Cholera, Vibrio cholerae O1 and O139, and Other Pathogenic Vibrios

Richard A. Finkelstein

General Concepts

Cholera and Vibrio cholerae

Clinical Manifestations

Cholera is a potentially epidemic and life-threatening secretory diarrhea characterized by numerous, voluminous watery stools, often accompanied by vomiting, and resulting in hypovolemic shock and acidosis. It is caused by certain members of the species Vibrio cholerae which can also cause mild or inapparent infections. Other members of the species may occasionally cause isolated outbreaks of milder diarrhea whereas othersthe vast majorityare free-living and not associated with disease.

Structure, Classification, and Antigenic Types

Vibrios are Gram-negative, highly motile curved rods with a single polar flagellum. They tolerate alkaline media that kill most intestinal commensals, but they are sensitive to acid. Numerous free-living vibrios are known, some potentially pathogenic. Until 1992, cholera was caused by only two serotypes, Inaba (AC) and Ogawa (AB), and two biotypes, classical and El Tor, of toxigenic O group 1 V cholerae. These organisms may be identified by agglutination in O group 1-specific antiserum directed against the lipopolysaccharide component of the cell wall and by demonstration of their enterotoxigenicity. In 1992, cholera caused by serogroup O139 (synonym "Bengal"; the 139th and latest serogroup of V cholerae to be identified) emerged in epidemic proportions in India and Bangladesh. This serovar is identified by 1) absence of agglutination in O group 1 specific antiserum; 2) by agglutination in O group 139 specific antiserum; and 3) by the presence of a capsule.

Pathogenesis

Cholera is transmitted by the fecal-oral route. Vibrios are sensitive to acid, and most die in the stomach. Surviving virulent organisms may adhere to and colonize the small bowel, where they secrete the potent cholera enterotoxin (CT, also called "choleragen").
This toxin binds to the plasma membrane of intestinal epithelial cells and releases an enzymatically active subunit that causes a rise in cyclic adenosine 5'-monophosphate (cAMP) production. The resulting high intracellular cAMP level causes massive secretion of electrolytes and water into the intestinal lumen.

**Host Defenses**

Gastric acid, mucus secretion, and intestinal motility are the prime nonspecific defenses against *V* cholerae. Breastfeeding in endemic areas is important in protecting infants from disease. Disease results in effective specific immunity, involving primarily secretory immunoglobulin (IgA), as well as IgG antibodies, against vibrios, somatic antigen, outer membrane protein, and/or the enterotoxin and other products.

**Epidemiology**

Cholera is endemic or epidemic in areas with poor sanitation; it occurs sporadically or as limited outbreaks in developed countries. In coastal regions it may persist in shellfish and plankton. Long-term convalescent carriers are rare. Enteritis caused by the halophile *V* parahaemolyticus is associated with raw or improperly cooked seafood.

**Diagnosis**

The diagnosis is suggested by strikingly severe, watery diarrhea. For rapid diagnosis, a wet mount of liquid stool is examined microscopically. The characteristic motility of vibrios is stopped by specific antisomatic antibody. Other methods are culture of stool or rectal swab samples on TCBS agar and other selective and nonselective media; the slide agglutination test of colonies with specific antiserum; fermentation tests (oxidase positive); and enrichment in peptone broth followed by fluorescent antibody tests, culture, or retrospective serologic diagnosis. More recently the polymerase chain reaction (PCR) and additional genetically-based rapid techniques have been recommended for use in specialized laboratories.

**Control**

Control by sanitation is effective but not feasible in endemic areas. A good vaccine has not yet been developed. A parenteral vaccine of whole killed bacteria has been used widely, but is relatively ineffective and is not generally recommended. An experimental oral vaccine of killed whole cells and toxin B-subunit protein is less than ideal. Living attenuated genetically engineered mutants are promising, but such strains can cause limited diarrhea as a side effect. Antibiotic prophylaxis is feasible for small groups over short periods.

**Other Vibrio Infections**

Other serogroups of *V* cholerae may cause diarrheal disease and other infections but are not associated with epidemic cholera. *Vibrio parahaemolyticus* is an important cause of enteritis associated with the ingestion of raw or improperly prepared seafood. Other *Vibrio* species, including *V* vulnificus, can cause infections of humans and other animals including fish. *Campylobacter* species (formerly included with vibrios) can
cause enteritis. *C. pylori*, now known as *Helicobacter pylori*, is associated with gastric and duodenal ulcers (see Ch. 23).

**INTRODUCTION**

Vibrios are highly motile, gram-negative, curved or comma-shaped rods with a single polar flagellum. Of the vibrios that are clinically significant to humans, *Vibrio cholerae* O group 1, the agent of cholera, is the most important. *Vibrio cholerae* was first isolated in pure culture by Robert Koch in 1883, although it had been seen by other investigators, including Pacini, who is credited with describing it first in Florence, Italy, in 1854.

