

Seminar

Cholera

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Intestinal infection with *Vibrio cholerae* results in the loss of large volumes of watery stool, leading to severe and rapidly progressing dehydration and shock. Without adequate and appropriate rehydration therapy, severe cholera kills about half of affected individuals. Cholera toxin, a potent stimulator of adenylate cyclase, causes the intestine to secrete watery fluid rich in sodium, bicarbonate, and potassium, in volumes far exceeding the intestinal absorptive capacity. Cholera has spread from the Indian subcontinent where it is endemic to involve nearly the whole world seven times during the past 185 years. *V cholerae* serogroup O1, biotype El Tor, has moved from Asia to cause pandemic disease in Africa and South America during the past 35 years. A new serogroup, O139, appeared in south Asia in 1992, has become endemic there, and threatens to start the next pandemic. Research on case management of cholera led to the development of rehydration therapy for dehydrating diarrhoea in general, including the proper use of intravenous and oral rehydration solutions. Appropriate case management has reduced deaths from diarrhoeal disease by an estimated 3 million per year compared with 20 years ago. Vaccination was thought to have no role for cholera, but new oral vaccines are showing great promise.

Detailed accounts of the history of cholera are available so only a brief summary is provided here.^{1,2} "Asiatic cholera", as it was sometimes called, has been endemic in south Asia, especially the Ganges delta region, from the time of recorded history. It was always much feared because it regularly occurred in epidemics with high mortality rates. In Kolkata, a cholera temple, Ola Beebe ("our lady of the flux"), was built for protection against the disease. In 1817, the first cholera pandemic began with spread of the disease outside the Indian subcontinent along trade routes to the west as far as southern Russia. A second pandemic started in 1826 and reached the major European cities by the early 1830s. In 1831, the pandemic reached the UK and the response was important in that it led to the establishment of local Boards of Health and a "Cholera Gazette", which served as a clearing house for tracking the epidemic.³

At that time cholera was thought to be spread by the "miasma" (like a fog) coming from the river, but the classic epidemiological study of John Snow in 1854 in London showed the association of the disease with contaminated drinking water even before any bacteria were known to exist.⁴ Three more pandemics, continuing up to 1925, involved Africa, Australia, Europe, and all the Americas. The causative agent, *Vibrio cholerae*, was not identified until 1884 in Kolkata during the fifth pandemic.⁵ Why the earlier pandemics began and how they ended is not known. However, cholera did not persist in any of the new geographical areas that it had invaded but continued as an endemic disease in the Ganges delta.

Because of the large numbers of cases and deaths during these pandemics, the disease was viewed as a major public-health disaster requiring governmental intervention. The New York cholera epidemic led to the first Board of Health in the USA in 1866,⁶ and cholera became the first reportable disease.

The current (seventh) pandemic now has involved almost the whole world. This pandemic began in Indonesia,⁷ rather than the Ganges delta, and the causative agent was a biotype of *V cholerae* serogroup O1 called El Tor. It was first isolated in 1905 from Indonesian pilgrims travelling to Mecca at a quarantine station in the village of El Tor, Egypt.² It was found again in 1937 in Sulawesi, Indonesia.⁸ Then in 1960, for unknown reasons, this strain began to spread around the world. It invaded India in 1964, Africa in 1970,⁹⁻¹¹ southern Europe in 1970,^{12,13} and South America in 1991.^{14,15} The disease has now become endemic in many of these places, particularly south Asia and Africa. Since 1973, a focus of El Tor *V cholerae* similar but not identical to the pandemic strain has persisted in the Gulf of Mexico of the USA causing sporadic cases of summertime, seafood-associated cholera.¹⁶

In 1992, a newly described, non-O1 serogroup of *V cholerae*, designated O139 Bengal, caused unusual cholera outbreaks in India and Bangladesh.^{17,18} Before the discovery of *V cholerae* O139 (the 139th serotype in the typing scheme for *V cholerae*), only serogroup O1 was known to cause epidemic cholera, so the O139 serotype was essentially a "new" cause of cholera.¹⁹ Serogroups O139 Bengal and O1 now coexist and continue to cause large outbreaks of cholera in India and Bangladesh. The O139 serogroup is likely to be the cause of the next

Lancet 2004; 363: 223-33

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Search strategy

We carried out a PubMed search with the terms "cholera" and "Vibrio cholerae" from 1966 onwards and selected references that were pertinent to this review. These articles were supplemented by additional references from the WHO and historical articles in our personal collections.



Figure 1: Bucket with typical rice-water stool from a patient with cholera

(eighth) pandemic of cholera. In spring 2002, serotype O139 caused an estimated 30 000 cases in Dhaka, Bangladesh, exceeding the number of cases associated with El Tor during a short period.²⁰

Epidemiology

Cholera is often described as the classic water-borne disease because it is commonly associated with water. This description oversimplifies the transmission of *V cholerae*, because the bacterium can be transmitted by contaminated food also; contaminated water is frequently mixed with food, allowing either to act as a vehicle. For more developed countries, contaminated food (especially undercooked seafood) is the usual vehicle for transmission, and contaminated water is more common in less developed countries.^{21–23}

Cholera has pronounced seasonality. In Bangladesh, where the disease is endemic, two peaks occur each year corresponding to the warm seasons before and after the monsoon rains.^{24–26} In Peru, epidemics are strictly confined to the warm season.²⁷ The seasonality seems to be related to the ability of vibrios to grow rapidly in warm environmental temperatures. Other than shellfish and plankton, there are no animal reservoirs. In endemic areas, annual rates of disease vary widely, probably as a result of environmental and climate changes. Better understanding of the relation to climate would allow better planning for epidemics by public-health officials.²⁸

