera emerged in Bangladesh and India. It has the potential to become the eighth pandemic strain. It has many similarities to El Tor cholera; it produces the same toxin (CT) and appears to be as virulent as El Tor strains. However, populations with immunity to O1 cholera are not immune to the O139 strains, since immunity is serogroup-specific (WHO, 1996). In the laboratory, the strains are recognized by agglutination with O139 antiserum and by antibiotic sensitivity pattern; otherwise, the two serogroups appear to be identical.

Transmission

Transmission of cholera is predominantly through faecally contaminated food and water; thus, it is usually a disease of developing countries or areas where clean water supply and adequate sanitation are lacking. Person-to-person transmission is extremely rare, probably because the inoculum needed to cause disease is high ($>10^5$ in most cases). In endemic areas such as Bangladesh, water appears to be the major vehicle, but in other regions food has been implicated. In fact, it is very difficult to separate the two mechanisms, since the water often contaminates the food. The bacteria are able to multiply in food, increasing the number of bacteria ingested and the probability of illness.

While contamination of water due to poor sanitation is largely responsible for transmission, this does not explain the seasonality of cholera. For example, the sanitation in rural Bangladesh is consistently inadequate, yet cholera is highly seasonal. If lack of sanitation were the only factor, the disease should occur year-round, whereas its incidence varies predictably during the year, suggesting a major role of the seasons and the environment in its transmission pattern. *V. cholerae* is known to persist for years in aquatic reservoirs such as shellfish and plankton, and the ecological changes associated with these reservoirs may explain the seasonality of the disease, the initiation of outbreaks, and the emergence of apparently new strains. Ecological reservoirs of cholera and their contribution to the epidemiology of the disease require further study.

Spectrum of illness

The disease is characterized by a short incubation period (8 to 72 hours) followed by acute watery diarrhoea, often associated with vomiting, muscle cramps, and complications related to severe dehydration and metabolic acidosis. Rehydration is the mainstay of cholera treatment, but antibiotics have been shown to be important and cost-effective adjuncts in severe cases and in epidemic situations. Under optimal treatment conditions, antibiotics are not considered life-saving, since individual patients can be adequately treated with only appropriate intravenous and oral rehydration fluids. However, antibiotics are part of the standard treatment of cholera because they reduce by about 50% the duration of illness, the diarrhoea volume, and the rehydration requirements. Shortening the duration and moderating the symptoms are particularly important when treating large numbers of cases; antibiotic treatment reduces the cost and effort required to deal with an outbreak.

There are few data correlating rates of antimicrobial resistance with treatment failure, morbidity and mortality. Since treatment failures due to antimicrobial resistance occur mainly in remote areas where data are not collected, the impact of resistance is difficult to determine. There is evidence, however, that resistance to first-line antibiotics was a contributing factor in the extraordinarily high death rates during the 1994 cholera epidemic in the Rwandan refugee camps in Goma, Zaire (Goma Epidemiology Group, 1995). In a prospective study of epidemiological characteristics of resistant and sensitive cholera strains in Bangladesh, secondary infection rates were higher and the duration of illness was longer in patients infected with resistant strains (Khan et al., 1986). There were no adverse clinical complications in patients treated with inappropriate antibiotics, but among patients treated with tetracycline, those infected with tetracycline-resistant strains had more severe, longer-lasting diarrhoea than those infected with sensitive strains (WHO, 1980).

In addition to the human health impact of drug resistance, the loss of effective first-line drugs carries a significant economic cost. Before widespread resistance developed to tetracycline and trimethoprim-sulfamethoxazole (TMP-SMZ), these were inexpensive, widely available, effective drugs for treating cholera. In some countries of the developing world, *V. cholerae* isolates are now sensitive only to expensive drugs such as fluoroquinolones, which are unavailable to local health centres. Without effective antibiotics, the length of hospitalization and of rehydration treatment for severe cases (and the associated cost) is more than doubled. The cost of the illness, in terms of treating the patient, lost wages for patient and family

members, and salaries of health care personnel can be substantial. While the concept that antibiotics are not life saving may thus be true for the individual patient, it is not true in the real world, where supplies of rehydration fluid may be limited and healthcare personnel may not be sufficiently skilled. The severe and prolonged fluid losses that characterize cholera challenge the ability of caregivers to provide the correct rehydration fluids. Additionally, the increased resources needed to manage epidemics may exhaust clinical supplies, resulting in shortages and inadequate treatment for many patients.

Disease incidence and trends

In areas with endemic cholera, such as the Ganges delta, cases appear regularly during predictable cholera seasons. The highest attack rates occur in children who lack acquired immunity to the organism. In contrast, epidemic cholera occurs in areas where populations have little or no previous exposure, and infection rates are more evenly distributed across age groups, although adult males are often preferentially affected.

Historically, cholera is believed to have originated in the Ganges delta region. Although it may have occurred in other parts of the world starting in ancient times, from 1817 until recent years cholera has spread from the Ganges delta to other continents in successive waves termed pandemics (Pollitzer, 1959). However, the current seventh pandemic, involving *V. cholerae* O1 biotype El Tor, began in 1961 in Sulawesi (Celebes), Indonesia and

has since spread throughout Asia and Africa, and in 1991 to Latin America. The spread to Peru and other Latin American countries was noteworthy because they had previously been free of cholera for over 100 years. Additionally, an endemic focus of cholera persists in the Gulf of Mexico area of the United States, apparently related to a marine reservoir of *V. cholerae*. Transmission to humans from this reservoir occurs via contaminated seafood. In 1992, a new stain of serotype O139 was recognized in Bangladesh and India, and it has spread to other countries in Asia. It is thought that it may eventually spread beyond Asia and become the eighth pandemic strain.

Prior to 1977, there were no reports of widespread, clinically significant resistance in cholera, although there were sporadic reports of plasmid-mediated resistance to tetracycline from several parts of the world. Strains with transferable, multiple drug resistance were first isolated in 1964-1965 in the Philippines (Kobari, Takakura & Nakatomi, 1970; Kuwahara et al., 1967). Multiply drug-resistant strains of cholerae O1 were isolated in the United Republic of Tanzania (Mhalu, Muari & Ijumba, 1979) and Bangladesh (Glass et al., 1980). Although in both cases resistance was mediated by conjugative plasmids, the resistance patterns differed. As the local epidemic subsided over time in Bangladesh, the resistant strains were replaced by sensitive strains. It has been hypothesized that widespread prophylactic tetracycline use in Tanzania and its availability over-the-counter in Bangladesh generated the selective pressure for these multiply resistant stains. Since the late 1970s, strains of *V. cholerae* O1 isolated from various locales (India, Bangladesh, East Africa, Thailand, Latin America) have shown plasmid-encoded high-level resistance to tetracycline, ampicillin, kanamycin, streptomycin, sulfonamides, TMP and gentamicin. Such **m**ultiply **a**ntibiotic-**r**esistant *V. cholerae* have been given the acronym MARV.

With the exception of data from several surveillance centres in Bangladesh and India, data on resistance rates in cholera are limited to cross-sectional studies performed during large epidemics. Resistance patterns for both O1 and O139 serotypes vary greatly depending on region, antibiotic use pattern, and point in time. In fact, the appearance of O139 was recognized, in part, by the change in the sensitivity pattern of the prevalent *V. cholerae* from tetracycline-resistant, to tetracycline-sensitive. Longitudinal surveillance studies at the International Centre for Diarrhoeal Disease Research,

FIGURE 1. **PREVALENCE (%) RESISTANT *V. CHOLERAE* O1 ISOLATES, ICDDR, B: DHAKA, 1992–1996**
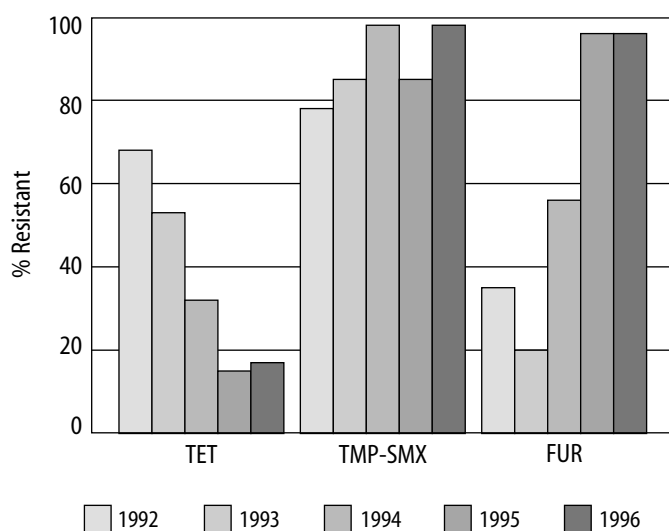
Bangladesh (ICDDR,B) reveal that susceptibility patterns fluctuate from year to year (see Figures 1 and 2). In some regions, resistance has emerged quickly to each new antibiotic used as the drug of choice for treating diarrhoeal disease. In Calcutta, resistance to TMP-SMZ quickly emerged over the course of a year during which it was heavily used; this was followed by an explosion of resistance to nalidixic acid (NA) when it became the first-line drug (Jesudason & Saaya, 1997). In several instances, such as in Bangladesh in 1979, rates of resistance to certain antibiotics rapidly increased concomitant with their use, but then declined without any change in antibiotic use patterns (Glass et al., 1983[1]). Currently, we lack an understanding of why such resistant strains should appear and then rapidly disappear while the antibiotics are still being intensively used. However, these phenomena suggest that there is an extensive pool of resistance genes and that strains with various resistance profiles will continue to appear, disappear, and reappear. One must also keep in mind the limited reliability of published data describing such events; they may not accurately reflect resistance rates in a region since there is a publication bias towards observations of high resistance rates. With these limitations in mind, the available data on resistance rates are presented by region.

Regional resistance trends

Bangladesh. Since the mid-1960s, the ICDDR,B has maintained surveillance of cholera at its field stations in Matlab and Dhaka. Prior to 1979, no resistant strains were found. In 1979, strains of *V. cholerae* O1 resistant to tetracycline, ampicillin, kanamycin, streptomycin and TMP-SMZ were isolated (Glass et al., 1980). The abrupt emergence of multiple drug resistance suggested that an R-plasmid was involved. Evaluation of 10 isolates revealed 3 distinct R-plasmids of the C incompatibility group mediating the resistance (Threlfall, Rowe & Huq, 1980). The strains disappeared after 5 months, without major changes in antibiotic use patterns (WHO, 1980). Two years later, in August 1981, a different MARV strain (ampicillin, kanamycin, sulfonamides, tetracycline and gentamicin) caused a small outbreak in Dhaka that quickly subsided (Threlfall & Rowe, 1982). Glass et al., 1983[1]) hypothesized that "the appearance and rapid disappearance of the strain in the second outbreak confirms the laboratory finding that, without antibiotic pressure, these strains have no

FIGURE 2. **PREVALENCE (%) RESISTANT *V. CHOLERAE* 0139 ISOLATES, ICDDR, B: DHAKA, 1993–1996**
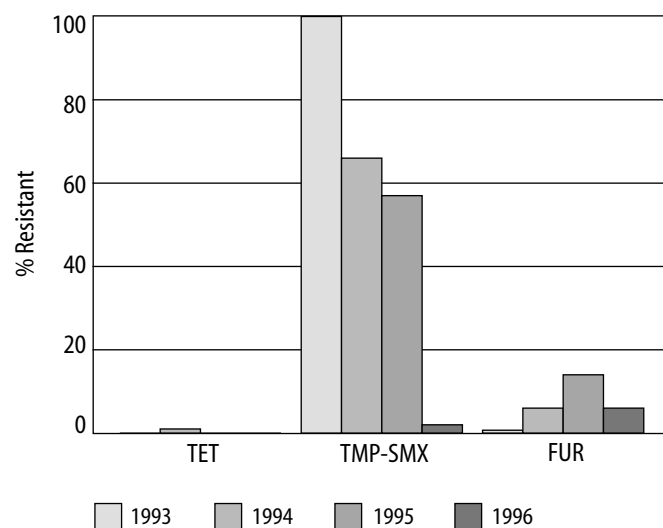
selective advantage and may continue to reappear and disappear on their own time". Dramatic increases in resistance to both tetracycline and TMP-SMZ were noted over the course of 1991 and 1992, rising from 2% to 90% for tetracycline and from 18% to 90% for TMP-SMZ (Khan et al., 1995). In another survey, tetracycline resistance among El Tor strains rapidly increased from 1.9% in 1990 to 7.6% in 1991, 61.1% in 1992, and 85.4% in 1993. As of 1994, all isolates in Dhaka were still sensitive to erythromycin, NA, pivmecillinam and the newer quinolones, although more than 90% of isolates were resistant to tetracycline, ampicillin, and TMP-SMZ (Bennish, 1994).

O139, a novel variant of cholera that was sensitive to tetracycline, erupted in Bangladesh and India in 1993 and has since spread to Thailand, Pakistan, and eight other South-East Asian nations. Fortunately, it has not spread beyond Asia. O139 strains from Bangladesh were found to be highly resistant to streptomycin and TMP-SMZ (although subsequently some isolates have been sensitive), moderately resistant to chloramphenicol and furazolidone, and susceptible to azithromycin, cephems, penems, minocycline, and the newer fluoroquinolones (Yamamoto et al., 1995). In a prospective study in Dhaka and Matlab comparing O1 and O139 strains, researchers found all O139 isolates to be sensitive to ciprofloxacin, all but one strain sensitive to erythromycin and doxycycline, and most (95% of O1 and 97% of O139) resistant to TMP-SMZ. However, the resistance patterns of O1 isolates seemed to fluctuate from year to year. Researchers attributed this fluctuation to the

instability of plasmids in *V. cholerae* (Sack et al., 1997).

India. TMP-SMZ had been widely used in India since it became available there in 1974. Subsequently, resistance emerged in a variety of pathogens, including *Salmonella typhimurium* in 1987 and *Shigella* spp. in 1988. Plasmid-mediated resistance to TMP-SMZ in *V. cholerae* appeared in Vellore in the summer of 1987. A rapid increase in resistance was documented, rising from 4.5% in July 1987 to 18.5% in August/September and 81.5% by October 1987 (Jesudason & John, 1990). With resistance to TMP-SMZ, NA became the drug of choice for the empiric treatment of gastroenteritis (when laboratory facilities were unavailable). However, *V. cholerae* O1 strains resistant to NA appeared abruptly in 1994, and have since increased in number in southern India (Mukhopadhyay et al., 1996).

Along with fluctuations in antibiotic resistance, serogroup fluctuation has been well documented in India. Epidemic *V. cholerae* O139 sensitive to tetracycline replaced the endemic strain of O1 in Calcutta between January and June of 1993. O1 reappeared in July 1993 and has subsequently predominated in this endemic area. There was marked variability in susceptibility patterns in O1 strains both before and after the epidemic of O139, although a higher proportion of MARV strains have been reported since the appearance of O139, with increasing resistance to TMP-SMZ, furazolidone, and NA (Mukhopadhyay et al., 1996) (see Figure 3). The appearance of resistance to NA has been linked to its widespread use in treating multiply resistant *S. dysenteriae* type 1 in Calcutta (Sen et al., 1988).

These findings are evidence for substantial mobility of genetic elements in *V. cholerae* (Mukhopadhyay et al., 1995). Continued surveillance revealed the resurgence of O139 in August 1996 in Calcutta, but with resistance patterns different from the O139 strains from 1993. Unlike the 1993 strains, the strains isolated in 1996 were sensitive to TMP-SMZ, chloramphenicol and norfloxacin but were more frequently resistant to tetracycline (25%), ampicillin (100%), and gentamicin (10%) (Mitra et al., 1996). The more recent O139 isolates were found to have acquired an extra rrn (ribosomal RNA) operon, demonstrating that rapid genomic changes are occurring in O139 strains (Khetawat et al., 1999). Resistance patterns of non-O1, non-O139 serogroups isolated from patients in Calcutta during 1993–1995 were very different from those of O1 and O139 strains, and included resistance to norfloxacin and ciprofloxacin (Mukhopadhyay et al., 1996). Trends towards fluoroquinolone resistance have also been noted in patients hospitalized with acute diarrhoea in Calcutta; a steady decrease in the size of the inhibition zones for norfloxacin and ciprofloxacin has been noted since 1996 (Mukhopadhyay et al., 1998).