Cholera is a life-threatening secretory diarrhea induced by an enterotoxin secreted by *V. cholerae*. Cholera and the cholera enterotoxin are increasingly recognized as the prototypes for a wide variety of non-invasive diarrheal diseases, collectively known as the enterotoxic enteropathies; of these, diarrhea due to enterotoxigenic strains of *Escherichia coli* (see Ch. 26) is the most important. Cholera remains a major epidemic disease. There have been seven great pandemics. The latest, which started in 1961, invaded the Western Hemisphere (for the first time this century) with a massive outbreak in Peru in 1991. There have since been more than a million cases in Central and South America as well as a few imported cases in the U.S. and Canada. *V. cholerae* serogroup O139, which arose in October of 1992 in India and Bangladesh, may become the cause of the 8th great pandemic of cholera.

Other vibrios may also be clinically significant in humans, and some are known to cause diseases in domestic animals. Nonpathogenic vibrios are widely distributed in the environment, particularly in estuarine waters and seafoods. For this reason, isolation of a vibrio from a patient with diarrheal disease does not necessarily indicate an etiologic relationship.

*Vibrio Cholerae*

**Clinical Manifestations**

Following an incubation period of 6 to 48 hours, cholera begins with the abrupt onset of watery diarrhea (Fig. 24-1). The initial stool may exceed 1 L, and several liters of fluid may be secreted within hours, leading to hypovolemic shock. Vomiting usually accompanies the diarrheal episodes. Muscle cramps may occur as water and electrolytes are lost from body tissues. Loss of skin turgor, scaphoid abdomen, and weak pulse are characteristic of cholera. Various degrees of fluid and electrolyte loss are observed, including mild and subclinical cases. The disease runs its course in 2 to 7 days; the outcome depends upon the extent of water and electrolyte loss and the adequacy of water and electrolyte repletion therapy. Death can occur from hypovolemic shock, metabolic acidosis, and uremia resulting from acute tubular necrosis.
Structure, Classification, and Antigenic Types

The cholera vibrios are Gram-negative, slightly curved rods whose motility depends on a single polar flagellum. Their nutritional requirements are simple. Fresh isolates are prototrophic (i.e., they grow in media containing an inorganic nitrogen source, a utilisable carbohydrate, and appropriate minerals). In adequate media, they grow rapidly with a generation time of less than 30 minutes. Although they reach higher population densities when grown with vigorous aeration, they can also grow anaerobically. Vibrios are sensitive to low pH and die rapidly in solutions below pH 6; however, they are quite tolerant of alkaline conditions. This tolerance has been exploited in the choice of media used for their isolation and diagnosis.

Until 1992, the vibrios that caused epidemic cholera were subdivided into two biotypes: classical and El Tor. Classical V cholerae was first isolated by Koch in 1883. Subsequently, in the early 1900s, some vibrios resembling V cholerae were isolated from Mecca-bound pilgrims at the quarantine station at El Tor, in the Sinai peninsula, that had been established to try to control cholera associated with pilgrimages to Mecca. These vibrios resembled classical V cholerae in many ways but caused lysis of goat or sheep erythrocytes in a test known as the Gréig test. Because the pilgrims from whom they were isolated did not have cholera, these hemolytic El Tor vibrios were regarded as relatively insignificant except for the possibility of confusion with true cholera vibrios. In the 1930s, similar hemolytic vibrios were associated with relatively restricted outbreaks of diarrheal disease, called paracholera, in the Celebes. In 1961, cholera caused by El Tor vibrios erupted in Hong Kong and spread virtually worldwide. Although in the course of this pandemic most V cholerae biotype El Tor strains lost
their hemolytic activity, a number of ancillary tests differentiate them from vibrios of the classical biotype.

The operational serology of the cholera vibrios which belong in O antigen group 1 is relatively simple. Both biotypes (El Tor and classical) contain two major serotypes, Inaba and Ogawa (Fig. 24-2). These serotypes are differentiated in agglutination and vibriocidal antibody tests on the basis of their dominant heat-stable lipopolysaccharide somatic antigens. The cholera group has a common antigen, A, and the serotypes are differentiated by the type-specific antigens, B (Ogawa) and C (Inaba). An additional serotype, Hikojima, which has both specific antigens, is rare. V cholerae O139 appears to have been derived from the pandemic El Tor biotype but has lost the characteristic O1 somatic antigen; it has gained the ability to produce a polysaccharide capsule; it produces the same cholera enterotoxin; and it seems to have retained the epidemic potential of O1 strains.

FIGURE 24-2 Vibrio cholerae (O group 1 antigen).

Other antigenic components of the vibrios, such as outer membrane protein antigens, have not been extensively studied. The cholera vibrios also have common flagellar antigens. Cross-reactions with Brucella and Citrobacter species have been reported. Because of DNA relatedness and other similarities, other vibrios formerly called "nonagglutinable" are now classified as V cholerae. The term nonagglutinable is a misnomer because it implies that these vibrios are not agglutinable; in fact, they are not agglutinable in antisera against the O antigen group 1 cholera vibrios, but they are agglutinable in their own specific antisera. More than 139 serotypes are now recognized. Some strains of non-O group 1 V cholerae cause diarrheal disease by means of an enterotoxin related to the cholera enterotoxin and, perhaps, by other mechanisms, but these strains have not been associated with devastating outbreaks like those caused by the true cholera vibrios. Recently, vibrio strains that agglutinate in some O group 1 cholera diagnostic antisera but not in others have been isolated from environmental sources. Volunteer feeding experiments have shown that these atypical O group 1 vibrios are not enteropathogenic in humans. Recent studies using specific toxin gene probes indicate that these environmental isolates not only are nontoxigenic, but also do
not possess any of the genetic information encoding cholera toxin, although some isolates from diarrheal stools do.