Although the typical clinical picture is severe diarrhoea, in fact, most individuals infected with *V cholerae* have no symptoms or only mild diarrhoea, indistinguishable from other mild diarrhoeal diseases. The ratio of cases to infections ranges from one in three to one in 100.^{25,29} The severity of the infection depends on many factors, especially including local intestinal immunity (from previous natural exposure or vaccination), the size of the

inoculum ingested, the adequacy of the gastric-acid barrier, and the patient's blood group. For unknown reasons, people of blood group O are at much higher risk of severe cholera from El Tor vibrios than are those of other blood groups.^{30–32} This susceptibility to cholera may be the reason for the lower than normal proportion of people with this blood group in the Ganges delta area.³¹

A high infectious dose (10^8 bacteria) is needed to cause severe cholera in healthy volunteers, but a much lower dose (10^7) is sufficient if given with antacids to neutralise stomach acid.^{33,34} Under natural field circumstances, the inoculum size to cause cholera may be even lower, because attack rates are lower than in volunteer studies, and many of the patients do have low gastric-acid production.³⁵

In cholera-endemic areas, the highest attack rates are in children aged 2–4 years;²⁵ in newly invaded areas, by contrast, the attack rates are similar for all ages. However, the illness is generally first seen in adult men on account of exposure to contaminated food and water.¹⁷ Water-use patterns in different areas affect spread of the disease. In some cities in Peru, cholera vibrios were spread through the municipal water system,³⁰ which resulted in very high rates of infection in the urban population. In rural areas, where rivers or open wells are used for drinking water, cases tend to cluster among people living close to and drinking from contaminated water. Secondary cases sometimes occur during funeral feasts as a result of traditional but unhygienic funeral practices in some parts of the world.³⁷

In contrast to *Salmonella typhi*, long-term carriers of *V cholerae* are extremely rare and are not important in the transmission of disease.³⁸

Since cholera outbreaks can become massive epidemics, they must be reported to national health authorities. If possible, cases of suspected cholera should be confirmed by bacteriology. Even without laboratory confirmation, cases should be reported if they meet the WHO definition: a cholera outbreak should be suspected if a patient older than 5 years develops severe dehydration or dies from acute watery diarrhoea, or if there is a sudden increase in the daily number of patients with acute watery diarrhoea, especially patients who pass “rice water” stools typical of cholera.³⁹

Clinical features

After an incubation period of between about 18 h and 5 days, symptoms are generally abrupt and include watery diarrhoea and vomiting. The most distinctive feature of cholera is the painless purging of voluminous stools resembling rice-water (figure 1). The stools are sometimes described as having a fishy odour. The vomitus is generally a clear, watery, alkaline fluid. In adults with severe cholera, the rate of diarrhoea may quickly reach 500–1000 mL/h, leading to severe dehydration. Signs of severe dehydration include absent or low-volume peripheral pulse, undetectable blood pressure, poor skin turgor, sunken eyes, and wrinkled hands and feet (as after long immersion in water). At first, patients are restless and extremely thirsty, but as shock progresses, they become apathetic and may lose consciousness. Many patients also show respiratory signs of metabolic acidosis with Kussmaul, gasping breathing. Most patients have no urine output until the dehydration is corrected. The fluid loss may be so rapid that the patient is at risk of death within a few hours of onset, and most deaths occur during the first day. However, if rehydration fluids are provided in insufficient quantities, the patient may survive temporarily, only to die a few days later.

Management of patients with suspected cholera

Assess for dehydration.

Rapidly rehydrate the patient with intravenous Ringer's solution for severely dehydrated patients or ORS for those with less severe dehydration; use rice-based ORS if possible.

Severely dehydrated patients require replacement of 10% of their bodyweight within 2–4 h.

Use cholera cot (if possible) to monitor stool output; monitor status of hydration and monitor severity of purging frequently.

Maintain hydration by replacing continuing fluid losses until diarrhoea stops.

Give an oral antibiotic (eg, doxycycline) to dehydrated patients as soon as vomiting stops.

Provide food as soon as patient is able to eat (within a few hours).

Several complications can occur with cholera, but these are generally from improper treatment. They include acute renal failure from protracted hypotension if insufficient fluids are given. Most cholera patients have low blood glucose concentrations, and a few have severe hypoglycaemia.⁴⁰ Electrolyte imbalance, especially hypokalaemia, can occur if the intravenous fluids are not appropriate.⁴¹ Miscarriage or premature delivery can occur in pregnant women as a complication of shock and poor perfusion of the placenta.⁴² With good hydration, these obstetric emergencies are becoming less frequent, but cholera treatment centres must be prepared for them. Severe muscle cramps of arms and legs are common. They are probably due to the electrolyte imbalance, although the exact explanation is not known. They subside within a few hours of treatment.

Treatment

Without treatment the case-fatality rate for severe cholera is about 50%. However, treatment is very effective and simple and is based on the concept of replacing fluids as fast as they are being lost (panel). Replacement fluids should have a similar electrolyte composition to the fluids being lost. Initially, the fluids must be given sufficiently rapidly to make up for the volume that has already been lost to restore circulating blood volume. Additional maintenance fluids must then be given to continue to replace continuing losses as they occur. If fluids are given promptly, nearly all deaths are avoided. However, effective treatment is not always available in remote areas where cholera occurs, and thus, cholera deaths are still common.

