# *Vibrio cholerae*[1]

## Description

### Taxonomy and serological classification

*Vibrio cholerae*, a member of the family Vibrionaceae, is a facultatively anaerobic, Gram-negative, non-spore-forming curved rod, about 1.4 – 2.6 mm long, capable of respiratory and fermentative metabolism; it is well defined on the basis of biochemical tests and DNA homology studies (Baumann, Furniss & Lee, 1984). The bacterium is oxidase-positive, reduces nitrate, and is motile by means of a single, sheathed, polar flagellum. Growth of *V. cholerae* is stimulated by addition of 1% sodium chloride (NaCl). However, an important distinction from other *Vibrio* spp is the ability of *V. cholerae* to grow in nutrient broth without added NaCl.

Differences in the sugar composition of the heat-stable surface somatic "O" antigen are the basis of the serological classification of *V. cholerae* first described by Gardner & Venkatraman (1935); currently the organism is classified into 206 "O" serogroups (Shimada et al., 1994; Yamai et al., 1997). Until recently, epidemic cholera was exclusively associated with *V. cholerae* strains of the O1 serogroup. All strains that were identified as *V. cholerae* on the basis of biochemical tests but that did not agglutinate with "O" antiserum were collectively referred to as non-O1 *V. cholerae*. The non-O1 strains are occasionally isolated from cases of diarrhoea (Ramamurthy et al., 1993a) and from a variety of extraintestinal infections, from wounds, and from the ear, sputum, urine, and cerebrospinal fluid (Morris & Black, 1985). They are ubiquitous in estuarine environments, and infections due to these strains are commonly of environmental origin (Morris, 1990). The O1 serogroup exists as two biotypes, classical and El Tor; antigenic factors allow further differentiation into two major serotypes — Ogawa and Inaba. Strains of the Ogawa serotype are said to express the A and B antigens and a small amount of C antigen, whereas Inaba strains express only

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the A and C antigens. A third serotype (Hikojima) expresses all three antigens but is rare and unstable.

Between 1817 and 1961, six pandemics of cholera were recorded. The classical biotype was responsible for the fifth and sixth pandemics and is believed to have been associated with the earlier pandemics as well, although there is no hard evidence. The causative agent of the seventh and current cholera pandemic, which began in 1961, is the El Tor biotype. The classical biotype has been completely displaced worldwide, except in Bangladesh where it reappeared in epidemic proportions in 1982 (Samadi et al., 1983), remained prominent there for a few years, and now seems to have become extinct again (Siddique et al., 1991). The simple distinction between *V. cholerae* O1 and *V. cholerae* non-O1 became obsolete in early 1993 with the first reports of a new epidemic of severe, cholera-like disease in Bangladesh (Albert et al., 1993) and India (Ramamurthy et al., 1993b). At first, the responsible organism was referred to as non-O1 *V.cholerae* because it did not agglutinate with O1 antiserum. However, further investigations revealed that the organism did not belong to any of the O serogroups previously described for *V. cholerae* but to a new serogroup, which was given the designation O139 Bengal after the area where the strains were first isolated (Shimada et al., 1993). Since recognition of the O139 serogroup, the designation non-O1 non-O139 *V. cholerae* has been used to include all the other recognized serogroups of *V. cholerae* except O1 and O139 (Nair et al., 1994a).

The emergence of *V. cholerae* O139 as the new serogroup associated with cholera, and its probable evolution as a result of horizontal gene transfer between O1 and non-O1 strains (Bik et al., 1995), has led to a heightened interest in the *V. cholerae* non-O1 non-O139 serogroups. There is evidence for horizontal transfer of O antigen among *V. cholerae* serogroups; Karaolis, Lan & Reeves (1995) reported that isolates of nearly identical *asd* gene (chromosomal housekeeping gene, which encodes aspartate semialdehyde dehydrogenase) sequences had different O antigens and that isolates with the O1 antigen did not cluster together but were found in different lineages. There has been elevated activity of the non-O1 non-O139 serogroups in the recent past, and localized outbreaks of acute diarrhoea caused by *V. cholerae* serogroups such as O10 and O12 have been reported (Dalsgaard et al., 1995; Rudra et al., 1996).

## Pathogenicity for humans, and virulence factors

The major features of the pathogenesis of cholera are well established. Infection due to *V. cholerae* begins with the ingestion of contaminated water or food. After passage through the acid barrier of the stomach, the organism colonizes the epithelium of the small intestine by means of the toxin-coregulated pili (Taylor et al., 1987) and possibly other colonization factors such as the different haemagglutinins, accessory colonization factor, and core-encoded pilus, all of which are thought to play a role. Cholera enterotoxin produced by the adherent

vibrios is secreted across the bacterial outer membrane into the extracellular environment and disrupts ion transport by intestinal epithelial cells. The subsequent loss of water and electrolytes leads to the severe diarrhoea characteristic of cholera.