The cholera vibrios cause many distinctive reactions. They are oxidase positive. The O group 1 cholera vibrios almost always fall into the Heiberg I fermentation pattern; that is, they ferment sucrose and mannose but not arabinose, and they produce acid but not gas. Vibrio cholerae also possesses lysine and ornithine decarboxylase, but not arginine dihydrolase. Freshly isolated agar-grown vibrios of the El Tor biotype, in contrast to classical V cholerae, produce a cell-associated mannose-sensitive hemagglutinin active on chicken erythrocytes. This activity is readily detected in a rapid slide test. In addition to hemagglutination, numerous tests have been proposed to differentiate the classical and El Tor biotypes, including production of a hemolysin, sensitivity to selected bacteriophages, sensitivity to polymyxin, and the Voges-Proskauer test for acetoin. El Tor vibrios originally were defined as hemolytic. They differed in this characteristic from classical cholera vibrios; however, during the most recent pandemic, most El Tor vibrios (except for the recent isolates from Texas and Louisiana) had lost the capacity to express the hemolysin. Most El Tor vibrios are Voges-Proskauer positive and resistant to polymyxin and to bacteriophage IV, whereas classical vibrios are sensitive to them. As both biotypes cause the same disease, these characteristics have only epidemiologic significance. Strains of the El Tor biotype, however, produce less cholera enterotoxin, but appear to colonize intestinal epithelium better than vibrios of the classical variety. Also, they seem somewhat more resistant to environmental factors. Thus, El Tor strains have a higher tendency to become endemic and exhibit a higher infection-to-case ratio than the classical biotype.

Pathogenesis

Recent studies with laboratory animal models and human volunteers have provided a detailed understanding of the pathogenesis of cholera. Initial attempts to infect healthy American volunteers with cholera vibrios revealed that the oral administration of up to 1011 living cholera vibrios rarely had an effect; in fact, the organisms usually could not be recovered from stools of the volunteers. After the administration of bicarbonate to neutralize gastric acidity, however, cholera diarrhea developed in most volunteers given 104 cholera vibrios. Therefore, gastric acidity itself is a powerful natural resistance mechanism. It also has been demonstrated that vibrios administered with food are much more likely to cause infection.

Cholera is exclusively a disease of the small bowel. To establish residence and multiply in the human small bowel (normally relatively free of bacteria because of the effective clearance mechanisms of peristalsis and mucus secretion), the cholera vibrios have one or more adherence factors that enable them to adhere to the microvilli (Fig. 24-3). Several hemagglutinins and the toxin-coregulated pili have been suggested to be involved in adherence but the actual mechanism has not been defined. In fact, there may be multiple mechanisms. The motility of the vibrios may affect virulence by enabling them to penetrate the mucus layer. They also produce mucinolytic enzymes, neuraminidase, and proteases. The growing cholera vibrios elaborate the cholera enterotoxin (CT or choleraagen), a polymeric protein (Mr 84,000) consisting of two major domains or regions. The A region (Mr 28,000), responsible for biologic activity of the enterotoxin, is linked by noncovalent interactions with the B region (Mr 56,000), which is composed of five identical noncovalently associated peptide chains of Mr
11,500. The B region, also known as choleragenoid, binds the toxin to its receptors on host cell membranes. It is also the immunologically dominant portion of the holotoxin. The structural genes that encode the synthesis of CT reside on a transposon-like element in the V cholerae chromosome, in contrast to those for the heat-labile enterotoxins (LTs) of E coli (Ch. 25), which are encoded by plasmids. The amino acid sequences of these structurally, functionally, and immunologically related enterotoxins are very similar. Their differences account for the differences in physicochemical behavior and the antigenic distinctions that have been noted. There are at least two antigenically related but distinct forms of cholera enterotoxin, called CT-1 and CT-2. Classical O1 V cholerae and the Gulf Coast El Tor strains produce CT-1 whereas most other El Tor strains and O139 produce CT-2. Vibrio cholerae exports its enterotoxin, whereas the E coli LTs occur primarily in the periplasmic space. This may account for the reported differences in severity of the diarrheas caused by these organisms.

Studies in adult American volunteers have shown that 5μg of CT, administered orally with bicarbonate, causes 1 to 6 L of diarrhea; 25μg causes more than 20 L.
FIGURE 24-3 Vibrio cholerae attachment and colonization in experimental rabbits. The events are assumed to be similar in human cholera. (A) Scanning electron microscopy during early infection. Curved vibrios adhering to epithelial surface.
Synthesis of CT and other virulence-associated factors such as toxin-coregulated pili are believed to be regulated by a transcriptional activator, Tox R, a transmembrane DNA-binding protein.