To facilitate clinical assessment and management of patients, dehydration is classified into three categories on the basis of clinical signs and symptoms: none, some (moderate), and severe (table 1). Signs of dehydration are not clinically apparent until the patient has already lost about 5% of his or her bodyweight. The degree of dehydration guides the therapy of the patient. A patient with severe dehydration requires emergency intravenous polyelectrolyte solution for rehydration followed by oral rehydration solution (ORS) for maintenance hydration. For milder cases, ORS is used for both rehydration and for maintenance. The principles of rehydration therapy are: rapid replacement of fluid deficits; correction of the metabolic acidosis; correction of potassium deficiency; and replacement of continuing fluid losses. These aims are all accomplished with appropriate rehydration fluids.

Feature	No dehydration	Some dehydration (two or more of these signs including one indicated by*)	Severe dehydration (two or more of these signs including one indicated by*)
General appearance	Well, alert	Restless, irritable	Lethargic or unconscious; floppy
Eyes	Normal	Sunken*	Very sunken and dry*
Tears	Present	Absent*	Absent*
Mouth and tongue	Moist	Dry*	Very dry*
Thirst	Drinks normally, not thirsty	Thirsty, drinks eagerly	Drinks poorly or not able to drink
Skin pinch	Goes back quickly	Goes back slowly	Goes back very slowly

In adults and children older than 5 years, other signs of severe dehydration are absent radial pulse and low blood pressure. The skin pinch is less useful in patients with marasmus (severe wasting) or kwashiorkor (severe malnutrition with oedema), or obese patients. Tears are a relevant sign only for infants and young children.

Table 1: Assessment of patients with diarrhoea for dehydration³⁸

Because of the acidosis, the serum potassium concentration may be normal or even high, so the potassium deficiency may not be apparent. As the acidosis is corrected, the serum potassium concentration will fall to dangerously low values unless additional potassium is provided.

Patients who are severely dehydrated are assumed to have lost 10% of their bodyweight, and this is the volume that needs to be replaced. For example, a 50 kg patient with severe dehydration will need immediate replacement of 5 L of intravenous fluids. Patients who have no pulse or blood pressure should receive the fluid as rapidly as possible and more than one intravenous line may be needed to infuse the fluid rapidly enough to restore the pulse. The entire amount should be given in 2–4 h. The most common error in the treatment of cholera is to give the intravenous fluid too slowly, allowing patients to remain in shock for a long period. If peripheral veins cannot be found, infusion via the femoral vein may be necessary.

For patients with lesser degrees of dehydration (the majority), ORS provides effective rehydration. The volume should also be calculated to replace the fluid deficit to ensure that sufficient volumes are given. For individuals with some dehydration, at least 5.0–7.5% of the bodyweight in ORS should be given, just to make up the deficit, and additional ORS should be given to compensate for the continuing losses.



Figure 2: A child, lying on a cholera cot, showing typical signs of severe dehydration from cholera

The patient has sunken eyes, lethargic appearance, and poor skin turgor, but within 2 h was sitting up, alert, and eating normally.

The use of a "cholera cot" is invaluable in managing severely purging patients. This is a simple camp cot with a hole in the middle and a plastic sheet that has a sleeve draining into a plastic bucket (figure 2). The cholera cot allows the patient to remain horizontal in bed while purging, and also allows for easy assessment of stool volumes so the carer can easily estimate the fluid requirements. Where cholera cots are not available, they can be constructed out of simple materials.

The intravenous fluid should be isotonic with respect to salts; it should also include a base and potassium (table 2). Ringer's lactate is the best commercially available intravenous fluid, though other polyelectrolyte solutions with additional potassium provide even better balance with the composition of the stool losses.⁴³ Since Ringer's lactate contains only 4 mmol/L potassium, ORS, which contains 20 mmol/L potassium, should be given as soon as the patient can drink. If no polyelectrolyte solution is available, normal saline can be used in emergency situations, but ORS should be provided as soon as possible to compensate for the acidosis and potassium deficiency. Dextrose and water does not provide the needed salts and is not appropriate.

ORS is the preferred therapy for patients who have no detectable dehydration or some dehydration. It is also used to maintain hydration to make up for continuing losses after correction of severe dehydration with intravenous fluids. Packets of oral rehydration solutes, containing carbohydrate and the correct salts are now widely available throughout the world. For cholera, ORS that uses rice rather than glucose is even better because it reduces the purging rate;^{44,45} this form is also available in packets to be mixed with water. The preferred formulation of ORS has changed lately; the sodium concentration has been lowered to 75 mmol/L. This hypo-osmolar solution is acceptable for cholera, although ORS solutions with sodium concentrations lower than this do not contain sufficient sodium and could result in severe hyponatraemia. If no ORS packets are available, ORS can be prepared by adding the following simple ingredients to 1 L water: 2.6 g sodium chloride, 2.9 g trisodium citrate, 1.5 g potassium chloride, and 13.5 g glucose (or 50 g boiled and cooled rice powder). The purest water that is available should be used when making ORS, and leftover solution should be discarded after 24 h.

Especially during the first 24 h, patients must be observed closely because the purging might continue at a high rate and some patients have difficulty drinking sufficient quantities of ORS, or vomiting can prevent sufficient oral intake. Such patients will become dehydrated and require intravenous infusion again.