The existence of cholera enterotoxin (CT) was first suggested by Robert Koch in 1884 and demonstrated 75 years later by De (1959) and Dutta, Pause & Kulkarni (1959) working independently. Subsequent purification and structural analysis of the toxin showed it to consist of an A subunit and 5 smaller identical B subunits (Finkelstein & LoSpalluto, 1969). The A subunit possesses a specific enzymatic function and acts intracellularly, raising the cellular level of cAMP and thereby changing the net absorptive tendency of the small intestine to one of net secretion. The B subunit serves to bind the toxin to the eukaryotic cell receptor, ganglioside GM1. The binding of CT to epithelial cells is enhanced by neuraminidase.

Apart from the obvious significance of CT in the disease process, it is now clear that the production of CT by *V. cholerae* is important from the perspective of a serogroup acquiring the potential to cause epidemics. This has become particularly evident since the emergence of *V. cholerae* O139. A dynamic 4.5-kb core region, termed the virulence cassette (Trucksis et al., 1993), has been identified in toxigenic *V. cholerae* O1 and O139 but is not found in non-toxigenic strains. It is known to carry at least six genes, including *ctxAB* (encoding the A and B subunits of CT), *zot* (encoding zonula occludens toxin (Fasano et al., 1991)), *cep* (encoding core-encoded pilin (Pearson et al., 1993)), *ace* (encoding accessory cholera enterotoxin (Trucksis et al., 1993)), and *orfU* (encoding a product of unknown function (Trucksis et al., 1993)). In the El Tor biotype of *V. cholerae*, many strains have repetitive sequence (RS) insertion elements on both sides of the core region; these are thought to direct site-specific integration of the virulence cassette DNA into the *V. cholerae* chromosome (Mekalanos, 1985; Goldberg & Mekalanos, 1986; Pearson et al., 1993). The core region, together with the flanking RS sequences, makes up the cholera toxin genetic element CTX (Mekalanos, 1983).

Recent studies have shown that the entire CTX element constitutes the genome of a filamentous bacteriophage (CTXf). The phage could be propagated in recipient *V. cholerae* strains in which the CTXf genome either integrated chromosomally at a specific site, forming stable lysogens, or was maintained extrachromosomally as a replicative form of the phage DNA (Waldor & Mekalanos, 1996). Extensive characterization of the CTXf genome has revealed a modular structure composed of two functionally distinct genomes, the core and RS2 regions. The core region encodes CT and the genes involved in phage morphogenesis, while the RS2 region encodes genes required for replication, integration, and regulation of CTXf (Waldor et al., 1997). Generally, CTXf DNA is integrated site-specifically at either one (El Tor) or two (classical) loci within the *V. cholerae* genome (Mekalanos, 1985). In El Tor strains, the prophage DNA is usually found in tandem arrays that also include a related genetic element known as RS1. The RS1 element contains the genes that enable phage DNA replication and integration, plus an additional gene (*rstC*) whose function is unknown but

that does not contain *ctxAB* or the other genes of the phage core region that are thought to produce proteins needed for virion assembly and secretion (Davis et al., 2000). CTXf gains entry to the *V. cholerae* cell by way of the toxin-regulated pili—the surface organelles required for intestinal colonization. Its genes are then incorporated into host chromosome, inducing the cell to secrete CT.

The *zot* gene increases the permeability of the small intestinal mucosa by an effect on the structure of the intestinal tight junctions (Fasano et al., 1991), while *ace* affects ion transport in the intestinal epithelium. Another factor whose gene resides outside the CTX genetic element and which is thought to contribute to the disease process is haemolysin/cytolysin (Honda & Finkelstein, 1979). In contrast to the watery fluid produced by CT, the haemolysin can cause accumulation in ligated rabbit ileal loops of fluid that is bloody with mucous (Ichinose et al., 1987). Although not fully characterized, other toxins produced by *V. cholerae* include the shiga-like toxin (O' Brien et al., 1984), a heat-stable enterotoxin (Takeda et al., 1991), new cholera toxin (Sanyal et al., 1983), sodium channel inhibitor (Tamplin et al., 1987), thermostable direct haemolysin-like toxin (Nishibuchi et al., 1992), and a cell-rounding cytotoxic enterotoxin known as the non-membrane-damaging cytotoxin (Saha, Koley & Nair, 1996; Saha & Nair, 1997).

## In vitro and animal studies, volunteer studies, dose–response modelling

Although natural infection with *V. cholerae* O1 does not occur in animals, some animal models have been developed for the study of cholera. A few of these models have yielded useful information relevant to human disease. The most widely used adult intact animal model for *V. cholerae* is the RITARD (removable intestinal tie-adult rabbit diarrhoea) model (Spira, Sack & Fröhlich, 1981), which allows massive and often fatal diarrhoea to occur within 1 – 5 days.

