Vibrio cholerae - A Review

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Abstract

Vibrio cholerae is a facultative anaerobic, Gram negative, non-spore forming curved rod, about 1.04-1.06 µm long. It is a facultative human pathogen found in coastal waters that causes the acute gastrointestinal disease, cholera, a major health threat in poor nations. It is widely acknowledged as one of the most important water borne pathogen of worldwide economic significance. Sea foods and water is the most common vehicle for this infection in humans. It has been isolated from wide variety of samples such as seawater, sediments, plankton, finfish and shellfishes of coastal and estuarine environments. Cholera pathogenesis is a complex process and involves synergistic action of several genes. CT is considered the most important epidemic marker among various toxins produced by *V.cholerae*. Detection of *V.cholerae* from food stuffs is problematic, since they are present at low level together with large number of competing microflora and also they may be injured by different food processing methods.

Keywords: Diarrhoea, Food, Water borne disease, Sea Food, Aerobic organism.

Introduction

Cholera, an enteric diarrheal disease caused by the gram-negative bacterium *Vibrio cholerae*, continues to be a worldwide health concern. Two distinctive epidemiologic features of cholera are its tendency to appear in explosive outbreaks, often starting in several distinct foci simultaneously, and its propensity to cause true pandemic that progressively affect many countries in multiple continents over the course of many years (Kaper *et al*., 1995). *V. cholerae*, the causative agent of cholera in humans, is classified into two serotypes: O1 and non O1 (Chatterjee and Maiti, 1984). The O1 serogroup of *V. cholerae* is further classified into two biotypes, namely, the classical and El Tor biotypes. In 1993, *V. cholerae* serogroup O139 made an explosive appearance and caused a severe epidemic in the Indian continent (Ramamurthy et al., 1993). The major surface antigen employed in characterization of *V. cholerae* is the O antigen. According to the typing scheme of Sakazaki and Shimada, there are 139 different O groups. *V. cholerae* O1 and O139 are known to be the dominating and pathogenic strains.

However, the pathogenicity of *Vibrio cholerae* is chiefly associated with the secretion of the Cholera toxin (CTX), which is a protein complex. CTX is responsible for the harmful effects of cholera infection. The structure of CTX is typical of the A-B subunit group of toxins in which each of the subunits has a specific function. The B subunit serves to bind the holotoxin to the eukaryotic cell receptor, and the A subunit possesses a specific enzymatic function that acts intracellular. Recent studies of the aquatic environment have shown that *V. cholerae*, including strains of O1and 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 (Huq *et al*., 1983).

Etiological studies on acute diarrhoeal diseases in gangetic plain areas have shown that gastroenteritis caused by *V. cholerae* ranks first in terms of incidence followed by *V. parahaemolyticus* in India (Sakazaki *et al.*, 1971) and other developing countries (Albert *et al.*, 1993). Cholera has been categorized as one of the emerging and re-emerging infections in developing countries (Satcher, 1995) and is classified as Category B bioterrorism by Centre for Disease Control and Prevention (WHO, 2008).

The Centre for Disease Control and Prevention (CDC) estimated that 76 million food borne illnesses occurred in the United States with 325,000 hospitalizations and 5,200 deaths (Mead *et al.*, 1999).

History and Epidemiology of the cholera

Cholera is one of the oldest and best understood of the epidemic- prone disease. The ancestral home of cholera is thought to be the Ganges delta on the Indian

subcontinent, where epidemic of cholera like disease were described as far back as the 16th century (Kindhauser, 2003). John Snows observations on cholera during 1848 London epidemic helped to establish concept of a living agent, as cause of disease and supported the theory of waterborne transmission of cholera (Winkelstein, 1995). In 1883, during the fifth pandemic Robert Koch, successfully isolated the agent of cholera and suggested the existence of CT in 1884 (Finkelstein, 2000). In 1953, SN De (De and Chatterjee, 1953), bacteriologist in Calcutta discovered the crude cholera toxin, responsible for stimulating fluid secretion from the small intestine.

There have been seven pandemic of cholera in recorded history. The classical *V. cholerae* O1was responsible for the fifth and sixth pandemic and is believed to have been associated with the earlier pandemic as well, although there is no hard evidence (Baruda, 1992). The causative agent of the seventh pandemic is the E1Tor biotype (Samadi *et al*., 1983). In December, 1992, a large epidemic of cholera began in Bangladesh. The new serogroup has been categorized as *V. cholerae* O139, with synonym Bengal to refer to it first isolation from areas surrounding the Bay of Bengal (Shimada *et al.*, 1993) possibly working the beginning of the eighth pandemic (Swerdlow and Ries, 1993).