Patients can be fed as soon as they are able to take food. There is no need to restrict food or fluids, and

babies can continue to breastfeed. There is no basis for "resting the gut" in any acute diarrhoeal disease.

Patients with clinically significant cholera should receive a 1–3-day course of antibiotic to shorten the illness and lessen the diarrhoeal purging.^{46,47} Antibiotics not only treat the illness; they also decrease the need for rehydration fluids and shorten the hospital stay. These effects are especially important because cholera outbreaks generally occur in areas where intravenous fluid and other supplies are lacking. In most cases, doxycycline is the antibiotic of choice (300 mg given as a single dose to adults).

During an outbreak, samples from representative patients should be tested for antibiotic sensitivity to select the most appropriate antibiotic, on the basis of current sensitivity patterns. For outbreaks due to tetracycline-resistant strains, other clinically effective antibiotics include erythromycin, co-trimoxazole,⁴⁸ ciprofloxacin,⁴⁹ and azithromycin.⁵⁰ Patients with mild diarrhoea need not receive antibiotics even during cholera outbreaks. Without antibiotic treatment (as long as rehydration is given), patients will recover in about 4–5 days. The recovery time is shortened to about 2–3 days with antibiotics.

Antibiotics should not be given to asymptomatic contacts. Prophylactic use of antibiotics greatly increases the risk of the development of resistance and is not cost-effective.⁵¹

Antimicrobial resistance

Widespread antibiotic resistance in *V cholerae* was unheard of before 1977, but conjugative-plasmid-mediated multiply antibiotic-resistant (including to tetracycline) *V cholerae* O1 (MARV) emerged as a major problem first in Tanzania⁵² then in Bangladesh.⁵³ During the past two decades, reports from several cholera-endemic countries of strains resistant to antibiotics including tetracycline, ampicillin, kanamycin, streptomycin, sulphonamides, trimethoprim, and gentamicin have appeared. Unlike *Shigella* spp, *V cholerae* O1 and O139 do not tend to accrue resistance to antibiotics but show spatial and temporal fluctuations, with periods of resistance fluctuating with periods of sensitivity, usually reflective of the antibiotics that are abused in any given region.⁵⁴

The molecular mechanisms underlying the emergence of MARV are becoming better known. Conjugative plasmids, conjugative transposons, and integrons are all vehicles of acquisition of resistance genes that facilitate the intracellular movement of genetic determinants of resistance to antimicrobial agents. Apart from the novel O antigen, *V cholerae* O139 strains that emerged in late 1992 carried a novel conjugative, self-transmissible, chromosomally integrating SXT element (a constin), which conferred resistance to sulphamethoxazole,

Fluid	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)	Bicarbonate (mmol/L)	Carbohydrate (g/L)	Osmolality (mmol/L)
Cholera stool						
Adults	130	100	20	44
Children	100	90	33	30
ORS						
Glucose (WHO)	75	65	20	10*	13.5†	245
Rice	75	65	20	10*	30–50‡	About 180
Intravenous fluids						
Lactate Ringer's	130	109	4	28§		271
Dhaka solution	133	154	13	48		292
Normal saline	154	154	0	0		308

*Trisodium citrate (10 mmol/L) is generally used rather than bicarbonate. †Glucose 13.5 g/L (75 mmol/L). ‡30–50 g rice contains about 30 mmol/L glucose depending on degree of hydrolysis. §Base is lactate. ||Base is acetate.

Table 2: Composition of cholera stools and electrolyte rehydration solutions used to replace stool losses

trimethoprim, chloramphenicol, and low levels of streptomycin.⁵⁵ Subsequent studies showed that there is much flux in the antibiotic-resistance genes found in the SXT family of constins.⁵⁶ Quinolones generally have excellent activity against *V cholerae*, but fluoroquinolone-resistant strains of *V cholerae* have lately been reported from Kolkata, India.^{57,58} In addition to mutations detected in the target genes *gyrA* and *parC*, proton-motive-force-dependent efflux is involved in quinolone resistance in *V cholerae*.⁵⁹

Integrans are a newly identified group of gene expression elements that incorporate open reading frames (gene cassettes) and convert them to functional genes.⁶⁰ These have been implicated as a major factor in the dissemination of drug resistance for *V cholerae*.^{61,62}

Clinical microbiology

V cholerae is a gram-negative, polar monotrichous, oxidase-positive, asporogenous curved rod that ferments glucose, sucrose, and mannitol and is positive in the lysine and ornithine decarboxylase tests. The organism is classified by biochemical tests and is further subdivided into serogroups based on the somatic O antigen. The O antigen shows enormous serological diversity, with over 200 serogroups.⁶³ Only the O1 and O139 serogroups cause epidemic and pandemic disease. Strains identified by biochemical tests as *V cholerae* that do not agglutinate with O1 or O139 antisera are referred to as non-O1 non-O139 *V cholerae*. Previously they were called non-cholera vibrios or non-agglutinable vibrios. The non-epidemic serogroups, though not involved in cholera epidemics, can be pathogenic,⁶⁴ and are infrequently associated with small outbreaks of diarrhoeal disease.^{65,66} They occasionally cause a variety of severe extraintestinal infections, including wound infections and acute sepsis, especially in people with liver disease or immunosuppression.⁶⁷

