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 strain 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 conjugal 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 multiply antibiotic-resistant *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,
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 & Saya, 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 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 \textit{V. cholerae} (Sack et al., 1997).  

\textbf{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 \textit{Salmonella typhimurium} in 1987 and \textit{Shigella} spp. in 1988. Plasmid-mediated resistance to TMP-SMZ in \textit{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, \textit{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 \textit{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 \textit{S. dysenteriae} type 1 in Calcutta (Sen et al., 1988). These findings are evidence for substantial mobility of genetic elements in \textit{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 \texttt{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).  

\textbf{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 \textit{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

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\caption{Prevalence (\%) resistant \textit{V. cholerae} O1 and O139 isolates, Calcutta, 1992–1996}
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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 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

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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 were sensitive 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 & Poudyal, 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, Tschepe & 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, Karolitis, 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 to 1985 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.