Volunteer studies with *V. cholerae* have yielded many insights into pathogenesis and host immune response. It has been shown (Cash et al., 1974; Levine et al., 1988) that more than $10^8$ *V. cholerae* cells are required to induce infection and diarrhoea. Administration of sodium bicarbonate ($NaHCO_3$) to neutralize gastric acid dramatically reduces the infectious dose to less than $10^4$, although lower inocula correlate with longer incubation periods and decreased stool volumes.

## Disease occurrence, outbreaks, sporadic cases, prospective studies

Cholera has re-emerged as a major infectious disease in the recent past, with a global increase in its incidence. In 1994 cholera cases were notified from 94 countries—the highest ever number of countries in one year (World Health Organization, 1995). Two particularly disturbing aspects of the global cholera picture in the 1990s have been the dramatic and unexpected reappearance in

January 1991 of epidemic cholera caused by *V. cholerae* O1 El Tor in Latin America after a 100-year absence from the region (Tauxe & Blake, 1992) and the unprecedented appearance in late 1992 in southern India of an epidemic strain of *V. cholerae* non-O1, classified as *V. cholerae* O139 Bengal (Ramamurthy et al., 1993b). The reasons for these phenomena are still being intensively researched.

### *Asia*

A new cholera epidemic erupted in Madras, southern India, in October 1992 and rapidly spread eastward (Ramamurthy et al., 1993b). The causative organism isolated from this outbreak was *V. cholerae* non-O1, which produces cholera toxin and is now known as the O139 serogroup. Within months of the Madras outbreak, *V. cholerae* O139 strains were isolated from Calcutta and Bangladesh (Albert et al., 1993; Ramamurthy et al., 1993b). The O139 serogroup has spread rapidly into several countries in south-east Asia since 1992, raising the concern that this may be the beginning of the eighth pandemic (Bhattacharya et al., 1993; Nair et al., 1994b). In 1994, however, a dramatic decline in the incidence of cholera caused by serogroup O139 was observed in areas where it had predominated in the preceding years. The O139 was replaced by strains of the O1 serogroup (Mukhopadhyay et al., 1996), but genetic studies showed them to be different from the O1 strains that were circulating before the emergence of the O139 serogroup (Faruque et al., 1997; Sharma et al., 1997; Yamasaki et al., 1997). In August 1996 there was a resurgence of the O139 serogroup—with an altered antibiogram—in Calcutta and other parts of India (Mitra et al., 1996), replacing the existing O1 serogroup to become the dominant serogroup in this part of the subcontinent. Molecular studies have again demonstrated that the O139 strains that re-emerged in August 1996 showed changes at the genetic level and were different from the O139 strains that appeared in 1992 (Sharma et al., 1997). A total of 50 921 cases and 1145 deaths were reported from 18 countries in Asia in 1995. The number of countries reporting thus declined from 26 in 1994. The case-fatality rate increased from 1.3% in 1994 to 2.2% in 1995 (World Health Organization, 1996).

Several lines of evidence suggest that the O139 serogroup closely resembles the O1 El Tor biotype. However, O139 contains a distinct O antigen and has been shown to express a polysaccharide capsule (Johnson et al., 1994; Weintraub et al., 1994). Accumulating data suggest that serogroup O139 Bengal arose from a serogroup O1 biotype El Tor by deletion of the genes responsible for O1 antigen biosynthesis (Manning, Stroeher & Morona, 1994; Waldor & Mekalanos, 1994; Comstock et al., 1995) and acquired DNA from another non-pathogenic serogroup (Mooi & Bik, 1997). In a cholera-endemic area, a newly emergent non-O1 serogroup has a selective advantage because of the absence of pre-existing immunity. These observations indicate the ability of *V. cholerae* to react to change and to adverse conditions (such as immunity).

### *Africa*

From 1970, *V. cholerae* O1 El Tor has gradually spread to most of the continent with case-fatality rates between 4% and 12%. From 1991 to 1996, the number of cases remained high and ranged between 70 000 and 160 000 (World Health Organization, 1997). The largest proportion of all reported cholera cases in 1994, and 42% of all cholera deaths reported globally that year, were in Africa (World Health Organization, 1995). The impact of war and political unrest on diarrhoeal disease is illustrated clearly by the cholera epidemic in Rwanda, which can be categorized into two phases. During the "stay" phase, nearly 2 million people resident in Rwanda moved to neighbouring countries because of the 1994 civil war between Hutu and Tutsi tribes. About 1 million Rwandans fled to Goma, Zaire (now the Democratic Republic of the Congo), and stayed in makeshift camps. Among these refugees, 12 000 died during epidemic outbreaks of cholera and shigellosis, caused mainly by poor water-supply and sanitation facilities coupled with inadequate use of oral rehydration therapy, use of inappropriate intravenous fluids, and inadequate experience among health workers (Siddique et al., 1995). Surveys conducted in the Goma region showed that the epidemic was caused by multidrug-resistant *V. cholerae* O1 biotype El Tor and *Shigella dysenteriae* type I (Islam et al., 1995). In the "return" phase in 1996, 8916 cases of diarrhoea were recorded among the 350 000 Rwandans returning from five camps,. The very low case-fatality rates were attributed to the rapid response by health officials in the cholera treatment centres (Brown et al., 1997). In 1995, a decrease of about 44% in the number of cases was observed compared with 1994. Efforts to repatriate refugees, improvements in surveillance, and control of diarrhoeal diseases by governments and collaborating agencies contributed to the decline in incidence and case-fatality rates. As of December 1996, 26 countries had reported cholera, with Nigeria, Senegal, and Somalia reporting more then 1000 cases each and very high case-fatality rates (World Health Organization, 1997).

