

Cholera Outbreaks in the El Tor Biotype Era and the Impact of the New El Tor Variants

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Abstract *Vibrio cholerae* O1, the causative agent of the disease cholera, has two biotypes namely the classical and El Tor. Biotype is a subspecific taxonomic classification of *V. cholerae* O1. Differentiation of *V. cholerae* strains into biotype does not alter the clinical management of cholera but is of immense public health and epidemiological importance in identifying the source and spread of infection, particularly when *V. cholerae* is first isolated in a country or geographic area. From recorded history, till date, the world has experienced seven pandemics of cholera. Among these, the first six pandemics are believed to have been caused by the classical biotype whereas the ongoing seventh pandemic is caused by the El Tor biotype. In recent years, new pathogenic variants of *V. cholerae* have emerged and spread throughout many Asian and African countries with corresponding cryptic changes in the epidemiology of cholera. In this chapter, we describe the outbreaks during the seventh pandemic El Tor biotype era spanning more than five decades along with the recent advances in our understanding of the development, evolution, spread, and impact of the new variants of El Tor strains.

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1 Introduction

Cholera, an intestinal infection spread by contaminated water and food, is a substantial health burden in countries where sanitation and availability of clean drinking water is limited. Recent years have witnessed a remarkable resurgence in the global incidence of cholera. The devastating and ongoing cholera outbreak in Haiti from 2010, for the first time in almost a century, placed this ancient scourge at the forefront of the global public health agenda. This dreadful diarrheal disease is caused by the Gram-negative toxigenic bacterium *Vibrio cholerae* O1 and O139. Based on certain phenotypic and genetic properties, *V. cholerae* O1 can be divided into two biotypes; classical and El Tor. From recorded history, till date, the world has experienced seven pandemics of cholera. Among these, the first six pandemics are believed to have been caused by the classical biotype whereas the ongoing seventh pandemic is caused by the El Tor biotype. In recent years, new pathogenic variants of *V. cholerae* have emerged and spread throughout many Asian and African countries with corresponding cryptic changes in the epidemiology of cholera. These strains include the Matlab variants from Bangladesh, the Mozambique variants, the altered El Tor type from various parts of the world, and very recently the Haitian variants of the El Tor biotype. These variants display a mix of phenotypic and genotypic traits from the two main biotypes, suggesting that they are genetic hybrids. Classical and El Tor biotypes have been the most epidemiologically successful cholera strains during the past century, and it is believed that the new variants are likely to develop successfully in a manner similar to these biotypes. Here, we describe the outbreaks during the seventh pandemic El Tor biotype era along with the recent advances in our understanding of the development, evolution, spread, and impact of the new variants of El Tor strains.

2 Biotypes of *Vibrio cholerae*

Biotype is a subspecific taxonomic classification of *V. cholerae* O1. Differentiation of *V. cholerae* strains into biotype does not alter the clinical management of cholera but is of immense public health and epidemiological importance in identifying the source and spread of infection, particularly when *V. cholerae* is first isolated in a country or geographic area. *V. cholerae* strains are classified into serogroups on the basis of epitopic variations in the cell surface lipopolysaccharide (Yamai et al. 1997), which identifies over 200 serogroups. However, the strains that have epidemic and pandemic potential belong to only two serogroups namely, O1 and O139. *Vibrio cholerae* O1 has two well-established biotypes, namely, classical and El Tor, that are differentiated primarily based on number of phenotypic traits like susceptibility to polymyxin B, chicken cell (erythrocytes) agglutination (CCA), hemolysis of sheep erythrocytes, Voges–Proskauer (VP) test, which measures the production of acetylmethylcarbinol, and phage susceptibilities (Faruque et al. 1998; Kaper et al. 1995). Conventionally, at least two or more of the phenotypic tests mentioned above should be included to determine the biotype, since results can vary between individual isolates. While El Tor strains are resistant to polymyxin B (50U), classical strains are susceptible to this antibiotic. El Tor strains yield a positive reaction in VP test indicating that the strains produce 2, 3-butanediol instead of producing organic acids as their fermentation end product and thus grow to much higher densities in media containing carbohydrates (Yoon and Mekalanos 2006); classical strains give negative VP reaction.