Africa. El Tor cholera appeared in Africa in the early 1970s and rapidly spread to more than 30 countries on that continent. Since then, acute watery diarrhoea caused by *V. cholerae* O1 has become endemic in the region, with seasonal outbreaks. A dramatic increase occurred in 1998, with 29 countries reporting cholera (WHO, 1999). Transmission of cholera has been linked to the migration of refugee populations, food- and waterborne outbreaks, and cultural practices such as the preparation of

FIGURE 3. **PREVALENCE (%) RESISTANT *V. CHOLERAE* O1 AND O139 ISOLATES, CALCUTTA, 1992–1996**
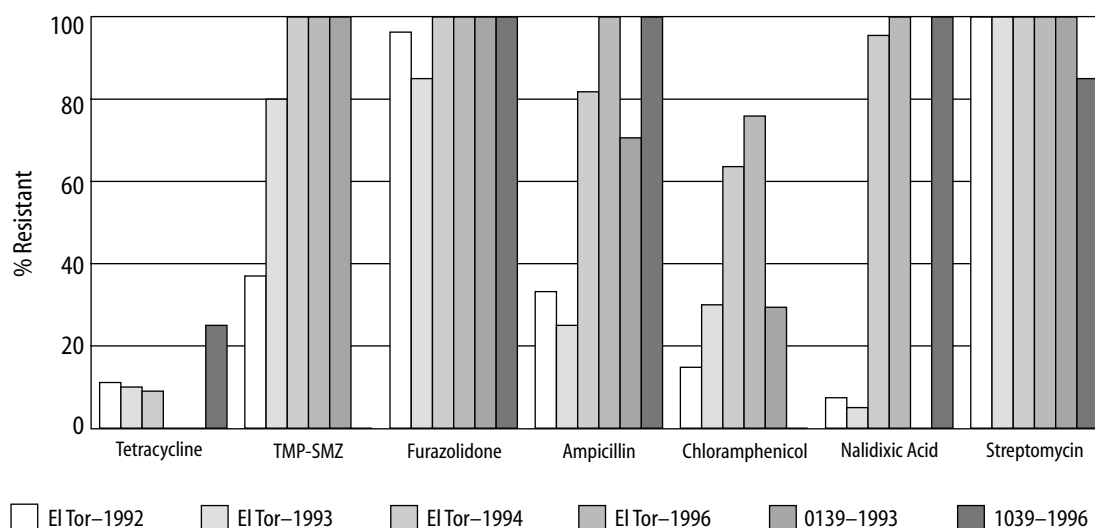
TABLE 1. **ANTIBIOTIC RESISTANCE TRENDS AMONG STRAINS OF *V. CHOLERAE* O1 IN AFRICA FROM 1994 TO 1996**

	Kenya	Sudan	Rwanda	Un. Rep. of Tanzania	Somalia
Tetracycline	0–20%	0–20%	100%	100%	10–35%
Chloramphenicol	15–>90%	30%	100%	100%	15–>90%
TMP–SMZ	15–>90%	20%	100%	100%	15–>90%
Nalidixic Acid	<5%	<20%	<5%	<20%	<5%
Erythromycin	<20%	<5%	<5%	<20%	<20%
Ampicillin	~100%	~80%	~100%	~100%	~100%
Furazolidone	~100%	~25%	~100%	~100%	~100%

bodies for burial. Surveillance during 1994–1996 revealed the resistance patterns shown in Table 1 (Materu et al., 1997).

An eight-year surveillance study in Somalia provided information about strains isolated before, during and after a 1985 epidemic that occurred in an area bordering Ethiopia. Both tetracycline and TMP-SMZ were used to treat patients and their contacts, and TMP-SMZ was also used in mass prophylaxis to control the epidemic at an early stage. Widespread use of these drugs may have influenced the emergence and spread of two resistant strains. However, despite continued use of these antibiotics, the prevalence of resistant strains began to decrease in early 1996 outbreaks. There is evidence that the decline in resistance over time was due to displacement of the resistant strain by susceptible strains rather than to loss of R-plasmids. Researchers found no evidence for an evolutionary link between strains having two distinct R-plasmids, suggesting rather that "*V. cholerae* O1 may acquire not only a resistance (R) plasmid locally, but also different R-plasmids in independent events very near in space and in time" (Coppo et al., 1995).

After a 14-year hiatus, epidemic cholera hit Burundi in 1992. Transmission was linked to bathing in and drinking contaminated water from the Great Rift Valley lakes. Most of the O1 isolates were resistant to chloramphenicol, doxycycline, TMP-SMZ, tetracycline, and ampicillin, and two isolates were resistant to NA (Birmingham et al., 1997).

The events of July 1994 in Rwanda led to one of the most noteworthy cholera outbreaks of this decade. Hundreds of thousands of refugees from Rwanda had fled to neighbouring Zaire. A cholera outbreak began on 20 July and peaked at 6000 cases on 26 July. The diarrhoeal disease case-fatality rate reached 22% at its peak, with about 40,000 people dying of cholera. The epidemic strain of O1 El Tor

was resistant to tetracycline, doxycycline, TMP-SMZ, ampicillin, and NA, but sensitive to furazolidone, erythromycin, and ciprofloxacin (Goma Epidemiology Group, 1995). The high case-fatality rate early in the outbreak was attributed to inadequate use of oral rehydration therapy (ORT), inappropriate use of IV fluids, and insufficient experience of the health care workers. In addition, several treatment centres prescribed tetracycline or doxycycline, which were not indicated given the sensitivity patterns in the region (Siddique et al., 1995). The neighbouring country of Tanzania reported 100% resistance to tetracycline, ampicillin and chloramphenicol (Materu et al., 1997), suggesting that awareness of the prevalent antibiotic resistance patterns could have been used to predict the need for alternative antibiotics.

Latin America. In January 1991, epidemic cholera emerged in Peru and rapidly spread to other Latin American nations (but, surprisingly, not to the Caribbean Island nations). From 1991 to 1994 there were over 1 million cases of cholera and 10 000 deaths. The source of the original outbreak in Peru remains unclear, but it was likely imported from Asia by way of ship's ballast and/or travellers. Molecular characterization studies have failed to find precisely the same strain in other parts of the world (Wachsmuth et al., 1993). Case-control studies linked transmission of cholera to waterborne mechanisms, food contamination (street vendors, leftover rice, unwashed vegetables) and seafood (uncooked ceviche and cooked crab) (Tauxe, Mintz & Quick, 1995).

Although the original isolates in Peru were sensitive, MARV strains appeared in Guayaquil, Ecuador later in 1991. A proportion of 36% of isolates from stool samples were resistant to multiple antibiotics, including chloramphenicol, doxycycline, kanamycin, streptomycin, sulfonamides, tetracycline

and TMP-SMZ (Weber et al., 1994). There was no clear cause of MARV emergence, but it may have been due to prophylactic use of antibiotics in this region. The recommendation was for adult family members of patients to receive 500 mg tetracycline every 4 hrs for 5 days and pregnant women and children to receive erythromycin or TMP-SMZ (Weber et al., 1994). Additional environmental pressure may have come from the use in the area of antimicrobials to control other bacteria in hatching shrimp, since fish, shellfish, and conch were implicated as vehicles of transmission. MARV strains of *V. cholerae* were also reported in Argentina and Honduras (Rossi et al., 1993; Dubon et al., 1997).

United States and Europe. Most cholera cases reported in the United States in the last four decades have been contracted during foreign travel. While, in 1992, 97% of imported strains were sensitive to all antimicrobial agents tested, resistance increased in 1993 and 1994, with the majority of isolates being resistant at least to sulfonamides, streptomycin and furazolidone. Since 1973, an endemic focus of *V. cholerae* O1 has emerged and caused sporadic cases in states bordering the Gulf of Mexico. These domestically acquired cases have remained susceptible to all antimicrobial agents tested (Mahon et al., 1996). Europe has had outbreaks of MARV cholera, with reports in southern Italy and Albania in 1994 of strains resistant to TMP-SMZ, chloramphenicol, tetracycline, doxycycline and streptomycin (Maggi, Carbonara & Santantonio, 1996).

Causes of resistance

The emergence and maintenance of drug resistance in cholera is governed by a complex series of biological, environmental, and behavioural factors. Transposons, plasmids, mobile gene cassettes and integrons mediate the rapid and broad dissemination of genetic information across species lines. Thus, we cannot look simply at resistance within the *V. cholerae* species, but rather consideration must be given to the relationship of vibrios with the environment and with other bacterial species in the environment.

Microbiological mechanisms of resistance.

Although antimicrobial resistance can result from the accumulation of chromosomal point mutations, the vast majority of clinically relevant resistance in

V. cholerae is due to exchange of genetic information among bacterial strains via plasmids and transposons. Laboratory experiments have shown that such exchange of bacterial genetic material can take place by conjugation, transduction or transformation (Ogg, Shrestha & Poudayl, 1978). In clinical settings, plasmid-mediated transfer has accounted for the emergence and dissemination of resistance genes in cholera.

Most plasmids isolated from *V. cholerae* O1 are cryptic, but some encode antibiotic resistance determinants (R-factors). These R-plasmids are large (110-170 kb), self-transmissible, and usually of the C incompatibility group. In the 1970s it was reported that, in the laboratory, R-plasmids were unstable in *V. cholerae* and were easily eliminated in drug-free conditions (Yokoto et al., 1972). A similar observation had been made earlier by Kuwahara et al. (1963) after in vitro transmission of plasmids from *Shigella* spp. to *V. cholerae*. However, stability of certain R-plasmids was reported some years later (Rahal, Gerbaud & Bouanchaud, 1978).

V. cholerae O1 R-plasmids have been found that carry genes encoding resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, spectinomycin, streptomycin, sulfonamides, tetracycline and TMP, with up to seven resistance determinants on a single plasmid (Threlfall, Rowe & Huq, 1980). It is thought that most of these genes were acquired from *Enterobacteriaceae*. Plasmids of the C incompatibility group are found in a wide variety of bacterial genera, including *Pseudomonas*, *Proteus*, *Klebsiella*, and *Serratia*. Bacteria may acquire resistance genes from other species of the normal intestinal flora under the selective pressure of antimicrobial use. *V. cholerae* R-plasmids have been shown to carry resistance determinants (e.g. for ampicillin and TMP) that are common in enteric bacteria (Young & Amyes, 1986). When Rahal, Gerbaud & Bouanchaud (1978) examined the transferability and maintenance of plasmids, they found that although plasmids of most incompatibility groups could be transferred from *E. coli* to *V. cholerae*, only those of groups C and J were maintained.

In recent years, light has been shed on the important role that transposons play in resistance. It has been found that the resistance of El Tor strains to TMP, spectinomycin, streptomycin and the vibriostatic agent 0/129 is due to a transposon inserted into the chromosome (Goldstein, Gerbaud & Courvalin, 1986). This was demonstrated by transferring the resistance determinants to a trans-

missible plasmid and then into the chromosome of *E. coli*. In *V. cholerae* O139 strains, resistance determinants for SMZ, TMP and streptomycin are carried on a 62-kb self-transmissible, chromosomally integrating genetic element that is similar to conjugative transposons found in *Bacteroides* (Waldor, Tschape & Mekalanos, 1996). In the same study, it was found that El Tor O1 strains that re-emerged after the O139 strain had declined had similar self-transmissible transposons carrying resistance genes, suggesting that these elements are widely disseminated in *Vibrio* spp. and confer some selective advantage to the bacteria.

Environmental determinants of resistance

Because *V. cholerae* can readily exchange genetic information among strains and with other bacterial species, controlling the emergence of resistance requires an understanding of the source of R-plasmids. The gut and other environments (soil, sewage, etc.) contain a variety of organisms that cannot be cultured. This makes it impossible to precisely track the transfer and dissemination of resistance genes in nature. Although not definitive, most available data suggest that other enteric bacteria, such as *E. coli* and non-O1, non-O139 *V. cholerae* serogroups in the environment have been the intermediate hosts.

Resistance genes are common and plentiful in normal bacteria in the environment, such as *E. coli*. Some of these genes may have first appeared millions of years ago. Since *E. coli* spend time cycling through animals, humans, sewage, water and soil, one can hypothesize that *E. coli* may have acquired resistance genes that evolved to provide protection against antibiotics produced naturally by soil bacteria. In the antibiotic era there is environmental selective pressure for the development of resistance. A longitudinal community-based survey of children from urban Mexico found persistent (13 weeks) faecal shedding of ampicillin-, tetracycline-, and TMP-SMZ-resistant *E. coli* in the majority of the cohort of healthy children, as well as some children that shed *E. coli* resistant to chloramphenicol, gentamicin, nitrofurantoin, and norfloxacin. The fact that the three most commonly used antibiotics in this Mexican community are ampicillin, TMP-SMZ and tetracycline suggests that overuse of these drugs for common illnesses exerts a selective pressure on the normal bowel flora, which then become an important reservoir of resistance genes that can potentially be transferred to pathogenic organisms such as *V. cholerae* (Calva, Sifuentes-

Osornio & Ceron, 1996). There is, in fact, evidence for the local acquisition of R-plasmids by *V. cholerae*. Most *V. cholerae* resistance plasmids are transferable to *E. coli in vitro* (Rahal, Gerbaud & Bouanchaud, 1978) and these plasmids can be transferred back into *V. cholerae* from laboratory strains of *E. coli* (Finch et al., 1988). *E. coli* and *V. cholerae* with identical resistance plasmids have been isolated from the same patient (Haider & Huq, 1986). Studies in Bangladesh demonstrated that family contacts of individuals infected with MARV were more likely than controls to have other multiply antibiotic-resistant bacteria, carrying the identical resistance plasmid, in their intestinal flora (Glass et al., 1983[1]).

To summarize, there is evidence supporting the hypothesis that resistance genes in *V. cholerae* can be acquired locally from enteric flora such as *E. coli*. If this is the case, antibiotic use for any purpose (other diarrhoeal disease, respiratory illness, STD control) will affect the reservoir of resistance genes in *E. coli* that are potentially transferable to *V. cholerae* should an outbreak occur.

There has also been increasing interest in the role that non-O1, non-O139 serogroups may play in the shifting dynamics of *V. cholerae* and its resistance patterns. On the basis of gene sequence variation analysis, Karaolis, Lan & Reeves (1995) suggested that the last two cholera pandemics were likely caused by independent clones that emerged from environmental, nontoxigenic, non-O1 *V. cholerae*. Because there is a high rate of genetic exchange among different *Vibrio* strains in the environment, non-O1, non-O139 strains may be important reservoirs of resistance elements. This is especially important since non-O1, non-O139 strains are increasingly resistant to ciprofloxacin and other fluoroquinolones, the only widely used drugs to which *V. cholerae* O1 remain universally sensitive.

Behavioural and economic factors in resistance

Because selection for resistance is thought to be a function of total antibiotic pressure in an area, drug use pattern is an important factor. In most developing countries there is uncontrolled use of inexpensive broad-spectrum antibiotics. There is often inappropriate prescribing by clinicians, or misuse by unskilled health workers or by traditional healers. A majority of the public may purchase antibiotics, without a prescription, from local pharmacies, as well as from street vendors or drug stalls. Because the unofficial retailers are not guided by any

regulatory criteria for rational antibiotic use, there is considerable inappropriate self-medication (Okeke, Lamikanra & Edelman, 1999).

As a public health measure, antibiotics are often prescribed on a large scale for prophylaxis during epidemics. This is controversial since, under such intense selective pressure, the benefit to individuals is usually offset by the rapid emergence of resistance. Experiences with mass prophylaxis in three different epidemic situations are described below.

1. **United Republic of Tanzania**. When mass tetracycline prophylaxis of close contacts was used for cholera control in 1977, widespread multiple drug resistance appeared within six months. During the first month of the epidemic, all isolates were sensitive to tetracycline, but after 5 months of extensive tetracycline use for therapy and prophylaxis (1788 kg used by the Ministry of Health [MOH]), 76% of the isolates were resistant to tetracycline and 52% to chloramphenicol (Mhalu, Muari & Ijumba, 1979).

2. **Cameroon**. Mass prophylaxis with sulfadoxine (fanasil) was used during a large outbreak in 1983. Multiply drug-resistant strains (sulfadoxine, tetracycline, chloramphenicol and TMP-SMZ) were isolated in 1984–1985 (Garrigue et al., 1986).

3. **Kenya**. Mass tetracycline prophylaxis campaigns were carried out from 1981 to 1988. Strains resistant to tetracycline, ampicillin and TMP-SMZ were isolated in 1982 (Ichinose et al., 1986). Resistance was found to be mediated by a single plasmid that differed from the plasmids found in other regions (United Republic of Tanzania, Nigeria, Bangladesh) (Finch et al., 1988). Studies of isolates from 1982 to1985 have demonstrated the persistence of the resistant strain. The fact that distinct plasmids persist in different geographical areas suggests that resistance plasmids are acquired locally as a result of local antibiotic pressure (Finch et al., 1988).