### *Latin America*

In 1991 cholera appeared in Latin America—the last part of the less-developed world to have remained untouched by the seventh pandemic of cholera. The epidemic in Peru began in three different foci along the Pacific coast (Ries et al., 1992). The Pan American Health Organization reported that 750 000 cases of cholera with 6500 deaths occurred between 1991 and 1992 (Tauxe et al., 1994). The epidemic spread in conventional fashion, following the trade routes into the interior of Peru, Ecuador, Colombia, Brazil, Chile, and then central Mexico. One year after the appearance of cholera in Peru in 1991, 18 south and central American countries had reported cases of cholera.

The appearance of cholera in Latin America remains an enigma. It could have been introduced by maritime traffic from the Pacific region, just as the Latin

American epidemic strain was introduced into the USA's Mexican Gulf coast in 1991 (McCarthy et al., 1992). Coincidental to cholera in Peru was a warm event related to El Niño in the tropical Pacific from 1990 to 1995 (Colwell, 1996). The Latin American isolates of *V. cholerae* were different from the endemic strain in the USA but share several similarities with most of the seventh pandemic isolates (Wachsmuth, Bopp & Fields, 1991; Faruque & Albert, 1992). All the Latin American strains subjected to multilocus enzyme electrophoresis exhibited the same pattern (Wachsmuth et al., 1993), indicating that they are clonal. However, when the Latin American clone of *V. cholerae* O1 was compared with three other known global clones—the seventh pandemic clone, the Mexican Gulf coast clone, and the Australian clone—the multilocus enzyme electrophoresis pattern of the Latin American clone was distinct from that of previously known clones of *V. cholerae* O1. It has been suggested that *V. cholerae* O1 was introduced into the aquatic environment off the Peruvian coast long before the outbreak flourished.

The Peruvian epidemic illustrates the importance of preparing for cholera epidemics. As many as 4500 cases a day occurred during the Peruvian epidemic, yet the *mortality rate* was less than 1% (Anon, 1991).

## International travel and cholera

During the nineteenth century cholera was a classic disease of long-distance travellers. More recently, international travel has often been the cause of sporadic cases of cholera in Chile and the USA. A history of seafood consumption is often obtained from travellers who acquire cholera during visits to areas in which the disease is endemic. Rarely, *V. cholerae* non-O1 non-O139 has been implicated as the cause of acute gastroenteritis among travellers with diarrhoea (Bhattacharya et al., 1992).

Some 38 cholera cases associated with consumption of shellfish from coastal waters in the Gulf of Mexico were reported in the USA in the 15 years from 1973 (Popovic et al., 1993). Shellfish was the vehicle of transmission of cholera from Latin America to the USA on five separate occasions in 1991. Isolation of toxigenic *V. cholerae* strains from commercial oyster beds in Mobile Bay, Alabama, raised concerns about contamination of shellfish beds in the Gulf of Mexico with Latin American *V. cholerae* strains (Depaola et al., 1992). However, molecular typing methods clearly showed that the Latin American isolates were different from previously described Gulf coast strains (Wachsmuth, Bopp & Fields, 1991). Furthermore, samples of the ballast, bilge, and sewage from several ships arriving from Latin American ports and docking in Gulf of Mexico ports has revealed the same toxigenic strains of *V. cholerae* O1 as those found in the contaminated oyster beds in the Gulf of Mexico (McCarthy et al., 1992). These data support the hypothesis that ships were responsible for the initial introduction of *V. cholerae* O1 to coastal waters of the Gulf of Mexico.

## Monitoring and assessment

### Sampling and sample preparation

For the investigation of surface waters, water samples should be collected in sterilized bottles following standard procedures. Plants should be collected in sterile polyethylene bags, and phytoplankton and zooplankton should be collected using plankton nets and kept in sterile glass bottles. Sediment should be collected by a core sampler and kept in sterile polyethylene bags. All field samples should be transported to the laboratory inside a cooled container (at about 4 – 10 °C) and processed within 6 hours (Donovan & van Netten, 1995).