Cholera has re-emerged as a major infectious disease in recent past, with a global increase in its incidence. In 1994, cholera cases were notified from 94 countries- the highest ever number of countries in one year (WHO, 1995). Cholera is one of the three diseases requiring notification to WHO under the International Health Regulation. It is endemic throughout much of the African continent, where it thrives under conditions of poor sanitation and waste disposal. In 2006, the total number of cases reported in Africa accounted for 99% of the global total. Africa has been the continent with the highest number of officially reported cholera cases since 1996 (Kindhauser, 2003). Cholera remains a global threat to public health, especially in developing countries (WHO, 2008).

The World Health Organization (WHO) (http://www.who.int/wer/2007) reported a 30% increase in cases of cholera worldwide between 2004 (101,383 cases) and 2005 (131,943 cases), and a further 79% increase between 2005 and 2006 (236,860 cases), whereas the number of countries reporting cases has remained constant. At the same time, the global case fatality rate rose from 1.72% in 2005 to 2.66% in 2006.

Pathogenicity for humans and Virulence factors

Infection due to *Vibrio cholerae* begins with the ingestion of contaminated water or food. After passage through the acid barrier of the stomach, the organism colonizes the epithelium of the small intestine by means of the toxin-co regulated pili (Taylor *et al*., 1987) and possibly other colonization factors such as the different haemagglutinins, accessory colonization factor and core encoded pilus, all of which are thought to play a role. Cholera enterotoxin produced by the adherent vibrios is secreted across the bacterial outer membrane into the extracellular environment and disrupts ion transport by intestinal epithelial cells. The subsequent loss of water and electrolytes leads to the severe diarrhea characteristic of cholera.

The major virulence-associated factors are present in cluster (Hacker *et al*., 1997) with at least three regions in *V.cholerae* chromosome. The first is the CTX genetic element (Mekalanos, 1985) which has been reported to compromise the genome of a filamentous bacteriophage (CTX ?) (Waldor and Mekalanos, 1996). The second region is a large pathogenicity island for *V.cholerae* (VPI) (Karaolis *et al.*, 1998) that encode a toxin regulated pilus (TCP) gene cluster, a type IV pilus that function as an essential colonization factor (Taylor *et al*., 1987) and act as CTX ? receptor (Waldor and Mekalanos, 1996). The third gene cluster, the RTX gene cluster in *V. cholerae* encodes the presumptive cytotoxin (rtxA), an acyltransferase (rtxC), and an associated ATP-binding cassette transporter system (rtxB and rtxD, two proteins for toxin transportation) (Lin *et al.*, 1999). *V. cholerae* virulence cassette or CTX element corresponds to genome of CTX ?, a lysogenic filamentous bacteriophage has given a way for emergence of new clones of toxigenic *V. cholerae* strains (Waldor and Mekalanos, 1996).

CT is clearly the most important causative factor in the disease cholera, CT deficient isolates of *V. cholerae* also elicit mild to severe diarrhea and other reactogenic symptoms in human indicating that other toxins are likely to contribute to pathogenesis of the disease (Coster *et al*., 1995). The signs and symptoms of cholera are caused by cholera toxin (CT) or choleragen, a protein enterotoxin (Kaper *et al*., 1995 and Albert and Morris, 1999) produced by pathogenic *V. cholerae* (Herrignton *et al*., 1988). The structure of CT is typical of A-B subunit group toxins. An active, (A1) subunit of 23500 Da and a bridging piece (A2) of 5500 Da that links A1 to the 5B subunits (Guidolin and Manning, 1987). The A subunit functions for adenylate

cyclase activation in small intestinal epithelial cells, leading to the loss of fluid and electrolytes. The 5 binding (B) subunits of 11500 Da each serves to bind the toxin to the epithelial cell surface receptor, GM1. The genes expressing A and B subunits are designated ctxA and ctxB, respectively, and are expressed as a single transcriptional unit (Mekalanos *et al*., 1983). Toxin production does not correlate with serotype (Kaper *et al*., 1981). The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface intestinal mucosal cells (Guidolin and Manning, 1987).