Another distinction among El Tor strains is the ability to agglutinate erythrocytes from several animal species like chicken, goat, or sheep, though classical strains are devoid of this capability. It has also been reported that the chicken blood cell-positive biotype of *V. cholerae* strains are observed to attach to the scattered erythrocytes and to propel them with a characteristic flipping motion when observed under a dark-field microscope (Mackowiak and Huq 1974). Based on phage-typing, El Tor strains are susceptible to El Tor-specific bacteriophage V, but are resistant to classical bacteriophage IV whereas classical strains show the reverse traits (Mukerjee 1963). El Tor strains were identified historically by its ability to hemolyze sheep erythrocytes, while classical strains were nonhemolytic. But by 1972, nearly all El Tor isolates become nonhemolytic worldwide (Barrett and Blake 1981). Exceptions are found in isolates from the U.S. Gulf Coast and from Australia (Barrett and Blake 1981). Thus, hemolysis of erythrocytes continues to be a useful phenotypic characteristic for differentiating the Gulf Coast clones of *V. cholerae* O1 El Tor from those isolated in the rest of the world, including Latin America.

Comparative genetic analyses have recently revealed a high degree of conservation among diverse strains of *V. cholerae* but have also shown genes that differentiate classical biotype from El Tor biotype (Dziejman et al. 2002). Molecular biotyping of *V. cholerae* O1 using multiplex PCR (*ctxA–tcpA*) exploits the nucleotide sequence differences of the major subunit protein of the toxin

coregulated pilus (TCP) gene (*tcpA*) to differentiate between classical and El Tor biotypes (Keasler and Hall 1993). Only in toxigenic *V. cholerae* O1 El Tor and O139 strains, cholera toxin prophage region (CTX Φ) is often flanked by an element termed RS1 containing *rstC* gene (Waldor et al. 1997). The only difference between RS1 and RS2 is the presence of *rstC* gene in RS1 alone (Waldor et al. 1997; Davis et al. 2000). Another virulence-associated protein known as repeat in toxin (RTX) encoded by a cluster of genes of 10 kb size, comprising four ORFs, *rtxABCD*, of which the *rtxC* gene has been observed only in El Tor biotype (Lin et al. 1999). Nucleotide sequence comparison of hemolysin encoding *hlyA* gene from classical and El Tor strains reveal the presence of an 11-base-pair deletion in classical strains that results in a truncated protein product of 27 kDa in classical strains rendering it nonhemolytic, whereas in El Tor strains the HlyA is intact 82 kDa and biologically active (Rader and Murphy 1988). On the basis of differences in the sequences of *hlyA* genes, a 19-base-pair oligodeoxynucleotide probe has been developed to distinguish between the two biotypes of *V. cholerae* serogroup O1 (Alm and Manning 1990). This gene marker was found to be very useful to differentiate the biotypes than the other commonly used methods, which are less reliable and often difficult to interpret (Alm and Manning 1990). Recently, comparative genomic studies using a *V. cholerae* DNA microarray on 11 epidemic isolates identified two regions, *Vibrio* seventh pandemic island I (VSP-I), encompassing VC0175–VC0185 and VSP-II, encompassing VC0490–VC0497, that were found exclusively among El Tor biotype isolates (Dziejman et al. 2002). Subsequently, it was shown that the VSP-II region actually encompassed a 26.9 kb region (VC0490–VC0516) in *V. cholerae* biotype El Tor and O139 serogroup isolates (O’Shea et al. 2004). Besides these phenotypic and genotypic differences, there are also dissimilarities in the infection pattern of disease caused by the two biotypes (Nair et al. 2008). Epidemiological studies proved occurrence of more asymptomatic carriers among El Tor cholera cases that outnumber active cases by a ratio of up to 50:1 (Sack et al. 2004), better survival of El Tor strains in the environment and in the human host, and more efficient host-to-host transmission of El Tor strains than of classical strains (Finkelstein 1996).

Cholera toxin (CT), the primary toxin produced by *V. cholerae* O1 and O139, is responsible for most of the manifestations of the disease cholera. Based on the B subunit of CT, two immunologically related but not identical epitopes have been designated: CT1 is the prototype elaborated by classical biotype strains and by U.S. Gulf Coast strains, while CT2 is produced by the El Tor biotype and O139 strains (Finkelstein et al. 1987). Another classification identifies three types of *ctxB* genes based on three non-random base changes resulting in changes in the deduced amino acid sequence. Genotype 1 is found in strains of the classical biotype worldwide and in US Gulf Coast, genotype 2 is found in El Tor biotype strains from Australia, and genotype 3 is found in El Tor biotype from the seventh pandemic and the Latin American epidemic strains (Olsvik et al. 1993). Thus, the *V. cholerae* O1 El Tor biotype of the ongoing seventh pandemic produces CT of the CT2 epitope and genotype 3, while the classical biotype CT belongs to the CT1 epitope and genotype 1.