The molecular events in these diarrheal diseases involve an interaction between the enterotoxins and intestinal epithelial cell membranes (Fig. 24-4). The toxins bind through region B to a glycolipid, the GM1 ganglioside, which is practically ubiquitous in eukaryotic cell membranes. Following this binding, the A region, or a major portion of it known as the A1 peptide (Mr 21,000), penetrates the host cell and enzymatically transfers ADP-ribose from nicotinamide adenine dinucleotide (NAD) to a target protein, the guanosine 5'-triphosphate (GTP)-binding regulatory protein associated with membrane-bound adenylate cyclase. Thus, CT (and LT) resembles diphtheria toxin in causing transfer of ADP-ribose to a substrate. With diphtheria toxin, however, the substrate is elongation factor 2 and the result is cessation of host cell protein synthesis. With CT, the ADP-ribosylation reaction essentially locks adenylate cyclase in its "on mode" and leads to excessive production of cyclic adenosine 5-monophosphate (cAMP). Pertussis toxin, another ADP-ribosyl transferase, also increases cAMP levels, but by its effect on another G-protein, Gi (Fig. 24-5). The subsequent cAMP-mediated cascade of events has not yet been delineated, but the final effect is hypersecretion of chloride and bicarbonate followed by water, resulting in the characteristic isotonic voluminous cholera stool. In hospitalized patients, this can result in losses of 20 L or more of fluid per day. The stool of an actively purging, severely ill cholera patient can resemble rice water the supernatant of boiled rice. Because the stool can contain 108 viable vibrios per ml, such a patient could shed 2 X 10^12 cholera vibrios per day into the environment. Perhaps by production of CT, the cholera vibrios thus ensure their survival by increasing the likelihood of finding another human host. Recent evidence suggests that prostaglandins may also play a role in the secretory effects of cholera enterotoxin. Recent studies in volunteers using genetically-engineered Tox- strains of V cholerae have revealed that the vibrios have putative mechanisms in addition to CT for causing (milder) diarrheal disease. These include Zot (for Zonula occludens toxin) and Ace (for accessory cholera enterotoxin), and perhaps others, but their role has not been established conclusively. Certainly CT is the major virulence factor and the act of colonization of the small bowel may itself elicit an altered host response (e.g., mild diarrhea), perhaps by a trans-membrane signaling mechanism.
FIGURE 24-5 Comparison of activities of cholera enterotoxin (CT) with pertussis toxin (PT). The α-subunits of Gs and Gi, with GTP-binding sites, are ADP-ribosylated, respectively, by A1 peptide of CT or by the A subunit of PT, preventing, respectively, the hydrolysis of Gs-GTP to GDP or the responsiveness of Gi to inhibitory hormones, both effectively producing increases in adenylate cyclase activity. (Modified from Gill DM, Woolkalis M: Toxins which activate adenylate cyclase. CIBA Found Symp 112:57, 1985, with permission.)

Various animal models have been used to investigate pathogenic mechanisms, virulence, and immunity. Ten-day-old suckling rabbits develop a fulminating diarrheal disease after intraintestinal inoculation with virulent V cholerae or CT. Adult rabbits are relatively resistant to colonization by cholera vibrios; however, they do respond, with characteristic outpouring of fluid, to the intraluminal inoculation of live vibrios or enterotoxin in surgically isolated ileal loops. Suckling mice are susceptible to intragastric inoculation of vibrios and to orally administered toxin. Adult conventional mice are also susceptible to orally administered toxin, but resist colonization except in isolated intestinal loops. Interestingly, however, germ-free mice can be colonized for months with cholera vibrios. They rarely show adverse effects, although they are susceptible to cholera enterotoxin. Dogs have been used experimentally, although they are relatively refractory and require enormous inocula to elicit choleraic manifestations. Chinchillas also are susceptible to diarrhea following intraintestinal inoculation with moderate numbers of cholera vibrios. Infections initiated by extraintestinal routes of inoculation (e.g., intraperitoneal) largely reflect the toxicity of the lipopolysaccharide endotoxin. The intraperitoneal infection in mice has been used to assay the protective effect of conventional killed vibrio vaccines (no longer widely used).

Various animals, including humans, rabbits, and guinea pigs, also respond to intradermal inoculation of relatively minute amounts of CT with a characteristic delayed (maximum response at 24 hours), sustained (visible up to 1 week or more), erythematous, edematous induration associated with a localized alteration of vascular permeability. In laboratory animals, this response can be measured after injecting a
protein-binding dye, such as trypan blue, that extravasates to produce a zone of bluing at the site of intracutaneous inoculation of toxin. This observation has been exploited in the assay of CT and its antibody and in the detection of other enterotoxins.

In addition, because of the broad spectrum of activity of CT on cells and tissues that it never contacts in nature, various in vitro systems can be used to assay the enterotoxin and its antibody. In each, the toxin causes a characteristically delayed, but sustained, activation of adenylate cyclase and increased production of cAMP, and it may cause additional, readily recognizable, morphologic alterations of certain cultured cell lines. The cells most widely used for this purpose are Chinese hamster ovary (CHO) cells, which elongate in response to picogram doses of the toxin, and mouse Y-1 adrenal tumor cells, which round up. Cholera toxin has become an extremely valuable experimental probe to identify other cAMP-mediated responses. It also activates adenylate cyclase in pigeon erythrocytes, a procedure that was used by D. Michael Gill to define its mode of action.