### Analytical methods: culture methods, immunological and molecular methods, methods performance

A qualitative enrichment procedure is normally performed for the detection of *V. cholerae* from food or environmental samples. Quantitative procedures, either direct plating or most probable number (MPN), are required only occasionally. Culture media that were developed for the isolation of *V. cholerae* from faeces in clinical laboratories have also generally been used for the isolation of *V. cholerae* from foods or the environment. Alkaline peptone water (APW) is the standard medium for enrichment of *V. cholerae*, although several nutrient-rich modifications of APW, such as blood – APW and egg – APW are also used (Donovan & van Netten, 1995). Thiosulfate – citrate – bile-salts – sucrose agar (TCBS) is a highly selective differential medium that is most commonly used for the isolation of *V. cholerae* ; its selective ingredients suppress the growth of most of the interfering organisms such as coliforms, pseudomonads, aeromonads, and other Gram-positive bacteria (Kobayashi et al., 1963). The advantage of TCBS is its sucrose – bromthymol blue diagnostic system, which distinguishes the yellow sucrose-positive colonies of *V. cholerae* from other colonies.

For isolation of *V. cholerae* from the environment the following procedures are recommended:

- 10 g of plant material are homogenized with 100 ml of normal saline in a blender.
- 10 ml of plankton sample should be homogenized in a PTFE-tipped tissue grinder using a stirrer.
- 1 ml of plant homogenate, 10 ml of plankton homogenate, 50 ml of water, and 1.0 g of sediment are enriched in either APW or bile – peptone broth overnight at 37 °C (Islam, Alam & Khan, 1995).
- All samples are then plated on TCBS agar or taurocholate – tellurite – gelatin agar and incubated at 37 °C for 18 – 24 hours.

Suspected *V. cholerae* strains transferred from primary isolation media can be identified by means of a standard series of biochemical media used for identifi-

cation of members of the Enterobacteriaceae and Vibrionaceae families. Both conventional tube tests and commercially available enteric identification tests are suitable for identifying *V. cholerae*. A crucial test for differentiation of *V. cholerae* from Enterobacteriaceae is the positive oxidase test. Other key traits for distinguishing *V. cholerae* from other species include fermentation of □-glucose with acid production (without gas), maltose, □-mannitol, sucrose, and trehalose. Most strains are also motile at 37 °C, metabolize lysine and ornithine, and show a positive string test (a mucoid "string" is formed when a large loop of growth from a noninhibitory agar medium is suspended in a drop of 0.5% aqueous selection deoxycholate and then drawn). The absence of arginine metabolism is also frequently used for differentiation. However, the most important test for identification of *V. cholerae* O1 or O139 is agglutination in antisera raised against O1 or O139.

Various simpler schemes for identification of *V. cholerae* are available for use in developing countries. One involves the inoculation of suspected *V. cholerae* colonies from the isolation plate into a single-tube, multitest medium which is based on the principles of triple sugar iron (TSI) and Kligler iron agar (KIA) medium (Kaper, 1979). Cultures yielding an alkaline slant (K) over acid (A) butt, with no gas or $H_2S$, are then tested for oxidase and reactivity with O1 or O139 antisera, using growth taken from the multitest medium. Extensive evaluation has revealed that 97.9% of the oxidase-positive strains that yield a K/A reaction in the multitest medium have biochemical reactions consistent with those of *V. cholerae* (Nair et al., 1987). Strains of *V. cholerae* that do not agglutinate in either O1 or O139 antisera should be labelled as non-O1 non-O139; if further serogrouping is deemed necessary it should be done at an International Reference Center for serogrouping of *V. cholerae*.

Specific probes for the A and B subunit genes of CT have been used to detect the location of these genes in the *V. cholerae* genome and in differentiating between clones of *V. cholerae* (Kaper et al., 1982; Wachsmuth, Bopp & Fields, 1991; Wright et al., 1992). Restriction fragment length polymorphism of the enterotoxin gene has been successfully used to identify the origin of *V. cholerae* strains involved in outbreaks (Yam et al., 1991). The DNA sequences of the structural genes of the CT subunit B show heterogeneity and have been classified into three genotypes: genotype 1 is found in strains of classical biotype worldwide and El Tor biotype strains associated with the USA Gulf of Mexico coast, genotype 2 in El Tor strains from Australia, and genotype 3 in El Tor biotype strains from the seventh pandemic and the recent Latin American epidemic (Ølsvik et al., 1993). Molecular diagnostic tests, such as PCR, are now being developed for both clinical and environmental monitoring of *V. cholerae* O1 and O139. Primer pairs corresponding to unique stretches in the genes of the *rfb* complex, which encode the O antigen, have been designed to develop PCRs for specific detection of O1 (Hoshino et al., 1998) and O139 (Albert et al., 1997) from stool specimens.

## Control

### Emission, transport, and survival in the environment

Most *Vibrio* species are ubiquitous in estuarine and marine environments and are also found in fresh water provided that there is a certain minimal level of sodium ions. The cholera vibrio, however, was long considered to be an exception, in that it was believed not to be an environmental organism (Feachem, Miller & Drasar, 1981) but associated with water only as a result of sewage contamination. Thus, until the late 1970s, *V. cholerae* was considered by most workers in the field to be an organism whose normal habitat was the human gut and to be incapable of surviving for more than a few days outside the gut. The reason for this belief was a general failure to isolate the organism from the water unless there were cholera cases in the immediate vicinity. During epidemics, toxigenic *V. cholerae* O1 or O139 can be isolated from the local fresh water as well as from patients (Khan et al., 1984; Ghosh et al., 1994) but disappears from the environment after the epidemic subsides.