However, over time, especially after the emergence of El Tor variant strains, many of the phenotypic and genotypic tests have proven to be inadequate for classifying strains of *V. cholerae* O1 strains into their biotype. Thus, revision of the conventional tests used for the identification of biotypes of *V. cholerae* O1 strains are necessary and an amendment of the existing biotyping scheme has been proposed (Raychoudhuri et al. 2008).

3 Origin of El Tor Biotype and Seventh Pandemics of Cholera

Although the Ganges delta has been considered as the cradle of cholera for many centuries, the seventh pandemic began in Sulawesi island of Indonesia (Barua 1972). This pandemic now has involved almost the whole world, and the causative agent was a biotype of *V. cholerae* serogroup O1 called El Tor. It was first isolated in 1905 from Indonesian pilgrims traveling to Mecca at a quarantine station in the village of El Tor, Egypt by a German physician, E. Gotschlich (Pollitzer 1959) and found again in an outbreak in 1937 in Sulawesi, Indonesia (Tanamal 1959). It caused four outbreaks in Sulawesi during 1937–1958, where it was endemic. During mid-1961, a few cases of cholera caused by the El Tor *V. cholerae* was reported in Java, and Samarang in Indonesia and then the infection spread out like a wild fire to neighboring countries, and went on a pandemic rampage (Barua 1992). The extent of the pandemic was due to the relative mildness (lower expression level) of El Tor and the disease has many more asymptomatic carriers (Sack et al. 2004).

4 Cholera Outbreaks in the 1960s

The factors that provoked the El Tor biotype of *V. cholerae* O1 to spread from its endemic focus in Sulawesi (Celebes), where it began to be more active than usual in January, 1961, will probably be never known. The increase in population movement due to political unrest and the availability of the faster transport systems may have contributed for the dissemination of the infection. The seventh pandemic El Tor cholera spread during 1961–1962 from Sulawesi to involve the other islands of Indonesia, including Java, Sarawak, and Borneo (Kamal 1974). El Tor cholera then spread to the Philippines, Sabah, Taiwan, and Irian Barat, thereby affecting virtually the entire Southeast Asian archipelago. In 1963, the pandemic strain reached Chittagong, Bangladesh, Cambodia, Thailand, Singapore, west Malaysia (Kaper et al. 1995). India was invaded in 1964 through the port of Madras, and within 1 year El Tor cholera had disseminated throughout the country. In 1965, the pandemic spread further westward and invaded West

Pakistan, Nepal, Brunei, Afghanistan, Iran and a limited area of Uzbekistan (USSR). Local outbreaks and sporadic cases of cholera followed subsequently in Uzbekistan (1968), Turkmenistan (1969) and some parts of Russia (1969). During this time a number of cholera strains were isolated, mainly from surface water in these areas as well as in Azerbaijan and in the Krasnodar region (Narkevich et al. 1993). From the time that El Tor cholera reached Pakistan, its spread became even more accelerated. In the space of a few months, Afghanistan, Iran, and nearby republics within the Soviet Union experienced outbreaks. Iraq reported El Tor cholera during the following year in 1966 (Kaper et al. 1995). A large outbreak of classical cholera was recorded in West Pakistan in 1968, and then spread with more vigor to the other countries. Laos reported cholera for the first time during this era. Hong Kong, Macao, and the Republic of Korea got affected again after remaining practically free from the disease since 1965. At the same time, Nepal, Malaysia, and Myanmar reported higher incidence of cholera than the previous years.