Treatment choices and development of resistance

The primary treatment for patients with cholera is rehydration with oral or intravenous fluids. Antibiotics are given to decrease the volume of purging and the duration of diarrhoea, and thus to decrease the cost of treatment (Lindenbaum, Greenough & Islam, 1967) by sparing limited supplies and personnel, shortening hospital stays and returning the patient to normal function sooner (i.e. shorter

period of lost wages). Inexpensive, effective antibiotics are very cost-effective as adjunct therapy in severe cases, since they reduce the hospital stay and decrease the volume of intravenous fluids and ORS needed for rehydration. While antibiotics rapidly eradicate organisms from the stool, they probably have minimal impact on the dynamics of cholera transmission in the community, as there are environmental reservoirs and because a large proportion of asymptomatic, or only mildly ill, infected individuals, who would not normally receive antibiotics, shed vibrios.

Tetracycline traditionally has been the antibiotic of choice (adult dose 500 mg every 6 hours for 48 to 72 hours; children's dose 50 mg/kg/day in 4 divided doses for 2–3 days), but resistance to this drug is widespread. Many authorities, such as WHO, now recommend doxycycline as the first-line drug, since a single 300 mg dose is effective for adults (Alam et al., 1990; Sack et al., 1978) and guarantees compliance without the need for follow-up, a significant logistical advantage. Strains that are sensitive to tetracycline are also sensitive to doxycycline, so there is no need to test specifically for doxycycline resistance. In fact, *in vitro* susceptibility to doxycycline may not correlate well with its *in vivo* activity, owing to the variable expression of inducible tetracycline resistance determinants (Khan et al., 1996).

TMP-SMZ is recommended as the first-line drug for children and furazolidone (100 mg) for pregnant women. Other effective drugs include ciprofloxacin, erythromycin and chloramphenicol. Sulfadoxine (fanasil), single dose, has also been used, but resistance is increasing in Africa and the drug may have serious side-effects (Stevens-Johnson syndrome).

In a study in Bangladesh, erythromycin and ciprofloxacin were both shown to be effective alternatives for the treatment of MARV strains. It was suggested that NA and pivmecillinam should be reserved for the treatment of shigellosis, since they did not have significant efficacy in symptomatic cholera (Khan et al., 1995).

Short- vs. long-course therapy

A three-day course of tetracycline has been the antibiotic regimen of choice for cholera although, as discussed above, doxycycline has some advantages. In a randomized double-blind study at ICDDR,B, single-dose ciprofloxacin (1 g) was effective in treating both *V. cholerae* O1 and O139 and was more effective than single-dose doxycy-

cline (Khan et al., 1996). Single-dose regimens are less effective in achieving culture-negativity of the stool (Islam, 1987). However, in cholera, antibiotics are used to accelerate the clinical improvement of the individual patient, not to control the spread of cholera, a purpose for which they are not effective. For this reason, the evaluation of antibiotic regimens should be based on clinical improvement of patients (decreased duration and volume of purging) rather than on eradication of vibrios from the stool.

Some clinicians are of the opinion that single-dose or short-course regimens will increase resistance. More research is needed to thoroughly evaluate this. However, data for *V. cholerae* suggest that resistance does not develop in an individual patient but rather that there are shifting populations of sensitive and resistant strains that are affected by overall antibiotic use. For these reasons, single-dose or short-course regimens which are clinically effective (e.g. doxycycline or ciprofloxacin) are preferred to longer course (3–5 day) regimens which are more likely to upset the enteric bacterial ecology of the patient and to favour resistant over sensitive strains. Additionally, the lower cost and ease of administration of short-course therapy outweigh the risk of slower eradication of vibrios from the stool.

The question of prophylaxis

During epidemics, there is often pressure to provide prophylactic antibiotics to household contacts of cholera patients, or even to entire communities. Early studies showed that tetracycline or doxycycline could prevent secondary cases and reduce vibrio excretion in household contacts (Gupta et al., 1978; Joint ICMR-GWB-WHO Cholera Study Group, 1971). McCormack et al. (1968) described the efficacy of 5 days of tetracycline prophylaxis in reducing secondary infection within the families of cholera patients. Khan (1982) later demonstrated that two 250 mg doses of tetracycline (short-course therapy) decreased the number of severe cases of diarrhoea and the hospitalization rate among contacts during a cholera epidemic in Dhaka. Treatment of close contacts has been considered more appropriate than mass chemoprophylaxis, since 10–25% of household contacts may become vibrio excretors as compared with less than 1% of community contacts. However, transmission rates are highly dependent on the local situation. WHO recommends that "selective chemoprophylaxis be considered only when surveillance has shown that, on average, at least one household member in five becomes ill after the first case has appeared" (WHO, 1992[2]). However, cholera outbreaks frequently occur quickly and in regions where such epidemiologically based decision-making is impractical.

The only situation in which prophylaxis seems warranted is during an epidemic of tetracycline-sensitive cholera, when a single dose of doxycycline can be given to immediate household members (e.g. those who share the same kitchen) within a two-day period after the case is diagnosed. Situations in which this can be accomplished are extremely rare, and attempts to follow this procedure frequently lead to wide-scale antibiotic use and abuse.

Mass antibiotic prophylaxis is not recommended because it has not been shown to be effective and because it contributes to the emergence of resistant strains. Additionally, there are problems such as delays in beginning prophylaxis, the fact that its effects last for a short time, non-compliance, and the occurrence of side-effects (especially with sulfonamides) (Hernborg, 1985). It is very difficult to selectively treat high-risk contacts without dispensing large amounts of antibiotics in the community; this generates selective pressure for resistant strains, and thus the possibility of being unable to effectively treat severe cases of disease.

It is important to realize that "prophylactic" antibiotic administration does not actually prevent infection. Rather, in the case of cholera, prophylaxis is intended to kill the bacteria at an early stage of infection; i.e. it is assumed that a large percentage of the contacts are already infected and are incubating the disease. Thus, these are not "secondary cases" infected by contact with the index case, but are rather "co-primary cases" who have not yet exhibited symptoms. Finding and treating them rapidly, appropriately and specifically is not practical in most cases.

Potential vaccines

Injectable vaccines for cholera have been used since the late 1800s, but with little lasting benefit. The currently available killed injectable vaccine is not recommended, since studies in the 1960s showed it to be only 60% effective for a period of 4–6 months (WHO, 1996). The recent availability of improved oral cholera vaccines, such as the recombinant oral B subunit killed whole-cell (rBS-WC) vaccine and the live attenuated CVD 103-HgR vaccine has led to renewed interest in the use of vaccines during cholera epidemics.

A recent analysis of results in sub-Saharan refugee camps showed that mass vaccination could be cost-effective in controlling cholera if the price of the vaccine was sufficiently low (Naficy et al., 1998). This study did not consider the potential role that a vaccine might play in preventing the occurrence of antibiotic-resistant infections. A vaccine that is effective in lowering the total number of cases will also lower the number of resistant infections and could thus represent an effective intervention measure to control antibiotic resistance in cholera. Additional research is necessary to define the role of vaccines as preventive measures in endemic regions such as the Ganges delta.

Recommendations

1. Antibiotics that are known to be clinically effective, and to which the bacteria are susceptible *in vitro*, are appropriate and cost-effective in the treatment of patients with moderate or severe cholera. In epidemics, antibiotics are likely to be life saving, providing better treatment to more patients.

2. If the predominant cholera strain is known to be resistant to an antibiotic, that antibiotic should not be used, as it would not be efficacious and is potentially harmful. Antibiotics that are not clinically effective for treating cholera (e.g. cephalosporins or gentamicin) should not be used even if *in vitro* tests show the strain to be sensitive.

3. A surveillance system is needed to collect a representative sample of strains, determine sensitivity patterns in a region, and continually monitor changes in sensitivity patterns. Susceptibility testing should be carried out in an established laboratory with stringent quality control (QC) standards. Strains with unusual susceptibility patterns should be sent to reference laboratories for confirmation. The results of surveillance should be used to guide antibiotic treatment of patients with suspected cholera. It is neither practical nor wise to base the choice of antibiotic on the sensitivity pattern in the individual patient, since antibiotics must be given early in treatment if they are to be effective.

4. If most strains are tetracycline-sensitive, then single-dose doxycycline is the preferred treatment. If the predominant strains are resistant to tetracycline, then alternative choices need

to be made based on antibiograms of locally representative strains.

5. If most strains are resistant to all recommended antibiotics, then treatment must be based on rehydration alone, and plans must be made to manage the most severe and refractory cases. Occasionally, ciprofloxacin is the only clinically effective antibiotic. An analysis of cost-effectiveness should guide the choice of this antibiotic, but in most areas it will be cost-effective. Ciprofloxacin should be used in a single-dose regimen.

6. Antibiotic pressure leads to the development of resistant *V. cholerae*; thus, inappropriate antibiotic use for any illness can lead to resistant cholera. Antibiotics should be carefully targeted to those conditions that truly warrant their use.

7. Antibiotics should not be used to prevent cholera, except in certain very unusual circumstances.

8. The use of antibiotics in agriculture should be limited to the treatment of individual animals; they should not be added to feed to promote the growth of animals and should not be added to environmental waters for fish or seafood farming. In particular, the use of tetracycline and fluoroquinolones in agriculture should be controlled.

9. MOHs, nongovernmental organizations (NGOs) and others should base their antibiotic use and procurement policies on data from established surveillance systems. Policies and procurement procedures need to be sufficiently flexible to adapt to changes in antibiotic susceptibility patterns.

10. Information on antibiotic sensitivity generated by surveillance systems should be readily available in the form of hotlines, web pages, bulletins, etc., so that it can be easily consulted by those responsible for drug policy and procurement decisions.

11. Ongoing epidemics, as well as expected cholera seasons, need to be posted in the same sites, since the number, timing and severity of the cases affect the urgency with which the health care system must prepare to treat patients with cholera. A geographical information system (GIS) format may be useful for reporting seasonal occurrences and antibiotic susceptibility patterns.

12. Cost-effectiveness analyses for alternative antibiotics must consider the total cost of illness and not simply the cost of the antibiotic. Overall costs include the price of the drug per patient treated (not per tablet), hospital time, nursing care, lost wages, etc.

13. Cholera control programmes that successfully limit the number of cases will also limit the number of antibiotic-resistant infections. Improved water, sanitation, and hygiene programmes, as well as the use of oral vaccines, all help to decrease disease incidence and antibiotic resistance, especially in high-incidence areas.

14. Representative strains having multiple antibiotic resistance should be characterized in molecular biology laboratories in order to better understand the mechanisms.

Research priorities

1. Epidemiological surveillance and communication of findings

   1.1 Establishment of a simple and inexpensive surveillance system for monitoring cholera incidence and antibiotic resistance patterns. One of the systems in use at the ICDDR,B may be adapted for other geographical locations. Any new surveillance system should be field-tested in two or three cholera endemic areas.

   1.2 The utility of rapid diagnostic tests (e.g. Smart Test or coagglutination tests) in surveillance should be studied. Such tests, if used appropriately, could help identify those specimens that should be sent to the central laboratory for further testing.

   1.3 Centralized or regional resource centres for cholera epidemiology and susceptibility testing need to be established so that MOHs and NGOs can have access to current information about resistance patterns. A resource centre should field-test its communications methods in order to optimize their effectiveness.

2. Factors affecting antibiotic resistance

   2.1 Studies are needed to document the effect of inappropriate antibiotic use on cholera sensitivity patterns, for example comparing the susceptibility patterns of geographical areas with high and low antibiotic use. The utilization of antibiotics in agriculture and aquaculture appears to be another important issue requiring investigation.

   2.2 Studies are needed to document the reappearance of sensitive cholera strains, following epidemics caused by resistant strains, in order to identify parameters that correlate with this phenomenon. Similarly, in areas where sensitive strains have not reappeared, explanations should be sought.

   2.3 Surrogate parameters that correlate with resistance in *V. cholerae* should be sought. It seems that *V. cholerae* follow the sensitivity patterns of *Enterobacteriacae*, so it is possible that surveillance of resistance patterns in normal *E. coli* will predict the patterns in *V. cholerae*.

   2.4 Antibiotic susceptibility trends of environmental non-O1, non-O139 *V. cholerae* need to be monitored in order to determine whether these will be predictive of patterns in O1 *V. cholerae* and whether resistance genes are exchanged among the different serotypes.

3. Studies of constraints on appropriate antibiotic use

   3.1 Case reports related to inappropriate antibiotic policy should be studied to help us understand why inappropriate antibiotics were recommended or used.

   3.2 The influence of non-medical factors (e.g. procurement methods, pharmaceutical sales methods, storage and distribution problems) on the choice of antibiotics and on essential drug programmes should be determined.

4. Intervention studies

   4.1 The impact on resistance trends of controlling antibiotic use should be quantitated and analysed in terms of lives saved and cost reduction.

   4.2 The impact of water and hygiene interventions on the incidence of antibiotic-resistant cholera should be determined.

   4.3 The impact of effective cholera vaccines on the incidence of antibiotic-resistant cholera should be studied.

5. Basic studies of antibiotic resistance

   5.1 The molecular mechanisms of antibiotic resistance need to be determined and the

different genes responsible for resistance catalogued.

5.2 The molecular mechanisms of transmission of resistance genes (plasmids, transposons, phages, etc.) need to be studied in order to better understand how transmission occurs and to which other bacterial species.

5.3 A better understanding of receptor mechanisms for transmissible genes is required in order to comprehend the range of bacterial species that contribute to resistance in *V. cholerae*.

Conclusion

V. cholerae can become multiply antibiotic-resistant via the acquisition of plasmids. Multiply resistant strains have repeatedly caused epidemics. Antibiotic resistance patterns change from time to time and place to place, requiring continual surveillance in order to provide optimal treatment. Susceptible strains sometimes reappear when antibiotic pressure is lifted, suggesting that cholera may be one disease in which we might expect relatively rapid reversion to sensitivity if antibiotic use is controlled.

Because epidemics due to antibiotic-resistant strains remain a threat, effective antibiotics are needed to reduce costs and treatment time, shorten the duration of illness, and save lives.

Review of *Shigella* spp.

Introduction

Like *Campylobacter* spp. and *V. cholerae*, *Shigella* spp. have managed to survive the antibiotic era via ingenious mechanisms of resistance. Unlike diarrhoea due to *C. jejuni*, shigellosis may occur in epidemic form, causing considerable morbidity and mortality in developing nations. Shigellosis is primarily a disease of resource-poor, crowded communities that do not have adequate sanitation or safe water, and where disease rates may be high. Because shigellae have acquired multiple antimicrobial resistances, the challenge for clinical management is identifying which drugs retain their activity and clinical effectiveness. In the United States, the Centers for Disease Control and Prevention (CDC) have recommended that sensitivity testing be performed to guide selection of appropriate antimicrobial therapy for shigellosis (CDC, 1987). However, testing requires several days to complete, resulting in treatment delay, and is generally not feasible in developing countries. Because antimicrobial susceptibility patterns of shigellae may vary greatly in different geographical areas and over time, monitoring resistance patterns is needed to guide selection of appropriate empiric treatment (Jesudason, Lalitha & Koshi, 1985; Munshi et al., 1987; Ashkenazi et al., 1995).

Organisms and syndrome

Shigella belongs to the family *Enterobacteriacae*. It is a small, unencapsulated, non-motile Gram-negative rod. There are four species of *Shigella*, classified on the basis of biochemical and serological differences: *S. dysenteriae* (Group A), *S. flexneri* (Group B), *S. boydii* (Group C), and *S. sonnei* (Group D). The first three are further divided into multiple serotypes; thus, the genus contains a large number of different pathogens. *S. dysenteriae* serotype 1 (also known as the Shiga bacillus) deserves special note because it is more likely to produce severe disease, to spread in epidemics, to be particularly antibiotic-resistant, and to produce Shiga toxin. Because organisms of the genus *Shigella* are the most common cause of bacillary dysentery, this

term has become synonymous with all clinical presentations of shigellosis, although these presentations range from asymptomatic carriage to mild, watery diarrhoea to overt dysentery (Levine et al., 1973; Levine, 1991).

Dysentery is a syndrome characterized by frequent, but small volume, loose stools, consisting largely of blood and mucus. Fever, pain, and tenesmus (unproductive straining) are frequently present. Unlike secretory diarrhoeas, this syndrome is a result of invasion of the distal small bowel and/or colon by bacteria. Because dehydration is not as severe as in secretory diarrhoeas, oral rehydration therapy does not significantly reduce the case-fatality rates for shigellosis (Keusch & Bennish, 1989; Bennish et al., 1990[2]).