Since the early epidemiological work in 1853 in London by John Snow and later laboratory investigation by Robert Koch (1884), it has been known that water is important in the transmission of cholera. Water from public supplies was implicated in the previous six pandemics. In the present (seventh) pandemic, properly treated public water supplies are not generally considered to be a risk factor. However, serious epidemics of cholera continue to occur in areas where treatment is poor or sporadic, such as in China and the Russian Federation, in Latin America, and in other developing countries. The recent epidemic in Latin America was said to have been exacerbated by the failure of the authorities to chlorinate water supplies; this omission was due in part to concern about the carcinogenic effect of chlorination by-products in drinking-water.

While there is no doubt that the faecal – oral route of cholera transmission is of primary concern because of its importance in the development of secondary cases and in the subsequent spread of the disease, it does not fully explain seasonal reccurrence of the disease in some areas or outbreaks that occur where faecal – oral transmission is unlikely. Traditional culture techniques for isolating *V. cholerae* from water are frequently unsuccessful. More advanced techniques, however, using direct immunofluorescence microscopy, DNA hybridization, PCR, and improved culture methods, have frequently isolated both O1 and non-O1 strains, even in the absence of traditional faecal indicator bacteria such as *Escherichia coli* and faecal streptococci. This suggests either that *V. cholerae* can survive longer in the environment than other faecal organisms or that *V. cholerae* is an environmental organism in its own right.

Several surveys have been conducted to study the environmental distribution of *V. cholerae* in diverse areas in the world. From their results it is clear that *V. cholerae* is widely distributed in temperate and tropical aquatic environments. Its distribution is affected by various abiotic factors including the inorganic and organic contents of water and sediments, pH, fluctuating temperature, salinity,

variations in oxygen tension, and exposure to the ultraviolet rays in sunlight. A linear correlation with salinity was observed, with more frequent isolations at sites with salinities between 0.2% and 2.0%. The effect of temperature was more strongly correlated with the frequency of isolations when the water temperature was above 17 °C. Studying the influence of water temperature, salinity, and pH on survival and growth of toxigenic *V. cholerae* O1 associated with live copepods, Huq et al. (1984) concluded that 15% salinity, 30 °C water temperature, and pH 8.5 supported increased attachment and multiplication of *V. cholerae* on copepods.

The cholera vibrio has been found in association with a wide range of aquatic life, including cyanobacteria (*Anabaena variabilis*) (Islam, Drasar & Bradley, 1989), diatoms (*Skeletonema costatum*) (Martin & Bianchi, 1980), in freshwater filamentous green algae (*Rhizoclonium fontanum*) (Islam, Drasar & Bradley, 1989), oysters (*Crassostrea virginica*) (Hood, Ness & Rodrick, 1981), water hyacinths (*Eichornia crassipes*) (Spira et al., 1981), the arthropod *Gerris spinolae* (Shukla, Singh & Sanyal, 1995), and blue crab (*Callinectes sapidus*) (Huq et al., 1986). *V. cholerae* produces a chitinase and is able to bind to chitin, a semi-transparent material, predominantly mucopolysaccharide, that is the principal component of crustacean shells (Nalin et al., 1979; Colwell & Spira, 1992). It colonizes the surfaces of copepods (Huq et al., 1983; Tamplin et al., 1990) with preferential attachment to the oral region and the egg sac. *V. cholerae* O1 also attaches to *Volvox* sp., a colonial form of phytoplankton, and the attachment appears as a "ring" pattern (Colwell et al., 1990).

Chitinases and mucinases facilitate the attachment of *V. cholerae* to aquatic organisms, while algae surface films enhance the growth of the pathogen (Epstein, Ford & Colwell, 1993). It has been suggested that *V. cholerae* can survive an inter-epidemic period and colonize the surfaces of algae, phytoplankton, and water hyacinth (Islam, Alam & Neogi, 1992). In an epidemic area like the Ganges river delta, copepods favour survival of *V. cholerae* because of the organism's production of chitinase and ability to use chitin as a source of nutrients (Nalin, 1976).

When present in the environment, *V. cholerae* may undergo a series of major physical and metabolic changes; while the altered cells are more difficult, or even impossible, to grow using conventional techniques, they have been shown to retain their virulence factors. Colwell et al. (1984) suggest that *V. cholerae* O1 can assume, or approximate, a state of dormancy in response to nutrient deprivation, elevated salinity, and/or reduced temperature. This finding was supported by others, who reported strong linear correlations between *V. cholerae* non-O1 and temperature and salinity (Miller, Drasar & Feachem, 1984). Nutritionally deprived *V. cholerae* shows an initial rapid decline in total lipids and carbohydrates and a more gradual decline in proteins and DNA (Hood, Guckert & White, 1986).