5 Cholera Outbreaks in the 1970s

The seventh El Tor pandemic gained its entry and caused explosive outbreaks of cholera in the Middle East and West Africa in 1970. El Tor cholera had touched the Arabian Peninsula, Syria, and Jordan by 1970 and a limited outbreak was recorded in Israel (Cohen et al. 1971). At this time, resurgence of El Tor Inaba serotype was documented in Iran and the southern Soviet Union. It is of interest that in Lebanon and Syria the epidemic strain was El Tor Ogawa, whereas in nearby Israel and Jordan and in Dubai and Saudi Arabia the epidemic organism was El Tor Inaba. The invasion of El Tor Ogawa cholera into the sub-Saharan West Africa was a momentous epidemiologic event (Goodgame and Greenough 1975). Following its introduction in Guinea in August 1970, probably by means of a returning asymptomatic traveler from the Asian continent, cholera subsequently spread along waterways along the coast and into the interior along rivers ((Swerdlow and Isaacs 1994; Isaacs et al. 1974). Subsequent dissemination into the interior of the Sahelian states occurred by land travel fostered by the movement of nomadic tribes (Kaper et al. 1995). It is estimated that the outbreak during 1970–1971 in West Africa sickened more than 400,000 persons. Within a year, 25 African countries were affected by cholera with a high case fatality rate (CFR) of 16 % (WHO 1991). In the following years (1972–1991), cholera swept in most of the African countries with case fatality ranging from 4 to 12 % (WHO 1991). Due to the lack of background immunity in the population, insufficient transport to move severe cases to treatment facilities, and inadequacies in the health care infrastructure, case fatality in West Africa was high (Kaper et al. 1995). According to WHO records, of the 36 countries that reported cholera in 1970, 28 were newly affected countries and 16 were in Africa. As epidemic cholera stormed in West Africa in 1970, epidemiologists and public health officials in South

America and elsewhere in Latin America restrained themselves and their communities for what was deemed to be the inevitable passage of cholera westward across the South Atlantic. The scenario of introduction of cholera into the Americas was considered particularly likely to occur once cholera hit Angola, since an estimated 40,000 Cuban troops were in that country. Yet, inexplicably, cholera did not cross the South Atlantic during the next 20 years.

In West Africa, cholera epidemic occurred in Togo during 1970–1973, affecting more than 1,000 people with CFR of 4–10 % (Bockemühl and Schröter 1975). Cameroon first reported cholera cases in 1971 when the current pandemic hit the African continent. More than 2,000 cases were reported in 1971 with a high case fatality rate (CFR) of 15 % (WHO 2012). Since the first outbreak in 1970 in Guinea, cholera recurred every 8 years till 1994 (Boiro et al. 1999). Based on the reported cases, cholera first appeared in Burundi, Zaire, and Congo during 1978–1979 (Yala et al. 1982) and in South Africa in 1980 (Küstner et al. 1981). *Vibrio cholerae* O1 Inaba was associated with African cholera for many years (Mugoya et al. 2008). Early cholera outbreaks (1971–1975) in Algeria were caused by the serotype Ogawa (Guechi and Mered 1978). In Zaria, Nigeria, Hikojima serotype that reacts with both Ogawa and Inaba antisera was prevalent from 1976 to 1978, but Ogawa became dominant from 1984 to 1986 (Onyemelukwe and Lawande 1991).

In USSR, cholera spread intensively between 1970 and 1977 and more than 80 regions of the country reported outbreaks. The peak occurred in 1970 when 3,989 cases and carriers were reported; this coincided with intensification of the global pandemic. The infection was introduced to Odessa, Kerch, Astrakhan (Pavlov 1976; Sergiev et al. 1981) and Batumi in 1970, followed by its spread from these foci to 38 cities in the same year, despite cholera control measures. Cholera was also imported to Azerbaijan from India, Jordan, and Iran (1970–1972), and to the Kemerovo region from Egypt (1975). Large-scale outbreaks occurred in 1970–1971 along the river Volga (5,584 cholera cases and carriers) and also in the Ukraine, Georgia, Azerbaijan, and Tajikistan. Subsequently, cholera spread into new territories, causing outbreaks in the north Caucasus, in the region of the rivers Volga and Viatka, and in western Siberia. The annual incidence of cholera in the USSR varied from 0.001 per 100,000 in 1977 to 0.8 per 100,000 in 1979. In India, the classical biotype was replaced by the El Tor from 1965 (Datta and Singh 1990). In some areas in India such as Raipur, the classical cholera prevailed till 1970 and the subsequent cholera outbreaks in 1975, 1977, and 1979–1981 were caused by El Tor vibrios (Darbari et al. 1982).

In Asia, Sri Lanka reported cholera for the first time since 1953; the Philippines reported 5,600 cases in 1972 and 2,075 in 1973, and Indonesia reported 44,300 cases in 1972; 52,000 cases in 1973; 41,000 cases in 1976, and 17,000 cases in 1977 (Barua and Burrows 1974).

In developed areas as Japan, Northern Europe, and North America, cholera has been introduced repeatedly in recent years, but had not caused devastating outbreaks. However, Japan also reported a limited outbreak in Wakayama Prefecture in 1977 and, in 1978, the United States 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 (Finkelstein 1996). In 1973, Italy suffered serious losses in its tourist trade when cholera broke out in August around Naples and Bari with some sporadic cases in other parts of the country (Baine et al. 1974). Cholera was reported again in 1979 in Italy and in eight districts of Spain.