Community data on shigellosis are incomplete, but most hospital-based studies suggest that the case-fatality rate is highest among children less than 5 years of age, particularly if there is malnutrition. In epidemic situations a mortality rate as high as 3.9% in children under age 1 and 19.3% for infants under 4 months of age has been reported. The case-fatality rate declines with increasing age (Bennish et al., 1990[2]; Bennish, 1991). An unusual finding from recent studies of epidemic shigellosis in Central Africa was high mortality rates in young adults. This region also has high HIV rates, so an interaction between the two infections is possible, but has not yet been studied. The most common acute complications, particularly in settings with limited health care services, include sepsis (caused by *Shigella* or other enteric organisms), intestinal perforation, toxic megacolon, dehydration, hyponatraemia, encephalopathy, haemolytic-uraemic syndrome, and pneumonia. Shigellosis may also be followed by persistent dysentery, protein-losing enteropathy, and rapidly worsening malnutrition. Because shigellosis often occurs in settings where treatment for some of the complications is not available, reducing mortality from this disease hinges upon its prevention (Bennish, 1991).

Geographical distribution

Shigella is highly adapted to man, with humans and primates in captivity being the only known natural hosts. The minimal infective dose is less than 200 cells, facilitating transmission in areas where there are crowding, poor sanitation and poor hygiene (Levine, 1991; DuPont et al., 1989). In endemic areas of the developing world, shigellosis is predominantly a paediatric disease, with the urban poor being hardest hit. In developed countries it occurs more commonly where there is overcrowding or poor sanitation (e.g. in institutionalized individuals, children in day-care centres, prisoners, military recruits and residents of Native American reservations) (Keusch & Bennish, 1998; Keusch, 1998; Pickering, Bartlett & Woodward, 1986; Rosenberg et al., 1976). Although these groups have higher rates of disease than the population at large, foodborne infection frequently occurs among individuals who are not in high-risk groups.

Shigella transmission is by the faecal-oral route, and may be via food or water, as well as by person-to-person spread. Contaminated hands often contaminate food that is then served to others; hand washing decreases rates of transmission. In some regions, disease rates increase somewhat during the monsoon season, perhaps due to increased faecal contamination of drinking-water (Hossain, Albert & Hasan, 1990). There is also evidence that flies, particularly *Musca domestica*, the common housefly, may serve as vectors for the transmission of shigellosis owing to the low inoculum needed to cause disease (Watt & Lindsay, 1948; Levine & Levine, 1991).

The geographical distribution and the pathogenicity of the four species of *Shigella* are different; the reasons for this are still unclear (Keusch & Bennish, 1998; Keusch, 1998). *S. flexneri* is the most commonly isolated species in the developing world and the most frequent cause of morbidity and mortality. *S. dysenteriae* infections also occur in less developed countries, often in epidemics, with periodic pandemic outbreaks. In industrialized countries (i.e. those with good water and sanitation) the predominant species is *S. sonnei*. In general, the illness caused by *S. sonnei* is less severe, but individual cases of infection with any of the *Shigella* species can be severe (Acheson & Keusch, 1995). Infection with *Shigella* protects against subsequent infection with the same serotype; however, because there are multiple serotypes, individuals may become infected several times.

Diagnosis and resistance detection

The physical signs and symptoms of shigellosis include abdominal cramps, fever and chills, malaise, diarrhoea and/or dysentery, and abdominal tenderness. Examination of the rectal mucosa shows it to be inflamed and friable, with ulcers present in severe cases. Blood and mucus in the stool are frequent manifestations of shigellosis, and abundant faecal leukocytes are generally noted on examination due to the inflammatory and invasive characteristics of the organism. Diagnosis is confirmed by culturing a stool specimen, although the organism may also be cultured from blood in some cases.

Media that inhibit the growth of Gram-positive bacteria, such as MacConkey, Shigella-Salmonella (SS) agar, or xylose-lysine-deoxycholate (XLD), are necessary for isolation of *Shigella* from clinical specimens. After overnight incubation at 37 °C shigellae appear as pale, lactose-non-fermenting colonies on MacConkey agar and as pink colonies on XLD medium (Shears, 1996). The identity of suspect colonies can then be confirmed using standard methods, including agglutination with species-specific antiserum.

Shigellae remain viable for a limited time outside the human body; therefore, stool specimens should be processed within a few hours after collection (Shears, 1996; Levine, 1991). If this is not possible, buffered glycerol saline or Cary-Blair medium can be used as a transport medium. Delayed detection of shigellosis epidemics has occurred in the past as a result of poor transport conditions or inadequate laboratory facilities. Because of this fragility during transport, more rapid and sensitive techniques for detecting *Shigella* have been developed. These methods utilize gene probes or polymerase chain reaction (PCR) primers directed towards virulence genes such as the invasion plasmid locus (*ipl*) or that encoding the IpaH antigen virulence factor. Although more sensitive than the conventional diagnostic methods, these techniques require a sophisticated laboratory and are not widely used in clinical laboratories (Keusch & Bennish, 1998; Keusch, 1998).

Antimicrobial susceptibility testing most often uses the disc diffusion (Kirby-Bauer) method due to its simplicity and to the possibility of testing a series of antimicrobial agents in one experiment (Bauer et al., 1966; Williams, 1990; Shears, 1996). The agar and broth dilution methods are also widely used. In these assays the medium contains fixed antibiotic concentrations, and a standard inoculum

of bacteria is used (Ackerman & Dello Bueno, 1996). Several newer methods are also available, including the epsilometer strip method (E-test), in which a plastic strip is impregnated with a series of antibiotic concentrations. Once the strip is placed on agar, diffusion creates a concentration gradient. Lines printed on the plastic strip permit accurate determination of the minimum inhibitory concentration (MIC) (Ackermann & Dello Buono, 1996; Brown & Brown, 1991; Olsson-Liljequist, 1992). The E-test is comparable to other methods for determining the MICs of antibiotics for a variety of pathogens, especially multi-resistant Gram-negative bacilli, including *Shigella* species (Huang et al., 1992; Kruse et al., 1992). However, it has several drawbacks, the most important of which is its high cost (Huang et al., 1992).

Pathogenesis

Several features of *Shigella* contribute to its invasiveness and pathogenicity. The bacteria are able to invade enterocytes in colonic and rectal epithelia and to lyse intracellular phagocytic vacuoles, thus escaping into the cytoplasm where they multiply and then invade adjacent cells (Shears, 1996; Keusch & Bennish, 1998; Keusch, 1998; Bernardini et al., 1989). The ability to invade epithelial cells is associated with the presence of large plasmids (120–140 Mdal) that encode outer membrane proteins such as invasion plasmid antigens or invasins (Ipa). Strains lacking this plasmid are avirulent (Sansonetti, Kopecko & Formal, 1982). The Ipa are involved in recognition of epithelial cells and induce a signal transduction cascade. Actin is polymerized in the cell, resulting in a bacterium-directed endocytosis of the organism (Sansonetti & Phalipon, 1996; Keusch & Bennish, 1998; Keusch, 1998; Shears, 1996; Adam et al., 1995). Intracellular movement is associated with IcsA, a protein linked to the formation of an actin tail behind the bacterium. Organisms with mutant IcsA are considerably less virulent (Sansonetti & Phalipon, 1996; Keusch & Bennish, 1998; Keusch, 1998). The ability of *Shigella* to colonize the epithelial layer protects the bacteria from exposure to the extracellular environment. Intracellular growth and multiplication ultimately result in the death of epithelial cells, with resultant ulceration and mucosal inflammation (Keusch & Bennish, 1998; Keusch, 1998).

In addition to the virulence factors discussed above, endotoxins contribute to the pathogenicity

of *Shigella* spp. and may be responsible for many of the systemic symptoms of shigellosis, such as fever, malaise, and body aches. *S. dysenteriae* type 1 produces a potent protein cytotoxin known as Shiga toxin. Studies in primate models and human volunteers have demonstrated that Shiga toxin is not needed to cause disease, although toxin-producing strains are more pathogenic than toxin-negative type 1 strains (Fontaine, Arondel & Sansonetti, 1988; Levine et al., 1973; Keusch & Bennish, 1998; Keusch, 1998). Shiga toxin has a subunit structure consisting of functional (A) and binding (B) subunits. The B subunit mediates binding to a specific cellular receptor known as glycolipid Gb3 (globotriaosylceramide) (Jacewicz et al., 1986; Acheson, Donohue-Rolfe & Keusch, 1991). Binding is followed by receptor-mediated endocytosis of the toxin. Once in the cytoplasm, the A subunit catalyses hydrolysis of the 28S RNA of the 60S ribosomal subunit, resulting in irreversible blockade of protein synthesis and cell death (Acheson & Keusch, 1995; Keusch & Bennish, 1998; Keusch, 1998). Shiga toxin appears to mediate the haemolytic-uraemic syndrome and thrombotic thrombocytopoenic purpura, which are also associated with infection by serotypes of *E. coli* that produce homologous toxins, but not toxin-negative strains (Acheson & Keusch, 1995; Hofmann, 1993; Keusch & Bennish, 1998; Keusch, 1998). The mechanism of these pathogenic effects may involve binding of the toxin to endothelial cells, leading to microangiopathic haemolysis and glomerular lesions (Keusch & Bennish, 1998; Keusch, 1998).

Therapy

As with other infectious diarrhoeal diseases, treatment of shigellosis includes both rehydration and antibiotics. Fluid losses in shigellosis are not as dramatic as in the secretory diarrhoeas, and dehydration is not usually as significant a problem. It can usually be managed with oral rehydration therapy. Most controlled clinical trials of antimicrobial chemotherapy have demonstrated that effective antibiotics shorten the duration of symptoms and eradicate shigellae from the stool more quickly (compared to placebo or to ineffective antibiotics) (Salam & Bennish, 1991). Therefore, adequate treatment for shigellosis depends upon the availability of effective antimicrobial agents.

A variety of antibiotics are effective for treatment of shigellosis, although options are becoming limited due to globally emerging drug resistance.

Originally, both sulfonamides and tetracycline were effective, but *Shigella* strains rapidly developed resistance to these agents. Ampicillin and TMP-SMZ were then used and continue to be effective in many industrialized countries. Unfortunately, in many parts of the world strains of all species of *Shigella* have become resistant to these low-cost agents, and neither can now be confidently used as empiric therapy for shigellosis (Tauxe et al., 1990; Haltalin et al., 1967; Nelson et al., 1976; Chang et al., 1977; DuPont & Steele, 1987; DuPont et al., 1987; Bennish & Salam, 1992; Bennish et al., 1992).

One of the few remaining, relatively inexpensive and effective drugs for shigellosis is the quinolone NA. In clinical trials, NA-treated groups achieved rates of clinical cure (absence of fever and of unformed stools by day 5 of treatment) and bacteriological cure (absence of *Shigella* from the stool by day 3 of therapy) comparable to the rates in individuals with ampicillin-sensitive infections treated with ampicillin (Salam & Bennish, 1988). Unfortunately, resistance to NA is also common; in regions where it was introduced to treat epidemic shigellosis due to Shiga bacillus, resistance developed within six months (Munshi et al., 1987). Although there is concern about the use of fluoroquinolones in children, because they cause arthropathy in immature animals, no evidence of arthropathy has been observed in children treated with NA (Corrado et al., 1987; Salam & Bennish, 1988). For a life-threatening infection like shigellosis, where no other treatment may be available, this potential side-effect is considered to be a very small risk. Newer fluoroquinolones, such as norfloxacin and ciprofloxacin, are more active than NA, with more rapid eradication of organisms (including NA-resistant strains) from the stool. (Rogerie et al., 1986). These antibiotics are more expensive, and emergence of resistance even to these drugs is likely if they are widely used; thus, there are few antibiotic options (Bennish & Salam, 1992; Bennish et al, 1992; Rogerie et al., 1986; Munshi et al., 1987; Hoge et al., 1998).

Clinical trials in Israel demonstrated that third-generation cephalosporins, such as cefixime and ceftriaxone, have better rates of bacteriological and clinical cure than ampicillin or TMP-SMZ and that they are safe for use in children (Ashkenazi et al., 1993; Varsano et al., 1991). However, in a Bangladesh trial, Salam et al. (1995) failed to confirm the effectiveness of cefixime in shigellosis. The reason for this disparity is unclear, but may involve the design of the clinical trials. Another agent that may be used in children and adults is pivmecillinam,

an oral pro-drug that is rapidly hydrolyzed to mecillinam (Kabir et al., 1984). Resistance to this antibiotic will likely occur if it is widely used, since the development of resistance during treatment has been reported.

Various antibiotics, as well as the optimal duration of therapy, have been carefully evaluated. Most controlled studies have used 5 days of treatment, although shorter courses of therapy have also been explored (Salam & Bennish, 1991). An early study by Gilman et al. (1981) demonstrated that single-dose ampicillin achieved rates of clinical cure comparable to the standard 5-day course of treatment with ampicillin, although rates of bacteriological cure were lower. A subsequent study demonstrated that a single dose of norfloxacin was as effective as 5 days of TMP-SMZ therapy for outpatient treatment of mild shigellosis (Gotuzzo et al., 1989). An Israeli study comparing two and five days of ceftriaxone for the treatment of severe shigellosis demonstrated comparable clinical and bacteriological cure rates (Eidlitz-Marcus et al., 1993). Additional studies are needed to further evaluate various regimens and their cost-effectiveness (Keusch & Bennish, 1989).

There is not a complete correlation between *in vitro* antibiotic susceptibility and clinical efficacy. Although the infecting organism must be sensitive to the antibiotic being used, several antibiotics that were active *in vitro* have been ineffective clinically, including the first-generation cephalosporins and gentamicin. Using an ineffective antibiotic, that is one to which the organism is resistant or that is clinically ineffective, may pose a risk. In addition to any potential systemic side-effects of the drug, it may affect the normal intestinal flora. There is evidence that the normal flora compete with the infecting shigellae; thus, an ineffective antibiotic may actually exacerbate the disease by selectively promoting the shigellae. In a rabbit model of shigellosis, animals must first be treated with tetracycline in order for orally administered tetracycline-resistant bacteria to cause illness. In this model, tetracycline apparently reduces the normal flora and encourages the growth of the shigellae, resulting in a lethal infection. It is not known whether administering inappropriate antibiotics exacerbates infection in humans, but case reports suggest that it may do so. Thus, giving an antibiotic "just in case the organism is sensitive" may be a dangerous strategy.

Drug resistance and trends

Analyses of disease incidence and resistance trends for shigellosis have faced difficulties because many cases are asymptomatic or present with atypical features such as watery diarrhoea. Additionally, in developing countries, laboratory services, appropriate transport of specimens, and access to health care services remain problematic (Keusch & Bennish, 1989; Keusch & Bennish, 1998; Keusch, 1998; Ries et al., 1994). Data may be subject to bias, as information often comes from hospital-based surveillance and therefore may reflect the more severe infections. Similarly, the data from epidemics may not reflect the situation during non-epidemic periods (Keusch & Bennish, 1989; Keusch & Bennish, 1998; Keusch, 1998). The most comprehensive longitudinal data on drug resistance in *Shigella* are from the ICDDR,B. As considerable geographical variation exists, these data may not reflect the situation in other countries (Keusch & Bennish, 1989; Keusch & Bennish, 1998; Keusch, 1998; Murray, Rensimer & DuPont, 1986). Antimicrobial resistance patterns vary even within Bangladesh, for example between Matlab and Dhaka, which are only 30 miles apart (Sack et al., 1997). Clearly, additional community-based longitudinal studies, using standardized laboratory and diagnostic methods, are needed to generate more meaningful resistance rates.

In the United States, sulfonamides were first used to treat shigellosis in 1945 (Hardy, 1945), followed by tetracycline; both agents were widely used until 1965. By 1967, due to high levels of resistance to sulfonamides and tetracycline, ampicillin became the drug of choice for treatment of shigellosis (Haltalin et al., 1967). However, by the early 1970s the prevalence of ampicillin resistance had markedly increased in the United States. In Washington, DC it increased from 5% to 95% over the period 1964 to 1971 (Ross, Controni & Khan, 1972). Similar increases, with concomitant treatment failures, were seen elsewhere in the country (Torrence, Owens & Cho, 1973; Lerman, Waller & Simms, 1973). Many of these strains were multiresistant (Ross, Controni & Khan, 1972). As a result, the combination antimicrobial agent TMP-SMZ became the drug of choice for treating shigellosis (Lexomboon et al., 1972; Nelson et al., 1976; Chang et al., 1977). Currently, TMP-SMZ is frequently used, but increasing resistance to this drug is being seen. A history of travel outside the United States and Europe is an important predictor of antibiotic resistance, reflecting the dramatic rise in antimicrobial resistance in developing countries (Tauxe, et al., 1990; Heikkila et al., 1990; Gross et al., 1984; Materu et al., 1997).