V cholerae survives well in faecal specimens if kept moist, but if there is a delay of more than a few hours, Cary-Blair transport medium should be used for transport to the laboratory. The faeces (either fresh or in the transport medium) should be plated onto TCBS (thiosulphate citrate bile salts sucrose) agar, a medium that inhibits most other normal faecal flora but supports the growth of the vibrios. In addition, the specimen should also be inoculated into alkaline peptone water, a high-pH enrichment broth, which preferentially supports the growth of vibrios. After 6–12 h of incubation, a second TCBS plate is inoculated. These plates are incubated for 18–24 h, and *V cholerae* colonies appear as smooth yellow colonies with slightly raised centres. Presumptive identification of *V cholerae* O1 or O139 can be made on the basis of typical colonies, which are oxidase-positive and agglutinate with O1 or O139 antiserum. Agglutination should be carried out with subcultures onto non-selective medium, because colonies can autoagglutinate from TCBS medium, giving false-positive results. Positive specimens should be reported immediately to the government health department and sent to the appropriate referral laboratory for confirmation.

Rapid tests include dark-field microscopy in which a wet mount of liquid stool is examined for the appearance of “darting” organisms that are halted by the addition of O1 or O139 antiserum.⁶⁸ Rapid immunoassays are also available.^{69,70}

The rapid immunological assays can be especially useful for monitoring of epidemiological patterns in remote areas where cultures are not readily available, but new outbreaks must be confirmed by cultures. Molecular methods, including PCR and DNA probes, are also

available but are not widely used and not practicable in many areas where cholera is common.

Subtypes of *V cholerae*

The O1 serogroup is divided into two biotypes, classical and El Tor, that can be differentiated by use of assays of haemolysis, haemagglutination, phage, polymyxin B sensitivity, and the Voges-Proskauer reaction. The latest approach, however, is to use biotype-specific genes (eg, *tcpA*, *rtxC*) to differentiate between the two biotypes. Each of the O1 biotypes can be further subdivided into two major serotypes, Ogawa and Inaba. Ogawa strains produce the A and B antigens and a small amount of C, whereas Inaba strains produce only the A and C antigens. A third serotype, Hikojima, produces all the three antigens but is rare and unstable.

V cholerae strains of the same biotype and serotype can be differentiated by a phage-typing scheme. There are 145 phage types for O1⁷¹ and five for O139.⁷² Multilocus enzyme electrophoresis can distinguish between classical and El Tor strains and has grouped the toxigenic El Tor biotype strains into four major clonal groups or electrophoretic types (ET) representing broad geographical areas.^{73,74} These include the Australian clone (ET1), the Gulf Coast clone (ET2), the seventh pandemic clone (ET3), and the Latin American clone (ET4).^{75–77} In addition, a standard ribotyping scheme for *V cholerae* O1 and O139 can distinguish seven different ribotypes among classical strains, 20 ribotypes and subtypes among El Tor strains, and six distinct ribotypes among O139 strains.^{78,79} These ribotypes have been especially useful for molecular epidemiological studies. For example, molecular analysis of epidemic isolates of *V cholerae* between 1961 and 1996 in Bangladesh revealed clonal diversity among strains isolated during different epidemics.^{80,81} These studies demonstrated the transient appearance and disappearance of more than six ribotypes among classical vibrios, at least five ribotypes of El Tor vibrios, and three different ribotypes of *V cholerae* O139. Different ribotypes showed different CTX genotypes resulting from differences in copy number of the CTX element and variations in the integration site of CTX element in the chromosome.⁸¹ Molecular epidemiological studies have shown that many strains are in circulation but most outbreaks are caused by a restricted number of clones.

Clinical pathophysiology

Ingested vibrios from contaminated water or food must pass through the acid stomach before they are able to colonise the upper small intestine. Colonisation is aided by way of fimbria, filamentous protein structures called toxin coregulated pilus (TCP) extending from the cell wall, that attach to receptors on the mucosa,⁸² and by the bacterium's motility, which helps to penetrate the mucus overlying the mucosa. *V cholerae* adhering to the M cells in rabbit intestine without causing any tissue damage are shown in figure 3. Concentrations of vibrios on the mucosal surface rapidly increase to 10⁷ or 10⁸ cells per g. With this high concentration of vibrios closely attached to the mucosa, enterotoxin can be efficiently delivered directly to the mucosal cells.

Formerly cholera was thought to cause sloughing of the intestinal mucosa by an inflammatory process. However, the intestinal mucosa is now known to remain intact and without inflammatory changes.⁸³ The previous findings were shown to be artifacts, based on autolytic post-mortem changes. Koch first postulated in 1884 that the bacteria produce a toxin and that this stimulates the massive outpouring of fluid from the intestine. De and



Figure 3: *V cholerae* adhering to M cells in rabbit intestine without causing any tissue damage

Note the typical comma-shaped bacteria from which the organism derives its name. Reproduced with permission from Yoshifumi Takeda, Faculty of Human Life Sciences, Jissen Woman's University, Tokyo, Japan and Junichi Takeda, Cine-Institute, Tokyo, Japan.