These assays and models also have been applied in the study of an expanding number of CT-related and unrelated enterotoxins. These include the LTs of E coli, which are structurally and immunologically similar to it and are effective in any model that is responsive to CT. The family of small molecular weight heat-stable enterotoxins (ST) of E coli, which activate guanylate cyclase, and which are rapidly active in the infant mouse and certain other intestinal models, are clearly unrelated to CT. CT-related enterotoxins have been reported from certain nonagglutinable (non-O group I) Vibrio strains and a Salmonella enterotoxin was shown to be related immunologically to CT. CT-like factors from Shigella and V parahaemolyticus have thus far been demonstrated only in sensitive cell culture systems. Other enterotoxins and enterocytotoxins, which elicit cytotoxic effects on intestinal epithelial cells, also have been described from Escherichia, Klebsiella, Enterobacter, Citrobacter, Aeromonas, Pseudomonas, Shigella, V parahaemolyticus, Campylobacter, Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, C difficile, and staphylococci. Escherichia coli, some vibrio strains, and some other enteric bacteria produce cytotoxins that, like Shiga toxin of Shigella dysenteriae, act on Vero (African green monkey kidney) cells in vitro. These toxins have been called Shiga-like toxins, Shiga toxin-like toxins, Vero toxins, and Vero cytotoxins. The classic staphylococcal enterotoxins perhaps should more properly be called neurotoxins, as they seem to affect the central nervous system rather than the gut directly to cause fluid secretion or histopathologic effects.

**Host Defenses**

Infection with cholera vibrios results in a spectrum of responses. These range from no observed manifestations except perhaps a serologic response (the most common) to acute purging, which must be treated by hospitalization and fluid replacement therapy; this is the classic response. The reasons for these differences are not entirely clear, although it is known that individuals differ in gastric acidity and that hypochlorhydric individuals are most prone to cholera. Whether individuals differ in the availability of intestinal receptors for cholera vibrios or for their toxin has not been established. Prior immunologic experience of subjects at risk is certainly a major factor. For example, in heavily endemic regions such as Bangladesh, the attack rate is relatively low among adults in comparison with children. In neoepidemic areas, cholera is more frequent among the working adult population. Resistance is related to the presence of circulating
antibody and, perhaps more importantly, local immunoglobulin A (IgA) antibody against the cholera bacteria or the cholera enterotoxin or both. Intestinal IgA antibody can prevent attachment of the vibrios to the mucosal surface and neutralize or prevent binding of the cholera enterotoxin. For reasons that are not clear, individuals of blood group O are slightly more susceptible to cholera. Breastfeeding is highly recommended as a means of increasing immunity of infants to this and other diarrheal disease agents.

Recovery from cholera probably depends on two factors: elimination of the vibrios by antibiotics or the patient's own immune response, and regeneration of the poisoned intestinal epithelial cells. Treatment with a single 200-mg dose of doxycycline has been recommended. As studies in volunteers demonstrated conclusively, the disease is an immunizing process. Patients who have recovered from cholera are solidly immune for at least 3 years.

Cholera vaccines consisting of killed cholera bacteria administered parenterally have been used since the turn of the century. However, recent controlled field studies indicate that little, if any, effective immunity is induced in immunologically virgin populations by such vaccines, although they do stimulate preexisting immunity in the adult population in heavily endemic regions. Controlled studies have likewise shown that a cholera toxoid administered parenterally was ineffective in preventing cholera. Probably the natural disease should be simulated to induce truly effective immunity although a parenterally administered conjugate vaccine consisting of the polysaccharide of the vibrio LPS covalently linked to cholera toxin has given promising results in preliminary studies. Studies in volunteers have shown that orally administered, chemically mutagenized or genetically engineered mutants which do not produce CT or produce only its B subunit protein can induce immunity against subsequent challenge. However, most of these candidate vaccines also produce unacceptable side effects primarily mild to moderate diarrhea. An exception is strain CVD103-HgR (a mercury resistant A-B+ derivative of classical biotype Inaba serotype strain 569B). This strain has minimal reactogenicity but does not colonize well and therefore has to be given in higher doses. Field studies with this strain are in progress. Combined preparations of bacterial somatic antigen and toxin antigen have been reported to act synergistically in stimulating immunity in laboratory animals; that is, the combined protective effect is closer to the product than to the sum of the individual protective effects. However, a large field study evaluating such nonviable oral vaccines in Bangladesh revealed that neither the whole-cell bacterin nor the killed vibrios supplemented with the B-subunit protein of the cholera enterotoxin induced sufficient long term protection, especially in children, to justify their recommendation for public health use. No clear-cut advantage of the inclusion of the B-subunit was demonstrated.

In any case, even if these vaccines were effective, the requirement for large and repeated doses would make them too expensive for use in the developing areas that are usually afflicted with epidemic cholera. Moreover, they were clearly less effective in children the primary target population in heavily endemic areas. Neither the killed whole cell vaccine nor strain CVD103-HgR could be expected to protect against the new O139 serovar.