In November 1979 an epidemic of multiply drug-resistant *S. dysenteriae* type 1 began in northeastern Zaire and quickly spread to Rwanda, Burundi, and the United Republic of Tanzania. The initial isolates were resistant to ampicillin, chloramphenicol and tetracycline, necessitating the use of TMP-SMZ in July 1981. By September 1981, strains resistant to TMP were increasingly isolated (Frost, Rowe & Vandepitte, 1982). In November 1981, some regions introduced NA as first-line therapy for shigellosis, which reduced the case-fatality rate. Predictably, by April of 1982, the first *S. dysenteriae* type 1 strains resistant to NA were

FIGURE 4. **AMPICILLIN RESISTANCE RATES IN *SHIGELLA* ISOLATES FROM THE ICDDR,B BETWEEN 1982 AND 1991.**
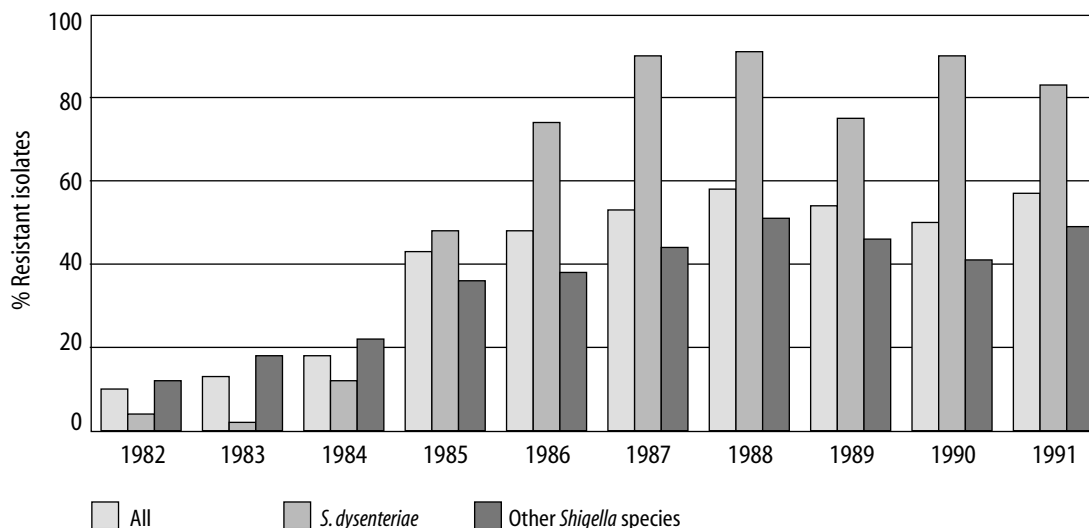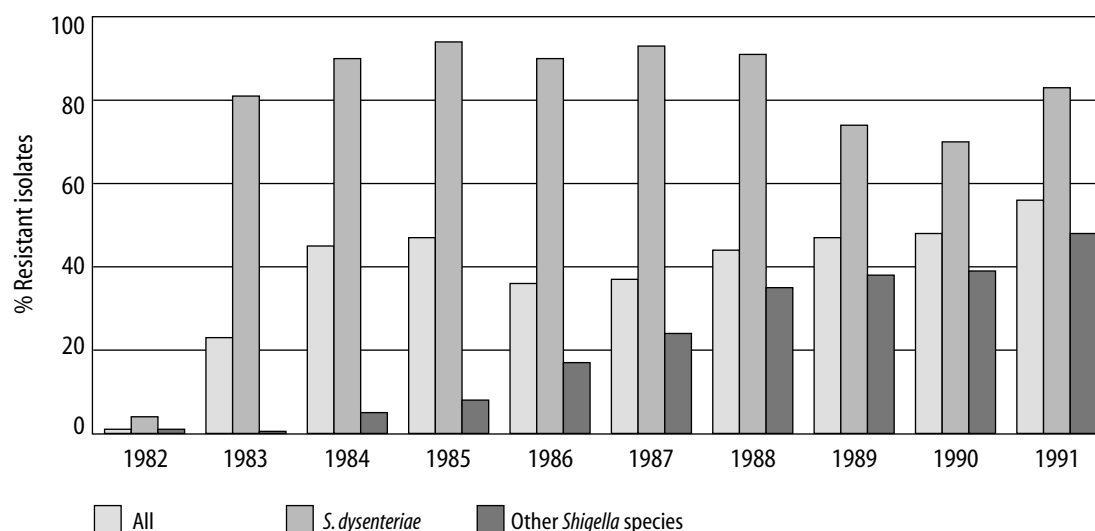
FIGURE 5. **TMP-SMZ RESISTANCE RATES IN *SHIGELLA* ISOLATES FROM THE ICDDR,B.**

reported (Frost et al., 1985; Malengreau, 1984). By 1985, 31% of strains isolated in southern Rwanda were resistant to NA (Mutwewingabo & Mets, 1987).

At the ICDDR,B, Islam et al. (1995) specified several laboratory and clinical tests to be used to identify patients who received ineffective therapy. Persistent fever (rectal temperature >37.8 °C), abdominal pain or tenderness on days 3 to 5 of therapy, and continuing anorexia on days 3 to 5 were significantly more frequent in these patients. In particular, continued fever, visible blood in the stool, and an insignificant reduction of shigellae in the stool were the best predictors of treatment failure. A simple, predictive laboratory test was a repeat microscopic examination of the stool; patients with faecal leukocytes >50/high power field (HPF), erythrocytes >50/HPF, and macrophages >5/HPF on day 5 of therapy were likely to have been inadequately treated. TMP-SMZ or ampicillin was used for empiric therapy at the ICDDR,B in the 1980s. If there was no clinical improvement by 48–72 hours, NA was substituted (Bennish et al., 1985). However, widespread NA resistance in *S. dysenteriae* type 1 occurred after NA replaced ampicillin as the drug of choice for shigellosis in 1986 (Bennish et al., 1992[1,2]).

The ampicillin and TMP-SMZ resistance rates in *Shigella* obtained from the ICDDR,B are shown in Figures 4 and 5. Ampicillin resistance increased from 10% of all *Shigella* isolates in 1982 to 57% in 1991; the resistance rate for *S. dysenteriae* increased from 4% to 83% during the same period. For TMP-SMZ, resistance increased from 1% to 56% of all

Shigella isolates, and from 4% to 83% of *S. dysenteriae*.

Mechanisms of resistance

Having recognized that many strains of *Shigella* were multiply resistant to antimicrobials, Ochiai et al. (1959) found that drug resistance could be transferred from *Shigella* to *E. coli* and vice versa by conjugation. In addition, it was noted that drug resistance did not segregate during the transfer and that there was no exchange of chromosomal DNA. This led to the initial descriptions of "resistance factors" or R-factors (Watanabe & Fukusawa, 1961; Mitsuhashi, 1969). R-factors were also found to be transferable to other enteropathogens such as *Salmonella*, either directly or indirectly via other enteric organisms such as *E. coli* (Watanabe & Fukusawa, 1961; Mitsuhashi, 1969). Transfer of antimicrobial resistance was thus determined to be mediated by mobile plasmids that encoded resistance and efficiently disseminated this information (Tanaka, Hashimoto & Mitsuhashi, 1983). Since the initial discovery of transmissible antibiotic resistance via R-plasmids in *Shigella*, similar plasmids have been described in other Gram-negative bacilli (Datta et al., 1971; Datta, 1965; Anderson & Datta, 1965). Rapid acquisition of R-factors by *Shigella* spp. has been documented during epidemics; initially sensitive organisms acquired R-factors mediating resistance to multiple antimicrobial agents, including TMP-SMZ and other sulfonamides, β-lactams, tetracycline, and streptomycin (Gangarosa et al., 1972; Olarte, Filloy & Galindo, 1976; Bremner,

1978; Chun et al., 1984; Kaliyugaperumal, Gupta & Mohapatra, 1978; Adeleye, 1992).

TMP resistance in *Enterobacteriaceae* was first found to be mediated by R-factors in 1972 (Fleming, Datta & Gruneberg, 1972). TMP is a diamino-pyrimidine compound that exerts its antimicrobial effect by selectively inhibiting bacterial dihydro-folate reductase (DHFR); this enzyme catalyzes the reduction of dihydrofolate to tetrahydrofolate, a cofactor required for the synthesis of nucleic acid precursors. Although TMP resistance can be chromosomally mediated, the most common mechanism in *Enterobacteriaceae*, including *Shig-ella*, is acquisition of an additional, plasmid-encoded, variant DHFR enzyme. The most common of these is known as DHFR I (Heikkila et al., 1990; Huovinen, 1987; Burchall, Elwell & Fling, 1982; Haider et al., 1990). Several plasmid-encoded DHFR variants have been characterized, all of which have reduced affinity for TMP (Huovinen, 1987; Hickey & Nelson, 1997). These enzymes are also produced at much higher levels than chromo-somal DHFR, effectively overwhelming the native enzyme (Amyes, 1989). DHFR I is often associated with transposons. Transposon 7 (Tn7), the first to be described, can transpose efficiently and spe-cifically into the *E. coli* chromosome. The ability to transpose to this ubiquitous enteric organism may explain its rapid and widespread dissemina-tion (Lichtenstein & Bremner, 1982; Fling & Richards, 1983). Tn7 also encodes resistance to streptomycin and spectinomycin, and therefore multi-drug resistance is common among isolates resistant to TMP (Heikkila et al., 1990; Amyes & Towner, 1990). Tn7 can jump from plasmid to plas-mid or onto the bacterial chromosome, leaving behind antimicrobial resistance determinants even after the plasmid it was initially carried by is spon-taneously lost (Amyes & Towner, 1990; Barth et al., 1976; Towner, Venning & Pinn, 1982). Further-more, genetic material may also be transferred to species outside the *Enterobacteriaceae* family. In 1984, high level TMP-resistant *V. cholerae* strains from an epidemic in the United Republic of Tan-zania were shown to have acquired a 113-kb resist-ance plasmid that contained Tn7 encoding the same DHFR-I enzyme found in *Shigella* (Young & Amyes, 1986).

SMZ blocks another enzyme involved in folic acid metabolism, dihydroopterate synthase (DHPS). Synergy between SMZ and TMP is based on their blocking sequential steps in this pathway. Similarly to TMP resistance, the most common mechanism for SMZ resistance in Gram-negative organisms is the acquisition of plasmids encoding an altered DHPS with reduced affinity for SMZ. Fortunately, fewer variants of this enzyme have been identified than for DHFR (Hickey & Nelson, 1997). Resistance to both TMP and SMZ implies resistance to the combination. Although the chance of a susceptible strain acquiring multiple resistances is small, the probability is markedly increased when a reservoir of transmissible plasmids exists (Then, 1982).

Once sulfonamide resistance became widespread in *Shigella* species, β-lactam antibiotics became the drugs of choice. β-lactam antibiotics target peni-cillin-binding proteins (PBPs), which carry out enzymatic functions essential for cell-wall structure. The binding of β-lactams to essential PBPs in susceptible species results in bactericidal activity (Georgopapadakou, 1993; Eliopoulos, 1988; Hickey & Nelson, 1997). Although resistance to β-lactams may arise in *Shigella* as a result of mutations that reduce the affinity of PBPs for the antibiotics, the most common and most clinically significant mechanism of resistance to this class of drugs in Gram-negative bacteria is the production of β-lactamases (Ghosh, Kar & Kundu, 1998). These enzymes hydrolyze the β-lactam ring, inactivating the drug. Since the introduction of ampicillin in 1962, β-lactamase genes have increasingly been found on bacterial plasmids, facilitating the rapid dissemination of resistance among bacterial species (Anderson & Datta, 1965; Amyes, 1989). β-lactamases are a diverse group of enzymes first rec-ognized in *E. coli* in 1940 and later evolving in response to the development of new β-lactam anti-biotics (Abraham & Chain, 1940; Eliopoulos, 1988; Hickey & Nelson, 1997). The genes may be located on chromosomes, plasmids, or transposons and may be constitutively expressed or inducible. β-lactamases vary in their spectrum of activity against penicillins and cephalosporins, as well as in their susceptibility to clavulanic acid inhibition (Hickey & Nelson, 1997). In Europe, Asia, and Africa the most common plasmid-encoded β-lactamase in resistant Gram-negative bacteria, in-cluding *Shigella*, is TEM-1 (Amyes, 1989; Schumacher et al., 1992). This enzyme can hydrolyze a number of penicillins and cephalosporins and is easily transferred to other members of the Enterobacteriaceae family as well as to *V. cholerae* (Amyes, 1989; Threlfall, Rowe & Huq, 1980).

Tetracycline resistance is common in many Gram-negative bacteria, including *Shigella*, the first organism in which it was described. Although the

precise mechanism of action of the antibiotic is still not well understood, tetracycline binds reversibly to the 30S subunit of the bacterial ribosome, preventing binding of aminoacyl-tRNA and inhibiting protein synthesis (Roberts, 1996). *Shigella* was also the first organism in which tetracycline resistance determinants were linked to R-plasmids and shown to be transferable to susceptible bacteria by conjugation (Roberts, 1996). Genes carried on transposons and/or plasmids encode transmembrane proteins that export the drug by an energy-dependent process (Hickey & Nelson, 1997; Speer, Shoemaker & Salyers, 1992).

Particularly in developing countries, fluoroquinolone-resistant *Shigella* is increasingly prevalent. Initially, low-level resistance to NA was seen. Later, chromosomal mutations were discovered that conferred greater resistance (Turnidge, 1995). The mutations affect either DNA gyrase (bacterial topoisomerase II) or the uptake and accumulation of quinolones (Turnidge, 1995; Yamagishi et al., 1981; Sato et al., 1986; Hooper et al., 1986; Hirai et al., 1986). Although quinolone resistance is not mediated by transferable plasmids, the acquisition of certain plasmids can contribute to quinolone resistance by increasing the spontaneous mutation rate (Ambler et al., 1993; Ashraf, Ahmed & Sack, 1991). These plasmids encode gene products involved in error-prone DNA repair responses, such as the SOS response in *E. coli*, which is induced by DNA damage. The organism's DNA is repaired, but with incorporation of a higher than normal number of incorrect bases. Populations of bacteria harbouring such plasmids may have an evolutionary advantage.

Many socioeconomic and behavioural factors contribute to increasing antimicrobial resistance. Shigellosis is more prevalent in individuals of low socioeconomic status and education level in developing countries, where there are frequently inadequate treatment and poor compliance (Haider, Malek & Albert, 1993; Okeke, Lamikanra & Edelman, 1999). In developing countries, healthy people commonly harbour resistant bacteria as part of their normal flora (Okeke, Lamikanra & Edelman, 1999; Calva, Sifuentes-Osornio & Ceron, 1996) and this resistance can be transferred at low frequency, via plasmids and transposons, to *Shigella* (Tauxe, Cavanagh & Cohen, 1989; Platt, Chesham & Kristinsson, 1986; Bratoeva & John, 1994; Murray, Rensimer & DuPont, 1982; Levine et al., 1983; Hickey & Nelson, 1997; Petrocheilou, Grinsted & Richmond, 1976). As is the case for *V.*

cholerae and *C. jejuni*, selective pressure due to excessive use of antimicrobial agents, whether misuse by health care providers (e.g. for childhood gastroenteritis or viral respiratory infections) (Thamlikitkul, 1988; Okeke, Lamikanra & Edelman, 1999; CDC, 1991; Guyon et al., 1994; Bojalil & Calva, 1994; Nizami, Khan & Bhutta, 1996; Paredes et al., 1996; Hui et al., 1997; Reyes et al., 1997), or unrestricted access to them, are responsible for much of the resistance seen in *Shigella* (Okeke, Lamikanra & Edelman, 1999; Haider, Malek & Albert, 1993).

In many developing countries antimicrobial agents are readily available without a prescription. In Nagpur, India, despite prohibition by the Indian Pharmaceutical Act, self-prescribing of antimicrobials was common, primarily for minor respiratory infections and non-specific complaints such as fever, diarrhoea, and abdominal pain (Dua, Kunin & White, 1994). In another study, in rural Bangladesh, 95% of the drugs consumed by the subjects were obtained from local pharmacies, with physicians having prescribed only 8% (Hossain, Glass & Khan, 1982). In addition, antimicrobial agents may be of poor quality, due to degradation, counterfeiting, or lack of bioequivalence in the case of generic drugs. Use of such products results in sub-optimal serum concentrations, which promotes selection for resistance (Okeke, Lamikanra & Edelman, 1999).

An increasingly mobile population provides opportunities for the rapid spread of multi-resistant organisms in regions where unrestricted antibiotic use is common, particularly in settings where many individuals may asymptomatically harbour pathogens such as *Shigella* (Bennish et al., 1985; Guerrero et al., 1994; Calva, Sifuentes-Osornio & Ceron, 1996). Overcrowding and improper sewage disposal promote the spread of resistant organisms among individuals and provide increased opportunities for genetic exchange among bacteria, facilitating the dissemination of antibiotic resistance determinants (Okeke, Lamikanra & Edelman, 1999). In many countries armed conflicts, corruption and mismanagement threaten the public health infrastructure, favouring the spread of infections and of drug resistance (Okeke, Lamikanra & Edelman, 1999).