Dutta were the first to demonstrate this toxin (now called cholera toxin) by use of culture filtrates in rabbits.^{84,85} The toxin was later purified and sequenced.^{86,87} It has a molecular mass of 84 000 kDa and consists of five binding (B) subunits and one active (A) subunit.^{88,89} As we now understand the mechanism of action, the B subunits are physiologically inactive but bind the holotoxin to the GM1 ganglioside receptors in the small-intestinal mucosa, and the A subunit is transported into the cell where it activates adenylate cyclase.^{90,91} This activation leads to an increase in cyclic AMP, followed by an increase in chloride secretion in the crypt cells, and inhibition of neutral sodium chloride absorption in the villus cells, which in turn leads to a massive outpouring of fluid into the small intestine.⁹² The volume secreted exceeds the normal absorptive capacity of the bowel and results in watery diarrhoea. Most of the secretions come from the small intestine, although the toxin also inhibits water absorption by the colon.⁹³ The diarrhoeal fluid contains large amounts of sodium, chloride, bicarbonate, and potassium, but little protein or blood cells.⁴³ The loss of electrolyte-rich isotonic fluid leads to blood volume depletion with attendant low blood pressure and shock. Loss of bicarbonate and potassium leads to metabolic acidosis and potassium deficiency. The stools of cholera patients contain high concentrations of cholera vibrios (up to 10^8 bacteria per g), and they are highly infectious. When passed into the environment, they can contaminate water sources and food and may seed an environmental reservoir.

Virulence factors

At the molecular level, the pathogenesis of cholera is a multifactorial process and involves several genes encoding virulence factors that aid the pathogen in its colonisation, coordinated expression of virulence factors, and toxin action. In *V cholerae*, the major virulence genes required for pathogenesis are in clusters and can apparently propagate laterally and disperse among different strains. Genetic analyses have revealed the presence of two important genetic elements that distinguish a pathogenic *V cholerae* from an innocuous one. These are the previously called CTX genetic element, which is the genome of a lysogenic bacteriophage designated CTX Φ that carries the genes encoding cholera toxin, and the

vibrio pathogenicity island (VPI), which carries genes for the pilus colonisation factor TCP.^{82,94}

The typical CTX Φ genome has a modular structure composed of two functionally distinct domains, the core and RS2 regions.⁹⁴ CTX Φ was originally perceived to be a transposon-like genetic element. The core region encodes cholera toxin, which does not contribute to virion formation, and the other genes encode proteins (Psh, Cep, OrfU, and Ace) that are involved in phage packing and secretion, and one (Zot) required for CTX Φ assembly.⁹⁴ The products of *zot* and *ace* genes also show enterotoxic activity and increase short-circuit current across rabbit intestinal tissue in Ussing chambers.^{95,96} The RS2 region encodes genes required for replication (*rstA*), integration (*rstB*), and regulation (*rstR*) of CTX Φ .⁹⁷

Within *V cholerae* cells, the CTX Φ genome can exist either as a replicating plasmid or as a prophage integrated into the chromosome.⁹⁴ Under appropriate conditions, toxigenic *V cholerae* strains can be induced to produce extracellular CTX Φ particles.^{94,98} Cultures of *V cholerae* harbouring the replicating form of CTX Φ produce high titres of the phage in their supernatants. Non-toxigenic environmental strains can be converted by phage transduction with CTX Φ ,⁹⁸ and this event could conceivably take place in the gastrointestinal environment, yielding new toxigenic strains.⁹⁹

TCP mediates bacterial colonisation of the intestine by facilitating microcolony formation via pilus-mediated bacterial interactions and perhaps direct attachment to the intestinal brush border.¹⁰⁰ The genes for TCP form part of the 40 kb VPI segment that is generally absent from non-epidemic strains.¹⁰¹ Biogenesis of TCP requires the activities of at least 11 accessory proteins, most of which are encoded by genes located in the TCP operon.¹⁰² The structural features of the VPI include the presence of groups of virulence genes, a regulator of virulence genes, a transposase gene, and specific (*att*-like) attachment sites flanking each end of the island. The presence of an integrase with homology to a phage integrase gene suggests that the VPI was also derived from a bacteriophage.^{103,104} As remarkable examples of evolutionary coadaptation, the CTX Φ virion uses TCP as a receptor during infection.¹⁰² Colonisation is a prerequisite to establishing a productive infection. Other colonisation factors such as the mannose-fucose-resistant cell-associated haemagglutinin, the mannose-sensitive haemagglutinin, and some outer-membrane proteins are suspected from findings in animals to have roles in increasing adhesion and colonisation.¹⁰⁵⁻¹⁰⁷ The exact roles of these factors in the virulence of *V cholerae* in human beings are still uncertain, but the mannose-sensitive haemagglutinin type IV pilus has been identified as one factor involved in the adherence to the chitin of zooplankton.¹⁰⁸

The entire genome sequence of *V cholerae* O1 (biotype El Tor) was recently described.¹⁰⁹ The genome consists of two circular chromosomes.^{109,110} The large chromosome contains most of the genes that are required for growth and pathogenicity, and some of the components of several essential metabolic and regulatory pathways are on the small chromosome.