Epidemiology
Humans apparently are the only natural host for the cholera vibrios. Cholera is acquired by the ingestion of water or food contaminated with the feces of an infected individual. Previously, the disease swept the world in six great pandemics and later receded into its ancestral home in the Indo-Pakistani subcontinent. In 1961, the El Tor biotype (a subset distinguished by physiologic characteristics) of V. cholerae, not previously implicated in widespread epidemics, emerged from the Celebes (now Sulawesi), causing the seventh great cholera pandemic. In the course of their migration, the El Tor biotype cholera vibrios virtually replaced V. cholerae of the classic biotype that formerly was responsible for the annual cholera epidemics in India and East Pakistan (now Bangladesh). The pandemic that began in 1961 is now heavily seeded in Southeast Asia and in Africa. It has also invaded Europe, North America, and Japan, where the outbreaks have been relatively restricted and self-limited because of more highly developed sanitation. Several new cases were reported in Texas in 1981 and sporadic cases have since been reported in Louisiana and other Gulf Coast areas. This now endemic focus appears to be due to a clone which is unique from the pandemic strain. In 1991, the pandemic strain hit Peru with massive force and has since spread through most of the Western Hemisphere, causing more than a million cases. Fortunately, mortality has been less than 1 percent because of the effectiveness of oral rehydration therapy. The vibrios surprised us again, in 1992, with the emergence of O139 in India and Bangladesh. For a while it appeared that O139 would replace O1 (both classical and El Tor) but it has exhibited quiescent periods when O1 reemerges.

Cholera appears to exhibit three major epidemiologic patterns: heavily endemic, neoepidemic (newly invaded, cholera-receptive areas), and, in developed countries with good sanitation, occasional limited outbreaks. These patterns probably depend largely on environmental factors (including sanitary and cultural aspects), the prior immune status or antigenic experience of the population at risk, and the inherent properties of the vibrios themselves, such as their resistance to gastric acidity, ability to colonize, and toxigenicity. In the heavily endemic region of the Indian subcontinent, cholera exhibits some periodicity; this may vary from year to year and seasonally, depending partly on the amount of rain and degree of flooding. Because humans are the only reservoirs, survival of the cholera vibrios during interepidemic periods probably depends on a relatively constant availability of low-level undiagnosed cases and transiently infected, asymptomatic individuals. Long-term carriers have been reported but are extremely rare. The classic case occurred in the Philippines, where "cholera Dolores" harbored cholera vibrios in her gallbladder for 12 years after her initial attack in 1962. Her carrier state resolved spontaneously in 1973; no secondary cases had been associated with her well-marked strain. Recent studies, however, have suggested that cholera vibrios can persist for some time in shellfish, algae or plankton in coastal regions of infected areas and it has been claimed that they can exist in "a viable but nonculturable state."

During epidemic periods, the incidence of infection in communities with poor sanitation is high enough to frustrate the most vigorous epidemiologic control efforts. Although transmission occurs primarily through water contaminated with human feces, infection also may be spread within households and by contaminated foods. Thus, in heavily endemic regions, adequate supplies of pure water may reduce but not eliminate the threat of cholera.

In neoepidemic cholera-receptive areas, vigorous epidemiologic measures, including rapid identification and treatment of symptomatic cases and asymptotically infected
individuals, education in sanitary practices, and interruption of vehicles of transmission (e.g., by water chlorination), may be most effective in containing the disease. In such situations, spread of cholera usually depends on traffic of infected human beings, although spread between adjacent communities can occur through bodies of water contaminated by human feces. John Snow was credited with stopping an epidemic in London, England, by the simple expedient of removing the handle of the "Broad Street pump" (a contaminated water supply) in 1854, before acceptance of the "germ theory" and before the first isolation of the "Kommabacillus" by Robert Koch.

In such developed areas as Japan, Northern Europe, and North America, cholera has been introduced repeatedly in recent years, but has not caused devastating outbreaks; however, Japan has reported secondary cases and, in 1978, the United State experienced an outbreak of about 12 cases in Louisiana. In that outbreak, sewage was infected, and infected shellfish apparently were involved. Interestingly, the hemolytic vibrio strain implicated was identical to one that caused an unexplained isolated case in Texas in 1973.