Lack of facilities and resources for susceptibility testing and surveillance, particularly in developing countries, results in inappropriate clinical decision-making, inappropriate treatment, and thus antibiotic resistance (Okeke, Lamikanra & Edelman, 1999; Hughes & Tenover, 1997). In some cases, even when most strains are known to be resistant,

inappropriate antibiotics are used under the misapprehension that any antibiotic is better than none. Regular, continuous surveillance is required since resistance rates, particularly in *Shigella*, vary geographically and also over time within a single country (Sack et al., 1997). When available, the results of accurate surveillance should be used to guide policies for antibiotic use.

Intervention strategies and research needs

1. **Programmes aimed at decreasing the total number of infections**.

   1.1 Resistant organisms thrive where rates of shigellosis are highest; thus, programmes targeting the overall rate of infection will also reduce the number of infections with resistant strains and the spread of such strains. Shigellosis is primarily a disease of crowded communities that do not have adequate sanitation or safe drinking-water. Therefore, the long-term strategy for control of this disease is still improvement of water, sanitation, and socioeconomic status (Shears, 1996; Bennish et al., 1990[2]). Theoretically, these public health measures are the most effective means of disease reduction, but practical considerations have limited their implementation. Development projects, such as the WHO Water and Sanitation Decade, are needed to improve the quality of water supplies and sanitation globally (Shears, 1996).

   1.2 Targeted interventions which have proved effective in reducing *Shigella* infection include: a) hand-washing programmes which make soap available and teach people how to use it effectively; b) encouraging breast-feeding of infants and small children (Ahmed et al., 1992); c) latrine programmes to reduce environmental contamination; and d) programmes to reduce the density of flies, which can deposit infectious inocula of *Shigella* on food (Ahmed et al., 1992; Levine & Levine, 1991; Acheson & Keusch, 1995; Watt & Lindsay, 1948). In a community-based trial in Bangladesh, Ahmed et al. (1992) demonstrated that breast-feeding protects children up to 3 years of age against shigellosis, particularly against disease due to multi-resistant organisms.

   1.3 An effective vaccine would prevent *Shigella* infections due to either sensitive or resistant strains. Much progress has been made towards an effective vaccine for shigellosis, but none is yet available for public health use. Vaccine development should be encouraged, as this will likely be the most effective way to achieve a significant reduction in transmission. Even if we need five more years to develop a successful vaccine for *S. flexneri* and *S. dysenteriae*, this is still shorter than the time needed to achieve significant improvements in water and sanitation systems. However, vaccine development and water/sanitation improvements should not have to compete for attention or resources; both programmes need to proceed simultaneously and be implemented appropriately. Since current evidence suggests that vaccines for shigellosis must be serotype-specific, priority must be given to those organisms that represent the principal public health problems, i.e. *S. dysenteriae* and *S. flexneri*.

   1.4 Is interrupting transmission by patients feasible? Antibiotic treatment of patients eradicates the organism from the stool. However, this may not be an important factor since, in most instances, only a small proportion of infected patients actually receive antibiotics. Individuals with asymptomatic or mild infections are responsible for most of the faecal contamination, and treatment of these individuals is both unrealistic and unwise. Thus, treatment of patients should be aimed at clinical cure rather than clearance of the organism.

2. **Limiting treatment to only those patients who really need the antibiotic**. Because shigellosis is often a self-limited illness, more research is needed to help identify those at greatest risk of dying; newer antimicrobial agents should be reserved for treating those individuals (Ries et al., 1994). There is little information available concerning the optimal duration of treatment, appropriate target populations, or the cost-effectiveness of different therapeutic options. Further research is needed in this area (Keusch & Bennish, 1989).

3. **Limiting the duration of treatment**. In those instances when antibiotics are needed, short but effective courses of treatment may be prudent. This would limit the environmental pressure for selection and dissemination of resistance genes among bacterial populations (O'Brien, 1997).

4. **Enforcement of appropriate antibiotic use policies**. Unfortunately, legislation has not always been effective in developing countries. In India, for example, pharmacists continue to sell drugs without requiring a prescription, despite the fact that they are forbidden to do so under the Indian Pharmaceutical Act (Dua, Kunin & White, 1994). In addition, there is often overzealous promotion of antimicrobials by pharmaceutical companies; measures to prevent this unethical practice should be implemented (Dua, Kunin & White 1994; Couper, 1997).

5. **Education**. Continuing education of health care providers and development of standard treatment guidelines for gastroenteritis and viral infections may reduce the inappropriate use of antibiotics, thus reducing the pressure that contributes to selection for resistance in *Shigella* (Couper, 1997).

Conclusion

Shigellosis is the most difficult of the three enteric organisms being reviewed, in terms of steady trends towards multiple resistance. Once they have become resistant, epidemic and endemic strains of *Shigella* have remained resistant; there have only been a few instances in which a sensitive strain has reappeared in a region. Additionally, shigellosis is one of the diseases most difficult to prevent because only a small number of bacteria are required to cause disease. Thus, intensive water and sanitation programmes and vaccine development would seem to be critical. It is not realistic to continue relying on the introduction of a new antibiotic every few years.

Review of *Campylobacter jejuni*

Introduction

C. jejuni and related species are increasingly recognized as important causative agents of enterocolitis. Although relatively recently (1972) recognized as a significant human pathogen, *C. jejuni* is now the bacterial pathogen most commonly responsible for diarrhoeal illness in the United States (Blaser, 1997; Griffiths & Park, 1990). According to the CDC, 46% of culture-proven bacterial gastroenteritis in the United States in 1996 was due to *Campylobacter* spp. *Salmonella*, *Shigella* and *E. coli* were less frequent causes of enteric disease (Altekreuse et al., 1999). *Campylobacter* is also a common cause of endemic diarrhoeal illness worldwide, although there are regional differences in the characteristics of the illness and the population affected (Taylor, 1992) The extent to which campylobacteriosis is responsible for travellers' diarrhoea varies regionally; this appears to be more common in Asia.

The bacteria now known as *Campylobacter* were first described in cattle and sheep, and were named *Vibrio foetus* because they caused abortion. *V. foetus* was isolated from immunocompromised patients as early as 1947, although its role in acute diarrhoea was not recognized until 1972 (Dekeyser et al., 1972). Based upon cell wall and DNA differences from other members of the genus *Vibrio*, *V. foetus* was reclassified in 1973 in the genus *Campylobacter*. This genus comprises an increasing number of species, but *C. jejuni* and *C. coli* are responsible for the majority of cases of human enterocolitis; *C. jejuni* accounts for 80–90% of enteric disease. Recent studies by the CDC found that in the United States, 99% of cultured *Campylobacter* spp. were *C. jejuni*. (Tauxe, 1992). The incidence of infection by *C. upsaliensis*, *C. lari*, *C. foetus*, and *C. hyointestinalis* is unknown, but these organisms seem to be much less common (Ketley, 1997). However, antibiotic-containing agar is used for primary isolation; this may select for *C. jejuni* and thus bias the frequency of isolation towards this species.

Microbiology

Campylobacters are slim, Gram-negative, curved rods. Located at one or both ends of the cell is a polar flagellum, making the organism highly motile. *C. jejuni* and *C. coli* are microaerophilic and thermophilic, growing best at 42 °C and requiring oxygen concentrations of 3–15% and carbon dioxide concentrations of 3–5%. The thermophilic characteristic is thought to be an adaptation to the environment of animal and bird intestines. *Campylobacter* spp. have a small genome of 1.6–1.7 Mbp, which may partially explain their complex nutritional requirements in laboratory culture (Griffiths & Park, 1990). Campylobacters use products of the Krebs cycle and amino acids for growth since they are incapable of fermenting carbohydrates. *C. jejuni* can be distinguished from other species by its ability to hydrolyze hippurate, benzoic acid and glycine. Extrachromosomal elements (plasmids) have been found in *Campylobacter* spp. and encode antibiotic resistance (Bopp et al., 1985; Taylor et al., 1981; Tenover & Elvrum, 1988; Prasad et al., 1994; Tenover et al., 1985).

Transmission

Campylobacter spp. are part of the normal intestinal flora of wild and domesticated animals and birds. Of particular importance to humans is their colonization of animals used in food production including poultry, cattle, sheep and swine (Blaser, 1997). Poultry comprise the most significant reservoir worldwide; *Campylobacter* spp. can be isolated from 30–100% of the birds in many domestic and wild avian species (Blaser & Reller, 1981). Household pets such as dogs, cats and birds are additional animal reservoirs. Although excretion of *Campylobacter* is not associated with symptoms in poultry, diarrhoeal illnesses have been documented in mammalian pets and livestock, and this contributes to the contamination of surface water. Post-slaughter processing does not reduce the extent of colonization of poultry and may, in fact, lead to cross-contamination of previously uncontaminated

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carcasses. In one study in England, the organism was isolated from 48% of randomly cultured fresh chickens (Hood, Pearson & Shahamat, 1988). Freezing the carcass does not eradicate the bacteria. Ingestion of contaminated water, interaction with colonized pets (especially puppies and kittens), and consumption of unpasteurized milk or undercooked poultry or meat are all associated with human disease (Blaser et al., 1978; Blaser & Reller, 1981; Blaser, 1997; Deming et al., 1987; Robinson, 1981). Human-to-human transmission, while uncommon, has been documented via faecal exposure, particularly from young, incontinent children (Blaser, 1997). A history of foreign travel is frequently documented in citizens of industrialized nations who contract *Campylobacter* enteritis. Although enterotoxigenic *E. coli* (ETEC) is generally the most common pathogen in travellers' diarrhoea, *Campylobacter* species represented 17% of the pathogens isolated from travellers to Thailand (much lower percentages are found in travellers to other countries) (Black, 1990).

Spectrum of illness

Clinical illness ranges from mild, self-limiting, non-inflammatory diarrhoea to severe, inflammatory, bloody diarrhoea with high fever and bacteraemia. In immunosuppressed patients, particularly those with HIV, the diarrhoeal illness may be relapsing or unremitting and may be accompanied by bacteraemia and extraintestinal illness (Tee et al., 1995; Tee & Mijch, 1998; Wang & Blaser, 1986). In an analysis of 24 isolates from extraintestinal campylobacter illness, these appeared to be similar to those that cause intestinal disease. However, 52% of patients with extraintestinal disease had underlying immunosuppression (Blaser et al., 1986).

Although asymptomatic bacterial shedding has been documented, a chronic carrier state has not been described in immunocompetent individuals. (Blaser & Reller, 1981) The annual incidence of *C. jejuni* diarrhoeal infection was estimated by the CDC to be 5–6 per 100 000 persons in the United States, although population-based studies suggest that it may be closer to 1000 per 100 000 (Blaser, 1997). Due to the broad range of clinical symptoms and the specific laboratory media and techniques required for culture, *Campylobacter* infections are thought to be underreported worldwide. The total number of infections in the United States could be close to 2.4 million/year, with 120–360 deaths (Taylor, 1992).

Curiously, the characteristics of the disease are different in different populations. In industrialized countries, where infection is relatively rare, its manifestations are more severe; in developing countries, infection is more common but symptoms are milder. Patients in industrialized countries usually experience an inflammatory diarrhoea with severe abdominal cramping and fever. Most (60%) stool samples from symptomatic, culture-positive *C. jejuni* gastroenteritis episodes contain blood and 78% of these have polymorphonuclear leukocytes (Blaser et al., 1979). *Campylobacter* infections are seasonal, peaking in late summer and fall. Sporadic illness and epidemic outbreaks associated with food- or waterborne exposures have been described, suggesting a general lack of immunity in the population. There is a bimodal age distribution, with one peak in children under the age of 1 year and the second peak in young adults between the ages of 15 and 34 (Blaser, 1997; Ketley, 1997). At present, rates of infection are rising, apparently linked to increased poultry consumption (Blaser, 1997; Deming et al., 1987).

In contrast, *Campylobacter* infection appears to be hyperendemic and non-seasonal in developing countries. The illness is characterized by watery, non-inflammatory, relatively mild diarrhoea. Infection rates are typically highest during the first two years of life and decrease with age (Taylor et al., 1988; Blaser, 1997; Glass et al., 1983[2]). There is also attenuation of the symptoms and reduction in convalescent-phase bacterial excretion with increasing age. Convalescent excretion occurred for 14 ± 2 days in Thai children less than 1 year of age and for 8 ± 2 days in children between the ages of 1 and 5 years (Taylor et al., 1988). These children experienced reinfection with different *Campylobacter* serotypes at a rate of 15% per week, demonstrating that the infection rate is high among children in developing nations. In Bangladesh, Glass et al. (1983[2]) found that healthy control patients had nearly the same frequency of *Campylobacter* infection as did patients presenting with diarrhoea. Asymptomatic infection rates may therefore be quite high in developing countries as compared to industrialized nations (Glass et al., 1983[2]; Black, 1988).

Although efforts have been made to characterize strains isolated in different regions in order to account for the differences in the symptoms, there is little evidence to suggest that *Campylobacter* strains isolated in developing countries are different from those found in industrialized nations with

respect to virulence factors (Taylor et al., 1988; Asrat et al., 1997). Travellers to developing countries develop an illness typical of their own country. Residents of developing nations average more than five infections with *C. jejuni* lifetime, as compared with an average of none to one in developed countries (Blaser, 1997). Therefore, the weight of the evidence is that immunity accounts for the attenuation of symptoms with increasing age in developing countries. Consistent with this interpretation are studies in Thailand and Bangladesh demonstrating that serum levels of *C. jejuni* cell surface-specific immunoglobulin A (IgA) rise with increasing age, implying acquisition of protective immunity with specific mucosal IgA (Blaser et al., 1985; Blaser et al., 1986; Blaser, Taylor & Echeverria, 1986). In each age group, and for each immunoglobulin subclass (IgA, IgG, and IgM), serum antibody to *C. jejuni* was found in significantly greater amounts than in Americans (Blaser et al, 1986; Blaser, Taylor & Echeverria, 1986). Thus, geographical differences in the nature of campylobacteriosis appear to be related to acquired immunity in developing nations (due to the hyperendemic nature of the disease) rather than to strain differences. However, much remains to be learned about the molecular biology of *Campylobacter* pathogenesis.

There is strong evidence linking *Campylobacter* infection to the development of Guillain-Barré Syndrome (GBS). Following intestinal infection, some patients develop an acute peripheral neuropathy, starting with ascending weakness or paralysis of the extremities and sometimes progressing to paralysis of the trunk and interference with breathing. The mechanism of this complication is thought to be related to a ganglioside, found on the surface of certain strains of *Campylobacter*, which mimics a ganglioside present on nerve cells. This "molecular mimicry" is thought to trigger an autoimmune response, leading to GBS (Moran, Appelmelk & Aspinall, 1996). Certain Penner serotypes (e.g. O19) are more commonly associated with GBS, but other serotypes may also lead to its development (Aspinall, McDonald & Pang, 1994). The true virulence marker is not known. Rapid methods for detecting Gm1 ganglioside on *Campylobacter* (Sack et al., 1998) may help identify strains more likely to induce GBS, but more work is needed to determine whether Gm1 is actually the virulence marker.

In the United States about 40% of GBS is associated with a prior *Campylobacter* infection (Altekreuse et al., 1999). GBS has not been well studied in developing countries; however, with the eradication of polio, it seems likely that some cases of acute paralysis that would previously have been diagnosed as polio will turn out to be GBS instead. Thus, *Campylobacter*-associated GBS may be even more important in developing countries, even though the diarrhoeal disease tends to be mild. It seems likely that GBS will be recognized as an "emerging disease," even though these new cases will probably represent disease that was previously misdiagnosed.

Pathogenicity

The incubation period after ingestion of *C. jejuni* is thought to average 24–72 hours, but periods of ≥1 week have been documented. The precise inoculum is unknown, but as few as 800 organisms have been shown to cause illness (Black, 1988). The rate of illness increases with increasing numbers of organisms ingested. Fever, chills, headache, and myalgia may precede diarrhoea symptoms. Acute abdominal pain coincides with diarrhoea and may be severe enough to mimic appendicitis. The diarrhoea may be severe, with frequent bloody bowel movements, upwards of eight per day have been reported. The symptoms generally peak by 24–48 hours and subside over a period of a week. Colonoscopy may reveal friable mucosa with oedema and petechial haemorrhages consistent with a diffuse colitis. Ileal and jejunal involvement have been noted as well.