V cholerae can activate or inactivate a set of genes including those encoding colonisation factors or toxins as an appropriate response to changing environmental conditions. ToxR, a 32 kDa transmembrane protein, binds to a tandemly repeated 7 bp DNA sequence found upstream of the *cxA*B structural gene and increases transcription of this gene resulting in higher expression of cholera toxin. The coordinated regulation of several genes

through the *toxR* regulon shows that the organism has developed a mechanism of sampling and responding to its environment. ToxR regulates the expression not only of *ctxAB* but also of at least 17 distinct genes that constitute the ToxR regulon.¹¹¹⁻¹¹³ Except for the *ctxAB* genes, other genes in the ToxR regulon are controlled through another regulatory factor called ToxT, a 32 kDa protein. ToxR controls the transcription of the *toxT* gene, which encodes one of the AraC bacterial transcription activators. The resulting increased expression of the ToxT protein then leads to activation of other genes in the ToxR regulon. Thus, ToxR is at the top of the regulatory cascade that controls the expression of several other genes, and the expression of ToxR itself remains under the control of environmental factors.^{114,115}

The emergence of the O139 epidemic strain of *V cholerae* resulted from horizontal gene transfer of a fragment of DNA from another serogroup into a strain of the seventh pandemic *V cholerae* O1 El Tor strain. This transfer occurred in the region that brings about O-antigen biosynthesis.¹¹⁶⁻¹¹⁸ DNA hybridisation analysis of the O-antigen biosynthesis gene in O139 showed that it has homology with the gene of several non-O1 serogroups, but especially with serogroup O22. Thus, O22 is the likely origin of the genes for O139 biosynthesis.^{119,120} Molecular epidemiological studies support these findings and show that O139 strains have genetic backbones very similar to those of the O1 El Tor Asian seventh pandemic strains.¹²¹⁻¹²³ However, unlike *V cholerae* O1, serogroup O139 has a capsule distinct from the lipopolysaccharide antigens and has 3,6-dideoxyhexose (abequose or colitose), quinovosamine, and glucosamine, and traces of tetradecanoic and hexadecanoic fatty acids.¹²⁴

Ecology of *V cholerae*

The general assumption, until quite recently, was that cholera was spread only by infected people to other susceptible individuals via faecal contamination of water and food and that global movement of populations accounted for the global movement of the disease. Recent studies of the aquatic environment, however, have shown that *V cholerae*, including strains of O1 and O139, are normal inhabitants of surface water, particularly brackish waters, and survive and multiply in association with zooplankton and phytoplankton quite independently of infected human beings.¹²⁵⁻¹²⁸ Because global climate changes affect the growth of plankton, growth of the vibrios associated with plankton could also be modified. The continuing presence of cholera in the Indian subcontinent and the re-emergence of cholera in other continents may be highly dependent on environmental factors.^{28,129} The movement of the bacteria in association with plankton has led to the suggestion that ship ballast may be a cause of its global spread.¹³⁰

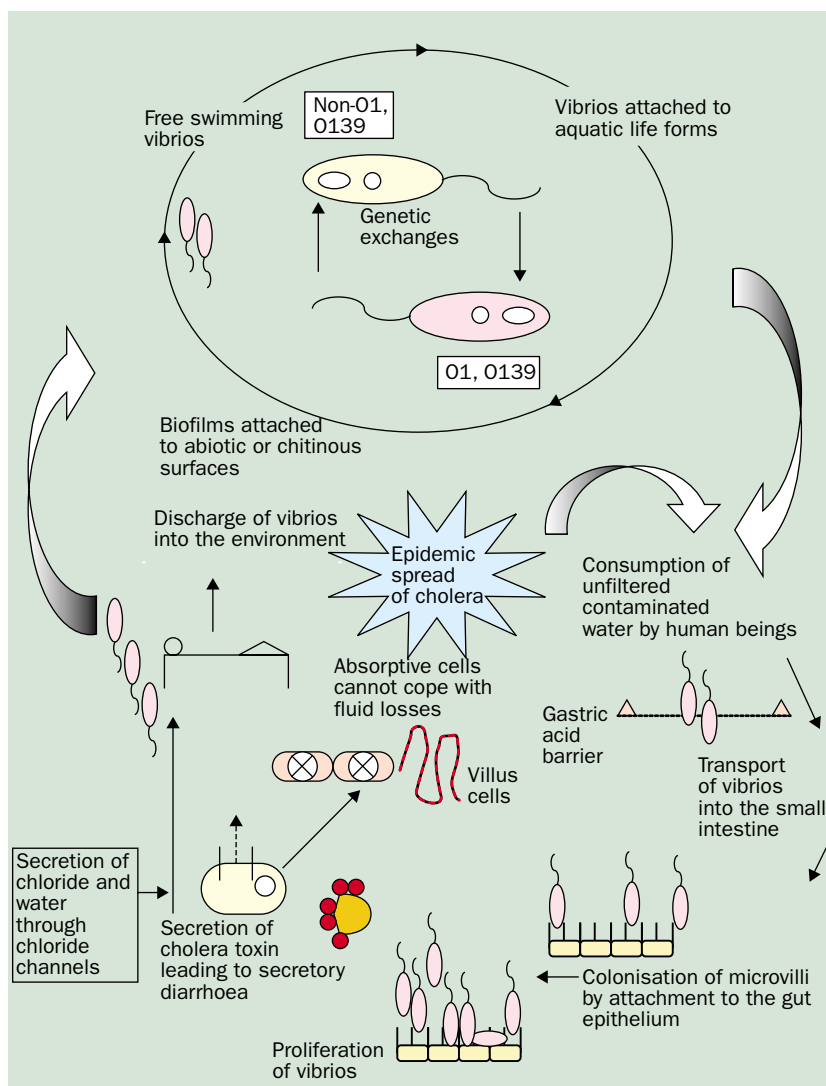


Figure 4: Life cycle of *V cholerae* involves both environmental and human segments, which sometimes intersect

The life cycle of *V cholerae* consists of two distinct phases (figure 4). Outside of the host and in the aquatic phase, *V cholerae* can be found as free swimming cells, attached to surfaces provided by plants, filamentous green algae, copepods, crustaceans, insects,^{129,131} and egg masses of chironomids.¹³² Biofilm formation¹³³ and entry into a viable but non-culturable state in response to nutrient deprivation¹³⁴ are thought to be important in facilitating environmental persistence within natural aquatic habitats during periods between epidemics.⁹⁹ Neither the genetic events that help the organism to lead a life in association with plankton nor the biofilm ecology of vibrios on abiotic surfaces are completely understood.