**Diagnosis**

Rapid bacteriologic diagnosis offers relatively little clinical advantage to the patient with secretory diarrhea, because essentially the same treatment (fluid and electrolyte replacement) is employed regardless of etiology. Nevertheless, rapid identification of the agent can profoundly affect the subsequent course of a potential epidemic outbreak. Because of their rapid growth and characteristic colonial morphology, V cholerae can be easily isolated and identified in the bacteriology laboratory, provided, first, that the presence of cholera is suspected and, second, that suitable specific diagnostic antisera are available. The vibrios are completely inhibited or grow somewhat poorly on usual enteric diagnostic media (MacConkey agar or eosin-methylene blue agar). An effective selective medium is thiosulfate-citrate-bile salts-sucrose (TCBS) agar, on which the sucrose-fermenting cholera vibrios produce a distinctive yellow colony. However, the usefulness of this medium is limited because serologic testing of colonies grown on it occasionally proves difficult, and different lots vary in their productivity. This medium is also useful in isolating V parahaemolyticus. They can also be isolated from stool samples or rectal swabs from cholera cases on simple meat extract (nutrient) agar or bile salts agar at slightly alkaline pH values. Following observation of characteristic colonial morphology with a stereoscopic microscope using transmitted oblique illumination, microorganisms can be confirmed as cholera vibrios by a rapid slide agglutination test with specific antiserum. Classic and El Tor biotypes can be differentiated at the same time by performing a direct slide hemagglutination test with chicken erythrocytes: all freshly isolated agar-grown El Tor vibrios exhibit hemagglutination; all freshly isolated classic vibrios do not. In practice, this can be accomplished with material from patients as early as 6 hours after streaking the specimen in which the cholera vibrios usually predominate. However, to detect carriers (asymptomatically infected individuals) and to isolate cholera vibrios from food and water, enrichment procedures and selective media are recommended. Enrichment can be accomplished by inoculating alkaline (pH 8.5) peptone broth with the specimen and then streaking for isolation after an approximate 6-hour incubation period; this process both enables the rapidly growing vibrios to multiply and suppresses much of the commensal microflora.
The classic case of cholera, which includes profound secretory diarrhea and should evoke clinical suspicion, can be diagnosed within a few minutes in the prepared laboratory by finding rapidly motile bacteria on direct, bright-field, or dark-field microscopic examination of the liquid stool. The technician can then make a second preparation to which a droplet of specific anti-V cholerae O group 1 antiserum is added. This quickly stops vibrio motility. Another rapid technique is the use of fluorescein isothiocyanate-labeled specific antiserum (fluorescent antibody technique) directly on the stool or rectal swab smear or on the culture after enrichment in alkaline peptone broth. For cultural diagnosis, both nonselective and selective (TCBS) media may be used. Although demonstration of typical agglutination essentially confirms the diagnosis, additional conventional tests such as oxidase reaction, indole reaction, sugar fermentation reactions, gelatinase, lysine, arginine, and ornithine decarboxylase reactions may be helpful. Tests for chicken cell hemagglutination, hemolysis, polymyxin sensitivity, and susceptibility to phage IV are useful in differentiating the El Tor biotype from classic V cholerae. Tests for toxigenesis may be indicated.

Diagnosis can be made retrospectively by confirming significant rises in specific serum antibody titers in convalescents. For this purpose, conventional agglutination tests, tests for rises in complement-dependent vibrioidal antibody, or tests for rises in antitoxic antibody can be employed. Convenient microversions of these tests have been developed. Passive hemagglutination tests and enzyme-linked immunosorption assays (ELISAs) have also been proposed.

Cultures that resemble V cholerae but fail to agglutinate in diagnostic antisera (nonagglutinable or non-O group 1 vibrios) present more of a problem and require additional tests such as oxidase reaction, decarboxylases, inhibition by the vibriostatic pteridine compound 0/129, and the "string test." The string test demonstrates the property, shared by most vibrios and relatively few other genera, of forming a mucus-like string when colony material is emulsified in 0.5 percent aqueous sodium deoxycholate solution. Additional tests for enteropathogenicity and toxigenesis may be useful. Genetically based tests such as PCR are increasingly being used in specialized laboratories.

Control

Treatment of cholera consists essentially of replacing fluid and electrolytes. Formerly, this was accomplished intravenously, using costly sterile pyrogen-free intravenous solutions. The patient's fluid losses were conveniently measured by the use of buckets, graduated in half-liter volumes, kept underneath an appropriate hole in an army-type cot on which the patient was resting. Antibiotics such as tetracycline, to which the vibrios are generally sensitive, are useful adjuncts in treatment. They shorten the period of infection with the cholera vibrios, thus reducing the continuous source of cholera enterotoxin; this results in a substantial saving of replacement fluids and a markedly briefer hospitalization. Note, however, that fluid and electrolyte replacement is all-important; patients who are adequately rehydrated and maintained will virtually always survive, and antibiotic treatment alone is not sufficient.

Recently it has been recognized that almost all cholera patients and others with similar severe secretory diarrheal disease can be maintained by fluids given orally if the solutions contain a usable energy source such as glucose. Because of this discovery, packets containing appropriate salts are distributed by such organizations as WHO and
UNICEF to cholera-affected areas, where they are dissolved in water as needed. One such formulation, called ORS for oral rehydration salts, contains NaCl, 3.5 g; KCl, 1.5 g; NaHCO3, 2.5 g (or trisodium citrate, 2.9 g); and glucose, 20.0 g. This mixture is dissolved in 1 L of water and taken orally in increments. Flavoring may be added. Improved versions of ORS, including rice-based formulations that reduce stool output and can be made at home, have been recommended. Unfortunately, this technique, which will save countless millions of lives in developing countries, has not yet been widely accepted by practicing physicians in developed countries.

The possibility of pharmacologic intervention (e.g., a pill that will stop choleraic diarrhea after it has started), has been considered. Two drugs, chlorpromazine and nicotinic acid, have been effective in experimental animals, although the precise mechanism of action has yet to be defined.

Like smallpox and typhoid, cholera under natural circumstances appears to affect only humans; therefore, V cholerae as an etiologic entity could conceivably disappear with the last human infection. Nevertheless, the spectrum of cholera-like diarrheal diseases probably will persist for some time.