The molecular pathogenesis of *C. jejuni* infections is multifactorial and complex, and seems to involve motility, adhesion, invasion, and toxin production. Campylobacters have the unusual ability to change from a thin, curved form to a spherical or coccoid shape. Along with this structural change, they become non-culturable. This viable, non-culturable state (referred to as the VNC state) may be a dormant state induced by environmental stress (Rollins & Colwell, 1986). It is not known if the VNC form is transmissible to humans (Ketley, 1997).

Their cell shape and flagella give campylobacters motility in the viscous environment of the gut. The flagellum loci have been well characterized and mapped to adjacent genes, *flaA* and *flab* (Guerry et al., 1991). Mutations in these genes affect the ability of *C. jejuni* to colonize the gut (Nachamkin et al., 1993), indicating that motility plays a role in the pathogenesis of the organism.

The roles of adhesion and invasion are incompletely understood. Fimbriae have recently been

described, and non-fimbriated mutants produce attenuated disease (Doig et al., 1996). The inflammatory nature of many Campylobacter infections suggests that the bacteria invade cells, but there is little *in vivo* evidence for this.

The role of toxin production in *Campylobacter* pathogenesis is highly debated. Cholera-like toxins (CLT) have been reported, and they may play a role in the enterotoxigenic, noninflammatory diarrhoea typical in the developing world. Enterotoxins are secreted proteins that are able to penetrate eukaryotic cells and trigger intracellular cyclic AMP production, leading to ionic changes that result in watery diarrhoea. There is immunological cross-reactivity among CLT, cholera toxin (CT) and *E. coli* heat-labile toxin (LT) (Wassenaar, 1997). A study in Costa Rica linked the production of CLT to non-inflammatory diarrhoeal symptoms, but studies performed elsewhere have produced inconsistent and conflicting data (Florin & Antillon, 1992). Multiple cytotoxins, including cytolethal-distending toxin (CLDT), haemolytic cytotoxins and Shiga-like toxins have also been described in *Campylobacter*. Cytotoxins are proteins that kill eukaryotic cells; *E. coli* Shiga-like toxin is an example. Although, theoretically, these toxins may play a role in the inflammatory type of diarrhoea commonly seen, this is incompletely understood (Ketley, 1997). While there have been significant advances in the molecular pathogenesis of *C. jejuni*, additional research is required to understand its ability to cause disease.

Diagnosis and identification

A confirmed diagnosis requires culture of the organism from faeces or blood. Faecal samples should arrive in the laboratory within a few hours after collection or, if a delay is likely, should be inoculated into a transport medium (e.g. Cary-Blair medium). In the laboratory, rich but selective media (containing antibiotics to reduce the growth of other species), microaerophilic incubation conditions (typically, a GasPak jar conditioned with a tablet to lower the oxygen concentration), and an elevated temperature (42 °C) are required. A variety of *Campylobacter*-specific media are produced commercially, including Skirrows medium, Butzler's Medium, and Campy BAP media. A technique for isolating *Campylobacter* spp. from faeces using antibiotic-free media involves allowing the bacteria to migrate through a microporous filter. This method appears to be more sensitive, espe-

cially for non-*jejuni* species that may be inhibited by the antibiotics used in standard isolation media. Enrichment broth is not normally used for faecal samples; however, it may be used when isolating *C. jejuni* from contaminated liquids such as surface water or milk (Griffiths & Park, 1990). Standard blood culture broth will recover *Campylobacter* from blood specimens without the need for specific selection or enrichment. Techniques for the identification of species and the typing of strains of *Campylobacter* are described in standard microbiology texts.

Additional experimental methods for characterizing strains may be useful for specialized purposes. The most common technique is serotyping, using either the Penner or the Lior system. The Penner system identifies a heat-stable somatic (O) antigen and the Lior system a heat-labile flagellar (H) antigen. Both systems can type over 90% of human and animal *Campylobacter* spp. (Lior et al., 1982; Penner & Hennessy, 1980).

The use of DNA probe analysis is limited by complicated protocols, the need for specialized equipment, and the short shelf-life of reagents (Griffiths & Park, 1990). PCR fingerprinting methods using the *gyrA* and *pflA* genes hold promise for determining the relatedness of *C. jejuni* isolates and streamlining the tedious restriction fragment length polymorphism (RFLP) analyses and pulsed field gel electrophoresis (PFGE) methods that are currently in use to characterize *Campylobacter* strains (Ragimbeau et al., 1998). Thus, while a number of techniques are available to speciate and type *Campylobacter*, their usefulness is limited by the technical requirements, and they must be performed by reference laboratories.

If both acute and convalescent sera are available, serodiagnosis of *Campylobacter* infection is possible using enzyme-linked immunosorbent assay (ELISA) with a glycine extract providing the antigen (Blaser et al, 1986; Blaser, Taylor & Echeverria, 1986; Griffiths & Park, 1990). Antibody response to *C. jejuni* antigens may include the production of IgA, IgM or IgG. Testing only a convalescent serum sample is not useful because an elevated titre may reflect previous rather than current infection.

Therapy

In immunocompetent individuals, *Campylobacter* enterocolitis is generally self-limited, with mild to moderate symptoms, and antibiotic therapy is not required for most patients. Supportive care with

oral rehydration is the preferred treatment. When antibiotics are used to treat severe or invasive illness, the definition of cure, by convention, is relief of diarrhoea. Shedding of organisms may continue for 2–4 weeks or longer (median <3 weeks) (Blaser, 1997). Antibiotics have been shown to reduce the excretion of *Campylobacter*; erythromycin reduces its duration from 16.8 to 2 days (Pai et al., 1983) and azithromycin eliminates excretion within 24 hours (Kuschner et al., 1995). Whether antibiotics actually reduce the duration of diarrhoeal symptoms is subject to debate. Pai et al. (1983) found no change in the duration of diarrhoeal symptoms when American children received erythromycin for *Campylobacter* enteritis. Goodman et al. (1990) found that, in patients in Chicago, empirical ciprofloxacin treatment for diarrhoea reduced the duration of symptoms from 3.4 days (with placebo) to 2.4 days. Others have found that antibiotic treatment reduced the duration of symptoms from an average of 90 hours to 30 hours (DuPont et al., 1987). In severe cases, and in immunosuppressed individuals (particularly those with HIV), relapsing or unremitting inflammatory diarrhoea requiring antibiotic administration may develop. *C. jejuni* bacteraemia occurs at rates in the order of 0.7% to 2% in immunocompetent hosts (Tee, Kaldor & Dwyer, 1986; Wang & Blaser, 1986). In contrast, 8.3% of 121 HIV-positive patients with *Campylobacter* enteritis became bacteremic and this was associated with 33% mortality (Tee & Mijch, 1998). Thus, antibiotic administration should be on a case-by-case basis, with antimicrobials reserved for serious infections.

C. jejuni is sensitive to several classes of antimicrobials. The drug of choice for *Campylobacter* enterocolitis is generally a macrolide. Erythromycin has traditionally been the first-line therapy, although newer macrolides such as azithromycin are quickly gaining popularity (Tee & Mijch, 1998; Sanchez et al., 1994; Kuschner et al., 1995). Bactericidal fluoroquinolones have become the drugs of choice for treating travellers' diarrhoea and are therefore used as first-line therapy of *Campylobacter* infections contracted abroad. Tetracycline, doxycycline and TMP-SMZ have been used as second-line agents, although increasing resistance limits their use. Gentamicin, kanamycin, imipenem and ampicillin-clavulanic acid are reserved for systemic, refractory illness.

Antimicrobial resistance

In the past, *Campylobacter* strains were rarely tested for antibiotic sensitivity because this was technically difficult, and because resistance was infrequently encountered. Due to increasing antibiotic resistance, susceptibility testing is becoming more important. Susceptibility can be determined using microdilution methods, agar dilution methods or the E-test (Baker, 1992). Agar dilution and the E-test give comparable results; broth microdilution is inaccurate, particularly for tetracycline (Baker, 1992). The tests performed better when incubation was at 42 °C for 16–18 hours. The need for microaerophilic and thermophilic conditions, and for including rigorous controls, limits the use of these assays to a few laboratories where *Campylobacter* testing is routinely performed.

Worldwide, a complex interplay of factors has created an environment fostering the rapid emergence of resistance in *C. jejuni* (see Table 2). Resistance to fluoroquinolones and tetracyclines has rapidly reduced the efficacy of these drugs in many parts of the world (Sanchez et al., 1994). Major factors exerting selective pressure have included the introduction of fluoroquinolones into veterinary medicine and the extensive and sometimes indiscriminate use of antimicrobials to treat a variety of conditions. Antibiotics have long been used as supplements in animal feed, to promote growth and to prevent infections associated with the crowded conditions in which mass-produced livestock are raised. Nearly 45% of the 2.5 million kilograms of antibiotics consumed in the United States is used in animal feed (DuPont & Steele, 1987). This heavy selective pressure leads to the carriage of resistant organisms by livestock, and there is concern that this leads to resistant infections in humans (Endtz et al., 1991). After the introduction of tetracycline-supplemented feed, tetracycline-resistant bacteria were found in the stools of 80% of farm personnel. Much lower rates (7%) were found in neighbours not exposed to these animals (Levy, FitzGerald & Macone, 1976). More recently (1987), enrofloxacin, a fluoroquinolone, entered the animal husbandry market.

Campylobacter infections are especially linked to poultry. Enrofloxacin has been used in broiler chickens, reproductive chickens and egg-laying hens to prevent enteritis and respiratory disease. Velazquez et al. (1995) has shown that antibiotic concentrations in poultry feed are high enough to select for NA resistance *in vitro*. Extensive studies in the Netherlands (Endtz et al., 1991) have documented

TABLE 2. **WORLDWIDE ANTIMICROBIAL RESISTANCE PATTERN OF *CAMPYLOBACTER* ISOLATES BY AUTHOR AND YEAR**

Location	Author[a]	Year	% resistant								
			Tet	Eryc	Az	Amp	Amp-clav	Clinda	Chlor	Nal acid	Cipro
North America											
Canada	Gaudreau & Gilbert	1988	55.7	0	–	–	–	–	–	13.9	12.9
	Lachance et al.	1990	–	–	–	60	0	–	–	–	–
USA	Bopp et al.	1984	42	0	–	16.1	–	0	6.5	–	–
	Tenover et al.	prior to 1985	43	2	–	–	0	–	0	–	0.003
Europe											
North											
Sweden	Sjogren, Lindblom & Kaijser[b]	1989	12.7	6.4	–	20.9	–	3.6	2.7	–	0.9
Norway	Kapperud et al.[c]	1990									
Imported			23.8	0	–	57.1	–	–	–	–	0
Domestic			0	0	–	39.5	–	–	–	–	0
Finland	Rautelin, Renkonen & Kosunen[d]	1990	17	3	–	–	–	–	–	–	9
South											
Spain	Ruiz et al.[c]	1994	45	5.3	–	76	2.6	5	2.6	88	88
	Velazquez et al.[c]	1993	44.3	–	–	18.9	0	–	–	–	30.4
	Sanchez et al.	1994	–	3.1	3.1	–	–	–	–	56.8	49.5
	Tajada et al.	1995	–	–	–	40	1	–	–	–	–
Asia											
Thailand	Kuschner et al.	1993	–	–	0	–	–	–	–	–	50
N. Thailand	Murphy et al.	1995	–	–	31	–	–	–	–	–	69
Thailand	Hoge	1995	–	–	7	–	–	–	–	–	84
India	Prasad et al.[c]	1994	6.7	2.2	–	22.2	–	–	–	4.4	4.4
China (Province of Taiwan)	Li CC et al.	1988	95	10	–	–	–	10	–	79	52

[a] Unless otherwise noted, resistance breakpoints are those of the National Committee for Clinical Laboratory Standards for agar dilution methods: Eryc ≥8 µ/ml, Tet ≥16 µ/ml, Cipro/Nal. Acid ≥4 µ/ml, Amp ≥32 µ/ml.

[b] Breakpoint for resistance as defined by Reference Group for Antibiotics in Sweden 1988-89 Eryc ≥8 µ/ml, Cipro ≥8 µ/ml, Doxy ≥4 µ/ml, Clinda ≥4 µ/ml, Amp ≥16 µ/ml, Chlor ≥16 µ/ml.

[c] Resistance criteria not given.

[d] Breakpoint for resistance as defined by Reference Group for Antibiotics in Sweden, March 1990: Eryc ≥8 µ/ml, Cipro ≥8 µ/ml, Doxy ≥4 µ/ml.

a significant rise in fluoroquinolone resistance (defined as MIC ≥4 (µ/ml) in *Campylobacter* isolated from poultry and human stools, coinciding with the introduction of norfloxacin and enrofloxacin, respectively into human and veterinary medicine. In the period from 1982 to 1989, resistance in *Campylobacter* isolates from poultry increased from 0 to 14%. During the same period, resistance in human isolates went from 0 to 11%. Increased resistance has also been seen in Spain; Sanchez et al., (1994) documented an increase in rates of resistance to ciprofloxacin from 8.6% in 1990 to 50.7% in 1991. Over the same time period, resistance to norfloxacin rose from 10.8 to 52.3%; to ofloxacin from 8.7 to 47.6%; and to NA from 17.4 to 57.7%. Other Spanish investigators have confirmed this phenomenon. Ruiz et al. (1998) reported 47.5%

ciprofloxacin resistance in 1991 and 88% in 1994. In Quebec, Canada, resistance rose from 0% in 1983 to 12.6% in 1997 (Gaudreau & Gilbert, 1998), and in China (Province of Taiwan), 52% resistance has been reported (Li CC et al., 1998). *Campylobacter* resistance is quite low in Scandinavia. This is thought to be due to strict regulation of the use of antimicrobials in livestock (Kapperud et al., 1992; Rautelin, Renkonen & Kosunen, 1991). The few resistant isolates found in Sweden were mainly in travellers (particularly to Thailand) (Sjogren, Lindblom & Kaijser, 1997).

In developing countries, the prevalence of fluoroquinolone-resistant *Campylobacter* varies, and its relationship to use in agriculture is less certain. In Thailand, ciprofloxacin resistance in *C. jejuni* was reported to be as high as 84% in 1995 (Hoge

et al., 1998). In contrast, as of 1994, a low frequency of resistance was reported in India (4.4% of human isolates and 0% of poultry specimens) (Prasad et al., 1994). In humans, ciprofloxacin is frequently used to shorten episodes of travellers' diarrhoea; however, the contribution of this use to the overall antibiotic pressure in a given region would seem to be marginal. However, this use may bias samples of isolates by selecting for resistant strains; this may have been the case in the Swedish study cited above. Nevertheless, all antibiotic use exerts selective pressure and contributes to the emergence of resistance; inappropriate use should be avoided.

In summary, quinolone resistance is emerging globally in *Campylobacter*. The heavy antibiotic pressure, both from the veterinary arena and from unregulated antibiotic use in humans, has severely reduced the effectiveness of this class of antimicrobials for the treatment of *Campylobacter* enteritis.

Although the quinolones are frequently used, macrolides are the recommended first-line therapy for *Campylobacter* enterocolitis. Erythromycin continues to be active, with only low-level resistance reported. Although erythromycin does not provide empirical coverage for travellers' diarrhoea, new macrolides such as azithromycin appear to be promising for this use, as they are active against *E. coli*, *Salmonella* spp., *Shigella*, and *V. cholerae* (Kuschner et al., 1995). Not unexpectedly, azithromycin resistance (MIC ≥8 (µ/ml) has now been reported in Thailand, with up to 31% of *Campylobacter* isolates resistant in some areas (Murphy et al., 1996). Nevertheless, an evaluation of azithromycin for the treatment of travellers' diarrhoea in United States military personnel in Thailand noted no treatment failures with a three-day course of therapy. Additionally, clearance of *Campylobacter* occurred within 24 hours after a single dose of azithromycin, suggesting that short-course therapy might be feasible (Kuschner et al., 1995). Azithromycin is characterized by a long half-life and high tissue levels, and it appears to adequately penetrate the intestinal mucosa. In Spain, susceptibility of *Campylobacter* to macrolides has remained relatively stable, with approximately 2.6% resistance to erythromycin and azithromycin in l988 and 3.1% in 1992 (Sanchez et al., 1994). Resistance to erythromycin is also low in India (1.3% in 1994) (Prasad et al., 1994). No resistance has been reported in Quebec, Canada as of 1997 (Gaudreau & Gilbert, 1998) and in the United States resistance is negligible (Tenover et al., 1985).

Tetracyclines were once effective in the treatment of campylobacteriosis, but resistance has now relegated this class of drug to second-line therapy. Taylor et al. (1988) reported that *Campylobacter* species caused 50% of the travellers' diarrhoea in United States military personnel in Thailand, who were taking doxycycline as prophylaxis for malaria. Tetracycline resistance is also increasing in non-malarial regions of the world, with 55.7% resistance (MIC ≥16 (µ/ml) in Quebec, Canada in 1997 (Gaudreau & Gilbert, 1998). The resistance rate in Spain is approximately 45% (Ruiz et al., 1998; Velazquez et al., 1995). In 1996, Taiwanese investigators, using the E-test, found 95% resistance in isolates from human stool specimens (Li et al., 1998).