Although not fully characterized, other toxins produced by *V.cholerae* include the shiga-like toxin (OBrien *et al.*, 1984), a heat-stable enterotoxin (Takeda *et al*., 1991), new cholera toxin (Sanyal *et al.*, 1983), sodium channel inhibitor (Tamplin *et al.*, 1987), thermostable direct haemolysin-like toxin (Nlishibuchi *et al*., 1992), and a cell-rounding cytotoxic enterotoxin known as the non-membrane-damaging cytotoxin (Saha and Nair, 1997).

Clinical Manifestations

Infection due to *V.cholerae* begins with the ingestion of food or water contaminated directly or indirectly with faeces or vomitus of infected person (CDC, 2003).

Infection of approximately 104-106 *V.cholerae* O1 organisms is likely to produce clinical cholera (Cash *et al.*, 1974). A dose of V.cholerae O1 (106 cells) was normally needed to cause diarrhea (Levine *et al*., 1981).

The incubation period for cholera ranges from a few hours to five days, usually two to three days followed by acute watery diarrhoea, often associated with vomiting, muscle cramps and complications related to severe dehydration. The water diarrhoea is speckled with flakes of mucus and epithelial cells (rice-water stool). Cardiac complications and circulatory failure occur due to loss of potassium. In severe cases, fluid loss of 500-1000 ml, an hour can occur, which may result in death in less than 24 hrs (Weinke *et al*., 2008) if untreated, with high (50- 60%) mortality rate (Guidolin and Manning, 1987).

Cholera enterotoxin is major virulence factor responsible for profuse diarrhoea, typically known as cholera gravis, characterized by profuse watery diarrhoea, vomiting and dehydration, often associated with leg cramps due to electrolyte imbalance leading to severe dehydration and death (Choopun *et al.*, 2002).

V. cholerae is non invasive enterotoxigenic organism causing gastroenteritis, whereas non O1 is associated with extraintestinal infection with septicemia, wound infection, ear infection, cellulitis, peritonitis, necrotizing fascitis, cholecystitis, endophthalmitis and meningitis (Yang *et al*., 2008). These symptoms are seen in patients with diabetes mellitus, chronic renal failure and dialysis, immunocompromized patient and post splenectomy.

Water borne infection

Cholera is usually transmitted by ingestion of contaminated water and sewage contaminated water remains the primary vehicle for cholera outbreaks (Tauxe and Blake 1992). Sewage contamination of ground water in Delhi was responsible for the epidemic of *V.cholerae* (Pathak *et al*., 1993). The presence of critical virulence genes in environmental strains of V. cholerae cultured from three different freshwater lakes and ponds in Eastern part of Calcutta, India (Chakraborty *et al*., 2000). Seawater and plankton samples from Peru were positive for *V. cholerae* O1 and found to contain ctx toxin. Mourino-Perez *et al*. (2003) found that dissolved organic matter during intense phytoplankton blooms has supported growth of *V. cholerae* in seawater. The toxigenic *V. cholerae* is a native flora of the aquatic environment which is transmitted through drinking water and still remains the leading cause of morbidity and mortality in many developing countries including Thailand (Chomvarin *et al*., 2007).

Food borne infection

a. Fruits and vegetables: In many countries, the practice of fertilizing gardens with untreated night soil and the habit of consuming uncooked vegetables have often resulted in cholera outbreaks. Vegetables may be contaminated during washing with polluted water. This can also occur when contaminated water is injected into fruits, such as watermelons, to preserve their weight and taste (Feachem, 1981). The pH of a specific fruit is an important factor that influences contamination by *V. cholerae*. Sour fruits such as lemons and oranges, with lower pH (below 4.5) do not support the growth of *V. cholerae*, and, thus, do not pose risk of cholera transmission. Fruit pulp and concentrate preserved in cans are also less likely to be contaminated if they have an acidic pH. Spices, including raw onions and garlic, can support the survival of *V. cholerae* for 2-3 days at ambient temperature (Felsenfeld, 1967).

b. Seafoods: The importance of fish and shellfish as a vehicle of cholera has been recognized by early observers. Fishes are likely to be contaminated by *V.*

cholerae when the surrounding water is contaminated by the sewage or other environmental sources of *V. cholerae* O1. It has been shown that *V. cholerae* can survive in seawater in association with zooplankton (copepods). Zooplankton secretes a self-protective coat of chitin that can be dissolved by chitinase, an enzyme produced by *V. cholerae* O1. Seafoods, including mollusks, crustaceans, crabs, and oysters, feed on plankton and can become infected with *V. cholerae*. Once infected, particularly clams, and oysters can harbour *V. cholerae* for weeks, even if refrigerated (Depaola, 1981). In crabs, the organisms can rapidly multiply at ambient temperature, and boiling for less than 10 minutes or steaming for less than 30 minutes does not completely kill *V. cholerae* (Huq *et al*., 1983).