The O1 and O139 strains may produce CT and other virulence factors; non-O1 non-O139 strains rarely possess these attributes. Laboratory studies have shown no loss of toxigenicity by *V. cholerae* O1 under conditions of low salinity, adverse pH, adverse water chemistry, low sodium, or long-term starvation, sug-

gesting that toxin-producing ability is unlikely to be lost when the organism is exposed to environmental stress (Miller et al., 1986). Survival of toxigenic *V. cholerae* O1 in water at different temperatures and with different values of salinity, pH, and cation concentration and composition also indicate its ability to survive for extended periods in warm water (25 °C) containing no nutrients, with a salinity of 0.25 – 3.0% and a pH of around 8.0 (Miller, Drasar & Feachem, 1984). Some strains have shown increased toxin production under certain conditions, such as when attached to various aquatic plants (Islam, 1990). The numbers of *V. cholerae* suspended in water are generally low, approximately $10^3$ cfu/litre for non-O1s and less than 50 cfu/litre for O1s. However, the organism can multiply rapidly in badly stored drinking-water and may be found in large numbers associated with aquatic species such as cyanobacteria, algae, zooplankton, and crustacea (including commercial species such as crabs). While counts of free organisms in the water may be low, copepods found in the same water may have $10^5$ organisms attached to their surface (Huq et al., 1983).

The continuing failure of attempts to isolate toxigenic *V. cholerae* O1 from natural aquatic environs remains unexplained. Although toxigenic *V. cholerae* has been isolated from surface waters, no study has yet demonstrated water as a reservoir of toxigenic *V. cholerae* in the absence of a person with cholera using that water. Organisms of the O1 serogroup have frequently been isolated from aquatic environs, but most of the environmental O1 isolates do not produce CT, the toxin to which the clinical state of cholera is principally attributed. Even in a hyperendemic area like Calcutta, toxigenic *V. cholerae* O1 could not be isolated from several aquatic bodies examined for a year (Nair et al., 1988).

The overall body of evidence suggests that faecal – oral transmission is of primary importance, and long experience has shown basic water-supply and sanitation measures to be effective in controlling secondary spread of the disease. Such measures are essential since toxigenic *V. cholerae* may be reintroduced to nonendemic areas by several mechanisms. It is also likely that environmental survival during inter-epidemic periods accounts for sudden multi-point outbreaks of cholera as occur, for example, in the Ganges delta area.

## Effects of drinking-water treatment

Ensuring safe drinking-water implies both securing a safe source and maintaining safety up to the point of consumption. This is equally true of sophisticated piped distribution systems, of water collected by householders from sources such as wells, and of water provided to the consumer by any other means.

Groundwater sources such as wells and springs are often believed to be of good quality with regard to bacterial pathogens transmitted by the faecal – oral route. However, such sources are readily contaminated by faecal material, especially where there are potential sources of contamination nearby or where contaminants may be carried by surface waters. Protection measures need to be properly applied (see for example World Health Organization, 1997).

Surface-water sources should generally be considered to be susceptible to faecal contamination and, therefore, to contamination by *V. cholerae*. However, the organism can be easily eliminated from drinking-water by appropriate treatment.

It has long been accepted that slow sand filtration is effective for removal of *V. cholerae* during drinking-water treatment. The biological processes that are responsible for water purification occur more slowly at low temperatures, and ice formation on filter surfaces has been associated with unacceptable deterioration in effluent water quality. The use of open filters should therefore be avoided in regions where temperatures can drop below 0 °C.

Other common treatment methods such as coagulation, flocculation, sedimentation, and rapid filtration will significantly reduce numbers of *V. cholerae*, but should be seen as preparatory treatments to be followed by disinfection.

Most chemical disinfectants effectively eliminate *V. cholerae* under normal operating conditions (principally concentration and time) provided that water is clear (i.e. free of particulates). The same is true of physical disinfection methods, such as the use of ultraviolet light.

Recontamination of "safe" water is a significant concern. When water has to be collected, there are several opportunities for recontamination, by recipients, and during handling or extraction from storage for use. In piped supply systems recontamination is also a significant risk, especially where the supply is discontinuous or of low pressure and where there is appreciable leakage. To minimize the health risks associated with recontamination, use of a residual disinfectant is recommended.

Boiling is generally advised but for poor populations this is not affordable (Barua & Merson, 1992). Use of potassium aluminium sulfate ($K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$), 500 mg/litre, has been claimed to kill *V. cholerae* (Barua & Merson, 1992) but the taste of water is unacceptable to many. Chlorine-releasing agents (such as calcium hypochlorite or bleaching powder) are very effective and less expensive. For domestic chlorination 1% stock solution is prepared by adding enough water to 4 teaspoons (16 g) of hypochlorite or 10 teaspoons (40 g) of bleaching powder to make 1 litre. Three drops of stock solution should be added per litre of water, which should be allowed to stand for 20 – 30 minutes before use (Clark, 1956). Various disinfecting solutions, containing about 1% chlorine, are available commercially, as are water purification tablets and liquid preparations containing chlorine. It is important that the stability of the disinfectant is checked frequently and that the disinfected water is properly stored. Iodine is also an excellent disinfectant for water: two drops of 2% tincture of iodine are sufficient for 1 litre of water. Various iodine-containing preparations are commercially available.