Cholera is essentially a disease associated with poor sanitation. The simple application of sanitary principles protecting drinking water and food from contamination with human feces would go a long way toward controlling the disease. However, at present, this is not feasible in the underdeveloped areas that are afflicted with epidemic cholera or are considered to be cholera receptive. Meanwhile, development of a vaccine that would effectively prevent colonization and manifestations of cholera would be extremely helpful. As indicated above, such vaccines are presently being tested. Antibiotic or chemotherapeutic prophylaxis is feasible and may be indicated under certain circumstances. It also should be mentioned that the incidence of cholera is significantly higher in formula-fed than in breast-fed babies.

Present information indicates that V parahaemolyticus enteritis could be almost completely prevented by applying appropriate procedures to prevent multiplication of the organisms in contaminated seafood, such as keeping it refrigerated continually.

Other Vibrio Infections

Other vibrios may be clinically significant also. These include non-O group 1 V cholerae. Vibrio parahaemolyticus, a halophilic (salt-loving) vibrio associated with enteritis is acquired by ingestion of raw or improperly cooked seafoods. Another halophilic vibrio, which ferments lactose and for this reason was called the L+ vibrio, has recently been identified as V vulnificus. It has been associated with wound infections as well as fatal septicemias. Other groups of vibrios, previously referred to as group F and EF-6, have recently been classified into species: V fluvialis, V hollisae, V furnissia, and V damsela. Vibrio mimicus is a recently described sucrose-negative species. Vibrio fetus, a group of anaerobic to microaerophilic spirally curved rods associated with venereally transmitted infertility and abortion in domestic animals, is now called Campylobacter jejuni and is considered to belong in the family Spirillaceae rather than in the family Vibrionaceae. Campylobacter jejuni has been associated with dysentery-like gastroenteritis, duodenal and gastric ulcers, as well as with other types of infection, including bacteremic and central nervous system infections in humans (see
Ch. 23). Another vibrio-like organism, Helicobacter pylori (formerly known as C pylori) causes gastritis and predisposes to duodenal ulcers and gastric cancer. Although some similarities in habitat and other properties occur, members of the family Vibrionaceae are separated taxonomically from members of the family Enterobacteriaceae. The oxidase test (vibrios are usually oxidase positive) is particularly useful. Other vibrios exist, and some of these may be responsible for diseases in fish and other lower animals. As vibrios are widely distributed in the environment, particularly in estuarine waters and in seafoods, reports of their isolation from patients with diarrheal disease do not necessarily always imply an etiologic relationship.

Cholera-like vibrios have been reported in Maryland's Chesapeake Bay but have not been associated with any human cases despite more than 15 years of extensive surveillance. These vibrios are probably nonpathogenic nonagglutinable (non-O group 1) vibrios, or the atypical O group 1 vibrios mentioned above, which do not contain the genes for toxin production, do not colonize, and are avirulent.

Relatively little is known about the epidemiology of nonagglutinable vibrios. When sought, these vibrios have been found widely in brackish surface waters (sewers, marshes, bogs, and coastal areas), and are generally more numerous in warmer months. They appear to be free-living aquatic organisms; whether particular subsets are potential pathogens is not yet clear. Strains isolated from humans with diarrheal disease more frequently give positive responses in assays for enterotoxins or enteropathogenicity, but the pathogenic mechanism of other isolates associated with shellfish remains undefined. An epidemiologic pattern is more evident with V parahaemolyticus, which is clearly part of the normal flora of coastal and estuarine waters throughout the world. Although originally recognized in Japan, V parahaemolyticus enteritis has been reported virtually worldwide within the last decade. Its reported frequency varies widely, partly because of inherent differences in distribution and partly because many laboratories do not use the appropriate culture medium (TCBS) to isolate these organisms. Two types of clinical syndromes, both usually self-limited, have been observed. The most common is a watery diarrhea, perhaps with associated abdominal cramps, nausea, vomiting, and fever, with a modal incubation period of 15 hours. A dysenteric syndrome with a short incubation period of 2 1/2 hours also has been described. In Japan, about 24 percent of reported cases of food poisoning are attributed to V parahaemolyticus. The disease occurs primarily during summer, possibly reflecting the increased presence of the organism in the marine environment during those months, as well as the enhanced opportunity for it to multiply in unrefrigerated foods. It appears to be transmitted exclusively by food, primarily raw or improperly prepared seafood. As growth of this organism is inhibited at temperatures below 15° C, rapid cooling and refrigeration of seafoods that are eaten raw would vastly reduce the incidence of disease. The organisms are killed by heating to 65° C for 10 minutes; therefore, properly handled cooked seafood should present no problem. The role played in virulence and pathogenesis by the thermostable direct hemolysin, which is responsible for the positive Kanagawa phenomenon (a hemolytic reaction around colonies growing on a particular blood agar medium), is not yet fully defined. This hemolysin is clearly associated with pathogenicity, but whether it is merely an associated marker or intimately involved in the disease process awaits further research. Be this as it may, only strains that possess the Kanagawa hemolysin are considered pathogenic. In laboratory studies, the isolated hemolysin has been reported to be cytotoxic, cardiotoxic, and lethal.
REFERENCES


Wachsmuth IK, Blake PA, Olsvik O. Vibrio cholerae and Cholera: Molecular to Global Perspectives. ASM Press, Washington, DC, 1994