c. Fish: Raw fish consumption was the cause for cholera outbreaks in Japan as early as 1886 (Donitz, 1892) and in Philippines in 1908 (Heiser, 1908). *V. cholerae* was isolated from salted fish responsible for cholera outbreaks in Guan during 1974 (Kuberski et al., 1979). Feldhusen (2000) reported that raw fish was initially implicated epidemiologically as a source of transmission of *V. cholerae* in the South American epidemic in 1991. *V.cholerae* O1 was present in 0.2% of raw fishery products, whereas *V. cholerae* non O1 was present in 26.3% of raw and 12.14% of frozen products in Kerala and Tamil Nadu coasts during 1986-87 (Verma *et al*., 1989). Fish samples from fresh as well as marine waters carry V.cholerae and it has been reported that fish intestine contained 5 X 103 *V. cholerae* cfu/g (Senderovich *et al*., 2010)

d. Shellfish: Ingestion of aquatic vegetation or sewage contamination of water are the main contamination sources of *V. cholerae* in shellfish (Colwell, 1990). Garate Lizarranga *et al.* (2006) reported that *V. cholerae* adhere strongly to shellfishs digestive tract and cannot be removed efficiently by rinsing the shellfish or depuration. Gholami *et al.* (1998) studied the risk factor of *V. cholerae* among renal diseased patients and reported that the infections were usually acquired by raw shellfish which has damaged the kidney.

e. Crabs: Inadequately steamed crabs infected with *V. cholerae* were responsible for outbreaks of cholera in Louisiana, USA (Blake *et al*., 1980). In crabs, the organisms can rapidly multiply at ambient temperature and boiling for less than 10 minutes or steaming for less than 30 minutes does not completely kill V. cholerae (Depaola, 1981).

f. Oysters: *V. cholerae* in oysters can survive for weeks during storage under refrigeration (Depaola, 1981). Huq *et al.* (1983) reported that oysters get the infection of *V. cholerae* due to feeding on plankton to which *V. cholerae* organisms are attached due to production of chitinase. Consumption of raw oysters correlated strongly with gastrointestinal infections in which *V. cholerae* has been implicated as causative agent (Rippey, 1994). It has been reported that *V. cholerae* exist in water with a salinity of 3-8% only (Sousa *et al*., 2004).

g. Dairy Products: *V. cholerae* O1 can survive for more than two weeks in different dairy products, including milk, milk products, soft deserts, and cakes. Addition of sugar and eggs enhances bacterial survival. Although *V. cholerae* is killed by pasteurization of milk, the organisms can persist in raw milk as long as four weeks, even if refrigerated (Felsenfeld, 1967).

h. Poultry and meat: Contamination of meat of animal origin occurs exogenously during processing, cooking, storage or consumption. It has been shown that *V. cholerae* can live and grow on cooked chicken, an increase in numbers of *V. cholerae* from 103 to 106 within 16 hours has been demonstrated (Kolvin and Roberts, 1982). An early observation by Seligmann indicates that consumption of improperly cooked horsemeat was incriminated in a small outbreak of cholera in Berlin in 1918 (Seligman, 1918). The meat had been prepared by an infected butcher who succumbed to cholera the next day. There are many other types of food that may be contaminated with *V. cholerae*. *V. cholerae* can survive on cooked rice, potato, eggs, and pasta for up to 5 days, and can also survive in spices, including pepper and cinnamon, for up to several days (PAHO, 1991).

Diagnosis

The conventional isolation procedure includes growth in enrichment broth like Alkaline Peptone water or Luria broth (LB) at 37 ºC for 18-24 hr to increase the ratio of *V. cholerae* to competitor organisms and plating on selective medium, TCBS i.e. Thiosulfate-Citrate-Bile salts-Sucrose agar (Finkelstein, 1988) for 24-48 hr at 37 ºC. *V. cholerae* ferment sucrose and produce yellow colonies. So, this medium is widely used for isolation of *V. cholerae*. These colonies are submitted to the oxidase test for the identification of *V. cholerae* complemented by biochemical and serological tests (Sack *et al*., 1980).

Prevention and Control

Since the infection primarily occurs via faeco-

oral route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine treated water and personnel hygiene measures like washing hands after toilet visits.

Therefore considering the severity of infections, *V. cholerae* should be given considerable preference in diagnosing the infections and eliminating the transmission.

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