Although *V cholerae* is part of the normal estuarine flora, toxigenic strains are mostly isolated from the environment in areas probably contaminated by infected individuals. Environmental isolates from areas that are distant from regions of infection do not generally have the cholera toxin genes.¹³⁵

There are two crucial sequential steps in the evolution of a pathogenic *V cholerae*. First, strains have to acquire the VPI (which most environmental strains do not have); second, having acquired the CTX Φ receptor, the TCP-positive strains are infected with and lysogenised by CTX Φ .^{94,98,136} Experiments in animals have shown that the

intestinal milieu is the site where strains can acquire these mobile elements efficiently.^{94,137} Thus, *V cholerae* can be visualised as an autochthonous marine bacterium that colonises and thrives in the human gut during phases of infection and spends the time between epidemics in its “original” habitat, the estuary.

Prevention of cholera and vaccines

Contaminated food and water are the main vehicles of transmission of *V cholerae* and much can be done to keep transmission rates to a minimum. The measures include ensuring a safe water supply, (especially for municipal water systems), improving sanitation, making food safe for consumption by thorough cooking of high-risk foods (especially seafood), and health education through mass media. Some important messages for the media during outbreaks include the importance of purifying water and seafood, washing hands after defecation and before food preparation, recognition of the signs of cholera, and locations where treatment can be obtained to avoid delays in case of illness. The long-term prevention of cholera will require improved water and sanitation facilities, but these improvements are not happening rapidly in most regions where cholera is prevalent.

A killed injectable vaccine was developed shortly after *V cholerae* was discovered in the 1880s, and it was widely used throughout the world. Vaccination was even a requirement for international travellers in the mistaken belief that it might prevent international spread of cholera. This vaccine was probably appropriate for those who could afford it during the early part of the 20th century when treatment was ineffective and sanitation standards were low. However, it was not cost-effective as a public-health intervention because protection was short-lived (6 months), it was associated with painful local inflammatory reactions, and it did not prevent the spread of disease.¹³⁸ Vaccination was not practicable and was too expensive for people might benefit from it. Those who could afford it no longer needed it, and they did not like the side-effects. Thus, the whole-cell injectable vaccine is no longer recommended for any purpose, though it is still licensed.

New oral cholera vaccines promise substantial protection without side-effects. A killed oral vaccine (Dukoral) consists of killed *V cholerae* organisms along with the cholera B subunit, and the vaccine therefore stimulates both antibacterial and antitoxic immunity. Two doses are given 1–6 weeks apart.¹³⁹ The other vaccine (Orochol) is an avirulent mutant of *V cholerae*, strain CVD103HgR, given as a single-dose, lyophilised oral vaccine.¹⁴⁰ Both are licensed in several countries, but not yet in the USA.

Dukoral was effective in field trials in less developed countries,^{141,142} and it is now recommended for use in refugee settings at risk of cholera.¹⁴³ Its cost-effectiveness in endemic areas is still not known. Orochol is highly protective in volunteer studies,^{140,144} though its use in endemic areas is uncertain.¹⁴⁵ Other live and killed oral vaccines are also being developed that may become useful in the future.^{146–150} A major problem in the development of these new oral vaccines will be to make them sufficiently inexpensive and to develop a formulation that can be readily distributed to huge populations at risk. Booster doses will probably be needed for each of the new oral vaccines, and the formulations will need to be sufficiently simple that the vaccine might even be self-administered at times of risk.

The new oral vaccines will not prevent all cases of cholera because local intestinal immunity can be

overcome with a high inoculum, but they should lower the risk by as much as 80% if used regularly. Also, a vaccine programme could work synergistically with sanitation programmes; the inoculum needed to cause disease would be raised and the numbers of pathogenic organisms entering the environment would be decreased. Thus vaccines and sanitation programmes should not be viewed as alternative preventive strategies but as complementary, perhaps even synergistic, ones.

Conclusion

At the beginning of the 21st century, cholera remains an epidemic or endemic disease in much of the world. Research has revealed much about the pathogenesis and the genetics of *V cholerae*, and has provided simple and effective methods for treatment. New epidemic strains are likely to develop, evolve, and spread. *V cholerae* cannot be eradicated; it is a part of the normal flora and ecology of the surface water of our planet. Thus, we have to learn to coexist with the vibrios. An understanding of the ecology of the organism should help to limit the times that human beings come into contact with this super-pathogen.

Conflict of interest statement

None declared.

Acknowledgments

Our work was supported by the a grant from the National Institutes of Health (R01 AI39129) and by a cooperative agreement from US Agency for International Development (HRN-A-00-96-90005-00) and by core donors to the ICDDR,B. Current donors providing unrestricted support include the aid agencies of the governments of Australia, Bangladesh, Belgium, Canada, Japan, Kingdom of Saudi Arabia, the Netherlands, Sweden, Sri Lanka, Switzerland, and the USA. The funding sources had no involvement in the writing of the paper or decision to submit it for publication.

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