Water from a relatively protected source cannot be guaranteed to be free from contaminating bacteria when actually consumed. In a typical urban setting in a developing country, where the water supply is intermittent, drinking-water is collected and stored, in various ways, for one or more days in the household. If the water is not handled correctly, the processes of collection and storage provide

ample opportunity for contamination. In addition, the residual chlorine in stored water is relatively low, and the viability of *V. cholerae* is thus extended. It has been recently reported that *V. cholerae* O1 and non-O1 can shift to a rugose form associated with the production of an exopolysaccharide which promotes cell aggregation (Morris et al., 1993). This rugose form resists both chlorine (even at levels exceeding 2 mg/litre) and other disinfectants in potable water and is likely to contribute to the waterborne transmission of cholera (Rice et al., 1992; Morris et al., 1993). Contamination of drinking-water can also occur as result of wastewater influx in old or damaged network systems. Network maintenance must therefore be considered as a further preventive measure against cholera.

Little is known about the regrowth ability for *V. cholerae* as a result of the uptake of assimilable organic carbon (AOC) or about the organism's occurrence or colonization in biofilms. In Africa, however, the short duration of the cholera epidemics in Goma and Uvira, which are connected by Lake Kiva, Lake Tanganyika, and the Rusizi river, suggest that large freshwater bodies do not provide a suitable environment for *V. cholerae* (Birmingham et al., 1997). This might be because of the low concentration of AOC (West, 1989). Cholera outbreaks in Burundi, including that in Rumonge, associated with natural water sources, were reportedly due to faecal contamination and high levels of AOC (Birmingham et al., 1997).

## Conclusions and recommendations

Cholera is usually transmitted by the faecal – oral route, with the infecting dose being around $10^8$. Individuals with reduced gastric acidity and blood group O are more susceptible to infection. In situations where poor environmental sanitation is coupled with poor domestic and personal hygiene, transmission of cholera is a result of faecal contamination of drinking-water. Events such as flood, famine and war, resulting in movements of refugees, favour the outbreak and spread of cholera. Refugees may be at particular risk for cholera because of secondary contamination, overcrowding, inadequate sanitary facilities and water supplies, and malnutrition. The situation is exacerbated by a number of other problems, such as inadequate staffing, frequent shortages of oral rehydration salts and late presentation of cases because of transport difficulties.

A study in Calcutta has shown that, at the family level, faecal contamination of stored water can be prevented by the use of narrow-necked pitchers.

It has been suggested that the control of cholera epidemics is too big a task for a national diarrhoeal diseases control programme. Although this may be true in the case of extensive epidemics, it should not be forgotten that large outbreaks of this kind are often the result of initial delays in detection and containment. A properly organized national control programme provides the framework for quick detection and prompt containment and is the best means of ensuring preparedness for cholera control. Some strengthening of existing human and material resources may be required, but this is much easier than ad hoc efforts to fight a large and widespread epidemic.

The essential features of a national diarrhoeal diseases control programme include a national epidemic control committee, a well-established survillance system, environmental sanitation and safe water supplies, health education, and hands-on training in clinical management (with adequate laboratory and logistic support). During an epidemic, the critical elements of cholera control are early identification of cases through surveillance and case-finding, notification to health authorities and WHO, establishment of treatment centres, health education, and proper disposal of human waste. Except in special circumstances, there is little value in chemoprophylaxis or mass vaccination. Restrictions on trade and travel offer few advantages, but travellers to epidemic areas should be extremely careful about what they eat and drink and scrupulous about personal hygiene.

Under the International Health Regulations, notification of WHO about cases of cholera is mandatory. National health authorities should report the first suspected cases to WHO at the earliest possible mement; laboratory confirmation of cases should also be reported immediately. Thereafter, health authorities should report confirmed cases to WHO on a weekly basis. Once the presence of cholera in a particular area has been confirmed, it is unnecessary to confirm all subsequent cases. Moreover, neither the treatment nor the notification of suspected cases of cholera requires laboratory confirmation of the presence of *V. cholerae* O1 (World Health Organization, 1993): the decision to initiate anti-epidemic measures must be taken regardless of the causative strain.

National risk management strategies should be planned and implemented by a national coordinating committee. This committee should be responsible for cholera preparedness, intersectoral cooperation, regional and interregional collaboration, collection and reporting of information on cholera cases and deaths, organization of any special training that may be required, procurement, storage and distribution of essential supplies, and implementation, supervision, monitoring and evaluation of control activities (World Health Organization, 1993).