Kanamycin, gentamicin, chloramphenicol and various β-lactams are also active against *C. jejuni*. Recently, multi-drug resistance has been observed, with case reports documenting the development of resistance over the course of prolonged, unremitting, HIV-associated enteritis. Tee et al. (1995) reported on three HIV-infected patients in whom the bacteria progressively acquired resistance to the antibiotics used during treatment (macrolides, quinolones, gentamicin and chloramphenicol). Although multi-drug resistance is still uncommon, when it does occur it poses problems in managing severe cases of campylobacteriosis.

In summary, because of regional variations in susceptibility and increasing resistance in *Campylobacter* spp. the choice of antibiotic therapy requires careful consideration. Empirical antimicrobial therapy is not recommended; therapy should be reserved for patients with evidence of invasive illness, such as unremitting or severe diarrhoea accompanied by fever and the presence of leukocytes and red blood cells in the faeces. Fluoroquinolones should be avoided as first-line therapy in much of Asia and Europe. Macrolides appear to have maintained their activity and therefore remain the first-line therapy for culture-proven campylobacteriosis throughout most of the world. Life-threatening illness, particularly in HIV-positive patients, may require a different approach; *in vitro* susceptibility testing should be used to guide therapeutic decisions in these cases. Ampicillin-clavulanic acid, aminoglycosides and imipenem appear to be effective for systemic illness, although treatment failures have been reported (Tee et al., 1995; Molina et al., 1995; Perlman et al., 1988; Tee & Mijch, 1998).

Mechanisms of resistance development

There are various mechanisms by which *Campylobacter* spp. become resistant to antimicrobial agents (see Table 3). Because a large number of genetic sites may be involved, a genetic approach for detecting resistance is too complex to be practical. Fluoroquinolone antimicrobials inhibit DNA gyrase activity by binding to the A subunit of the enzyme, interfering with the DNA supercoiling process and thus blocking DNA replication. High-level resistance to fluoroquinolones in *Campylobacter* spp. is associated with a nucleotide substitution near the 5' end of the *gyrA* gene (Charvalos et al., 1996; Ruiz et al., 1998). By direct sequencing, restriction fragment length polymorphism, and sequence-specific oligonucleotide probe hybridization, the point mutation determining an amino acid change from Thr-86 to Ile in the quinolone-resistance determining region (QRDR) of *gyrA* was shown to be responsible for high-level fluoroquinolone resistance (ciprofloxacin MIC ≥16 (μ/ml). Low-level ciprofloxacin resistance has been selected in the laboratory with mutations at Ala-70 or Asp-90 (Ruiz et al., 1998). Charvalos et al. (1996) detected ciprofloxacin resistance mutations by non-radioisotopic single-strand conformation polymorphism and by direct DNA sequencing. The emergence of high-level ciprofloxacin resistance (MIC ≥32 (μ/ml) during the course of treatment has been documented (Segreti et al., 1992). Additional mechanisms of resistance to fluoroquinolones in Gram-negative bacteria include reduced outer membrane permeability and efflux pumps that remove antimicrobials from the cell via an energy-dependent system. Charvalos et al. (1996) found evidence that efflux pumps represent a mechanism of multi-drug resistance in *C. jejuni*.

Transmissible plasmids are thought to play an important role in mediating high-level tetracycline resistance (MIC >64 (μ/ml) in *Campylobacter* spp. (Bopp et al., 1985; Tenover et al., 1985; Taylor et al., 1981). Bopp et al. (1985) evaluated the antimicrobial susceptibility, plasmid profiles and serotypes of outbreak strains of *Campylobacter*. All strains that were resistant to tetracycline contained 38-MDal plasmids having common nucleic acid sequences. Other investigators have also reported 38-MDal plasmids mediating tetracycline resistance (Taylor et al., 1981; Tenover et al., 1985). In addition, a smaller plasmid was associated with tetracycline resistance in Indian isolates (Prasad et al., 1994). One-third of the outbreak strains of *C. jejuni* evaluated by Bopp et al. (1985) and the CDC had plasmid-mediated tetracycline resistance, suggesting that analysis of plasmid profiles may be useful in outbreaks. A high degree of DNA homology has been noted among plasmids of *Campylobacter* spp., but there is no homology with plasmids of *Campylobacter* that mediate tetracycline resistance (Tenover et al., 1985). Tetracycline resistance plasmids of *Campylobacter* cannot be transferred to *E. coli* (Taylor et al., 1981). It is important to note that, while not all plasmid-containing *Campylobacter* isolates are resistant to tetracycline, all tetracycline-resistant *Campylobacter* strains carry plasmids (Tenover et al., 1985).

Kanamycin resistance is also plasmid-mediated. There are at least two different resistance genes in

TABLE 3. **MECHANISMS OF *CAMPYLOBACTER* RESISTANCE BY ANTIBIOTIC CLASS**

Antibiotic class	Mechanism of action	Mechanism of *Campylobacter* resistance
Fluoroquinolones	Inhibit DNA gyrase subunit A and prevent supercoiling of DNA	Point mutation of Thr86 to Ile nucleotide in QRDR*
Tetracyclines	Inhibit 30 S ribosomal subunit	Plasmid-mediated; 38 mDal and 23 kb plasmids described
Kanamycin	Inhibits 30 S ribosomal subunit	Likely plasmid-mediated
Macrolides and Clindamycin	Inhibit 50 S ribosomal subunit	Chromosomally mediated
Trimethoprim	Inhibits dihydrofolate reductase (DHFR)	Endogenous Possibly transposon-borne *dfr* gene
β-lactams	Inhibit transpeptidation step in cell-wall construction	Multi-factorial: Reduced affinity of penicillin binding proteins; porin mutations limit entry by charge and MW°; and β-lactamases selectively inactivate

* QRDR: Quinolone resistance determining region
° MW: Molecular weight

Campylobacter spp. Kanamycin resistance plasmids show considerable size variation, ranging from 41 to 132 kb, with a high proportion of them also encoding the *tetO* gene (Tenover & Elvrum, 1988).

Campylobacter spp. are resistant to most β-lactams (Tajada et al., 1996). Resistance appears to be multifactorial in nature, including reduced affinity of penicillin-binding proteins (PBPs), β-lactamase production, and reduced penetration into the cell due to changes in porins (Lachance et al., 1991; Tajada et al., 1996). According to Tajada et al. (1996), resistance in *Campylobacter* is primarily mediated by PBPs. Penicillin G and most narrow-spectrum cephalosporins do not bind to the PBPs of *Campylobacter* but imipenem, amoxicillin (particularly when combined with clavulanic acid) and cefepime (a broader spectrum cephalosporin) are active against *Campylobacter* spp. *in vitro*, which may reflect increased binding to PBPs. Reportedly, 83–92% of *C. jejuni* isolates produce a β-lactamase (Lachance et al., 1991). β-lactamase-positive organisms are significantly more resistant to ampicillin, amoxicillin and ticarcillin than are β-lactamase-negative bacteria. Among β-lactamase inhibitors, tazobactam was the most potent in inhibiting the enzyme, followed by clavulanic acid and sulbactam (Lachance et al., 1991). Susceptibility testing, in contrast, showed clavulanic acid to perform the best in combination with amoxicillin, ampicillin or ticarcillin (Lachance et al., 1991; Tajada et al., 1996). The activities of piperacillin, most cephalosporins, and imipenem are not affected by the *Campylobacter* β-lactamase. Imipenem is highly active against the majority of isolates; where resistance to this agent occurs in *C. jejuni* it has been linked to changes in porins (Page et al., 1989).

The outer membrane of Gram-negative bacteria is an effective barrier to antibiotics. The main route for penetration of many antibiotics is via pores in the outer membrane. *C. jejuni* pores are thought to be smaller than those of *E. coli* and therefore to limit passage to solutes with a molecular weight of 340 Dal or less. Additionally, the charge of a molecule affects diffusion through the pores; molecules that are uncharged or have zero net charge diffuse better. Molecules smaller than 360 Dal and with neutral charge are the most likely to penetrate the *C. jejuni* pore (Page et al., 1989). Imipenem, ampicillin, amoxicillin, quinolones and nitrofurans all penetrate the *C. jejuni* outer membrane well.

Data regarding the mechanism of erythromycin resistance are sparse, but it is believed to be chromosomally mediated in *Campylobacter*. There is cross-resistance with spiramycin, lincomycin, clindamycin and azithromycin (Taylor, 1992). Neither enzymes capable of modifying macrolides nor efflux mechanisms appear to contribute to resistance. Fortunately, erythromycin resistance appears to be rare in *C. jejuni*, although resistance rates are higher in *C. coli* isolates from livestock.

Trimethoprim, although used as empirical treatment of travellers' diarrhoea, is ineffective for the treatment of *C. jejuni* enteritis since all strains are resistant. Studies in Sweden reported a high prevalence of foreign genetic material encoding a resistant dihydrofolate reductase. The genes in question are known as *dfr1* and *dfr9*. There is evidence that they were acquired by transposon-mediated genetic exchange. Heavy trimethoprim use in agriculture was hypothesized to exert pressure leading to the emergence of resistance (Gibreel & Skold, 1998).

In summary, antibiotic resistance is a global threat to the ability to effectively treat *C. jejuni* gastroenteritis. The complex interplay of veterinary antibiotic use, unregulated human antibiotic use, and the erosion of geographical barriers to microbial migration due to the ease of international travel, have contributed to the emergence and spread of resistance, a process which is unpredictable and continually evolving. Vigilant monitoring of resistance is required worldwide. WHO, recognizing the impact of antimicrobial use in animal feed, met in Berlin and formulated recommendations to address this global problem (WHO, 1997). International cooperation by a variety of organizations is needed to address it.

Prevention

Campylobacteriosis is a disease that occurs worldwide, costing millions of dollars per year in the United States alone (Skirrow & Blaser, 1992). Childhood diarrhoea globally, travellers' diarrhoea, and epidemic and sporadic diarrhoea in the industrialized world could be dramatically reduced by appropriate intervention. *Campylobacter*-associated GBS may be the most important factor in terms of cost, at least in industrialized countries, because of the severe and prolonged nature of this complication. The incidence of GBS in developing countries remains to be determined. Research continues on many fronts, but simple hygiene remains the most effective means of preventing *Campylobacter* enteritis.

The reservoirs of *C. jejuni* are well described and include contaminated surface water, pets and, most

importantly, livestock (including poultry, swine and cattle). Primary prevention of *Campylobacter* enteritis involves improving personal hygiene, providing clean fresh water supplies, and improving food processing and handling. Poultry figures prominently in human illness, so its production and handling have been a focus of efforts to reduce *Campylobacter* colonization and transmission. Attempts to limit contact between farm personnel and chicken flocks, to introduce hygienic measures to limit campylobacter transmission within flocks, or to modify slaughtering methods to reduce cross-contamination have made little impact on the frequency of colonization. Chlorinating their drinking-water did little to reduce the extent of colonization of broiler flocks (Stern et al., 1995). Irradiation of poultry carcasses is effective, but has not been widely used due to concerns on the part of the public about its safety. Thus, measures short of vigilant culture and destruction of infected flocks have done little to curb the ubiquitous colonization by *Campylobacter* in large-scale poultry farms.

Colonization of poultry occurs quickly and uniformly; this may be due to the fact that the birds are coprophagic. Introduction of *Campylobacter* spp. into 4 chicks resulted in 100% of the flock becoming colonized within 2–3 days (Jacobs-Reitsma, 1998). Interventions could, therefore, involve prophylaxis to prevent colonization or treatment once colonization occurs. Tsubokura et al. (1997) administered anti-*Campylobacter* antibodies orally to chicken flocks and studied the effect on colonization. Feed was supplemented with bovine and chicken immunoglobulin preparations (derived from the milk or eggs of immunized livestock). In uncolonized flocks, this treatment produced a greater than 99% reduction in colonization upon subsequent oral challenge with *Campylobacter*. However, these preparations did not eliminate *Campylobacter* from pre-colonized flocks, producing only a transient reduction, by 50–80%, in the number of bacteria, as compared to controls. This difference disappeared within 3 days after treatment. Reduction in *Campylobacter* colonization of poultry will, presumably, lead to reduction in its transmission to humans. Passive immunity may reduce the bacterial load without selecting for resistance, in contrast to the use of feed supplemented with antimicrobials.

There is a significant incidence of locally acquired *Campylobacter* infection, both in industrialized and developing countries and, additionally, campylobacteriosis represents a large proportion of travellers' diarrhoea. In the latter infections, antibiotic resistance is emerging. For these reasons, efforts are under way to develop a vaccine. Progress has been limited by the antigenic variability of *Campylobacter* spp. and the lack of information about its pathogenicity. A whole-cell killed vaccine and live attenuated vaccines are being studied (Scott, 1997). The oral route of administration is being targeted, with the rationale that stimulation of intestinal immunity should provide adequate defence against *Campylobacter* enteritis. Since infection with *Campylobacter* spp. is associated with reactive arthritis and GBS, the vaccines must not trigger immune responses that would lead to these complications. More information is needed about the specific antigens that trigger GBS in order to eliminate them from vaccines.

Intervention strategies

As is the case for *V. cholerae*, well-located regional reference laboratories with standardized methods of data collection are needed to create a worldwide surveillance network. The ramifications of antimicrobial use in animal husbandry are only beginning to be understood, although it appears that the level of fluoroquinolone resistance in humans can, in part, be directly linked to additives in poultry feed and water. The ease of acquisition of antimicrobials and the great amount of air travel mean that regional initiatives would offer little more than stopgap measures of prevention. A global surveillance network with the authority to monitor trends on a worldwide scale and to recommend guidelines for human and veterinary antibiotic use worldwide would be a means of combating the rapidly increasing resistance in enteric organisms.

Conclusion

In 25 years, *C. jejuni* has progressed from a newly recognized pathogen to being understood as the most significant cause of diarrhoea in the industrialized world. It is a common cause of diarrhoea in children and travellers internationally, causes endemic diarrhoea and outbreaks of diarrhoea in industrialized countries, and appears to be the most common cause of Guillain-Barre Syndrome, a devastating and costly illness. Despite the large amount of information available, the mechanisms of pathogenicity in *Campylobacter* are still unclear. Thus, molecular pathogenesis studies represent the next research frontier. Controlling the spread of the or-

ganism in livestock, ensuring appropriate food han-
dling practices, and limiting the use of antibiotics
in agriculture are critical to reducing overall dis-
ease incidence and resistance. The development of
vaccines, both for humans and animals, will greatly
enhance our ability to contain the spread of
Campylobacter. Emerging drug resistance is a glo-
bal problem; therefore, international cooperation
will be required to address it.

Conclusion

In order to develop comprehensive policies, any strategy for controlling antimicrobial resistance must consider ecological factors and be based on data for a wide variety of pathogens. Given the high rate of genetic exchange among species, interventions aimed at a single enteric organism will be fruitless. The United States Agency for International Development (USAID) Infectious Disease Initiative intends to use data generated for individual organisms to create a more general global strategy to combat antimicrobial resistance. *V. cholerae*, *Campylobacter* and *Shigella* are but pieces of a larger puzzle. In many regions, financial and technical resources are needed to establish adequate laboratories and to network them within the areas they serve as well as with international centres. Support from WHO, CDC, and other donors will be essential in providing resources, training local health care workers and laboratory personnel, and educating the public.

The greatest obstacles to implementation will be at the local level. Changing the antibiotic prescribing behaviour of health care providers and their use by lay people is a daunting task, requiring communication strategies specific to the particular region's culture, habits and socioeconomic situation. Additionally, there are currently no policies in place to control the unregulated sale of antibiotics in developing countries. Without regulation of antibiotic availability there is little hope of implementing rational and effective guidelines for antibiotic use.

Implementation of recommended actions should take place under the umbrella of infectious disease control and surveillance programmes. There is an urgent need to set up low-technology regional laboratories throughout the developing world to deal with diagnosis and surveillance; these structures should also serve other infectious disease control programmes. Local antimicrobial resistance initiatives could be assisted by the "Surveillance Standards for Antimicrobial Resistance" currently being prepared (WHO, 2001) and the WHONET software (WHO, 1999, developed by the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance). Regional centres could collect samples, routinely test these for antimicrobial sensitivity, and log the data into a regional laboratory sample survey system. Such surveillance systems would be useful for the early detection of epidemics and in choosing antimicrobial therapy based on prevalent resistance patterns. These programmes would allow laboratories to analyse and share antimicrobial resistance data and facilitate the coordination of control efforts (Institute of Medicine, 1998).

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