6 Cholera Outbreaks in the 1980s

Intermittent appearance of classical cholera was recorded during 1979–1981 (Samadi et al. 1983a, b). Classical cholera appeared in the form of large epidemic starting from Matlab, Comilla, and Dhaka during late 1982 and spread to other districts replacing the El Tor biotype (Khan et al. 1984; Samadi et al. 1983a, b). Phenotypically, the new classical strains were identical to the one that prevailed a decade earlier and the virulence features and seasonality resembled that of El Tor strains prevailing at that time. It was hypothesized that the classical strains of *V. cholerae* O1 were indigenous to Bangladesh (Huq et al. 1983). Cholera due to classical biotype was predominant (79 %) in southern regions of Bangladesh during 1988–1989 (Siddique et al. 1991).

A 33-year (1966–1998) data analysis provided much information from Bangladesh (Longini et al. 2002). Between 1966 and 1988, both classical and El Tor biotypes had alternated and persisted and by 1988, the classical biotype disappeared. Both the Ogawa and Inaba serotypes circulated the entire time. The serotype prevalence during 1988–1989 was interesting as El Tor belonged to Inaba, whereas the classical strains to Ogawa type (Longini et al. 2002). Studies conducted from 1985 to 1991 in Bangladesh indicated that the incidence of cholera was among children below 5 years (24 %) and children below 2 years of age accounted for 10 % of the cases (Siddique et al. 1992). The overall case fatality during epidemics was 4.0 %. China also reported cholera in 1980. Large number of cases was reported by Indonesia, Iran, Jordan, and Yemen during in the early 1980s.

In Africa, large epidemics raged during 1984–1986 in the drought-affected countries of Burkina Faso, Niger, Mali, Mauritania, Senegal, Ghana, and the United Republic of Tanzania. Cameroon was also affected by the epidemic in 1985. A cholera epidemic in South Africa occurred in the 1980s, with over 20,000 culture-confirmed cases being documented. KwaZulu-Natal was the worst-affected province, although cases were described in Limpopo and Mpumalanga. This epidemic was primarily due to *V. cholerae* O1 Inaba (Küstner and Du Plessis 1991). This followed an outbreak of cholera in Maputo in Mozambique between 1980 and 1981 (Swerdlow and Isaäcson 1994).

Vibrio cholerae O1 was introduced in northern Somalia from Ethiopia during the early 1980s (Coppo et al. 1995). Recurrent cholera epidemics were reported

during rainy hot seasons since 1987 in Angola (Colombo et al. 1993). In Malawi, cholera cases were reported with high attack rate (2.6 %) with CFR of 3.3 % during 1988 (Moren et al. 1991). This outbreak was related to consumption of contaminated shallow waters.

Seventh pandemic of cholera extended to Swaziland in 1981, the Trust Territory of the Pacific islands in 1982, and Equatorial Guinea in 1984 bringing the total number of countries affected by the seventh pandemic to 93 (Barua and Burrows 1974). Somalia reported a large number of cases in refugee camps in 1985 and 1986. Guinea-Bissau was affected in 1986 and Yugoslavia in Europe in 1989. During 1982–1987, the number of countries reporting cholera to WHO has varied between 30 and 37 each year as compared to 40 and 42 in 1980 and 1981.

In 1981, for the first time the health authorities in the Mecca pilgrimage waived the requirement of a cholera vaccination certificate. In 1986, for the first time, India also did not enforce the use of cholera vaccine during the country's largest religious congregation at Kumbh Mela, instead relied heavily on the success of surveillance and sanitation measures taken by the authority.

7 Cholera Outbreaks in 1990s

The 1990s was a significant decade in the history of cholera, as there was a remarkable upsurge in the global incidence of the disease. The number of cases and number of countries reporting cholera to the WHO showed a precipitous increase. In the July 1998 issue of the WHO Weekly Epidemiological Record, it was reported that “almost every developing country is now facing either a cholera outbreak or the threat of an epidemic” (WHO 1996). The striking events of the 1990s included the dramatic reappearance of epidemic cholera caused by *V. cholerae* O1 El Tor in Latin America in 1991 after its absence for 100 years in that continent (Tauxe and Blake 1992); the genesis in late 1992 in the Indian sub-continent of a new epidemic strain of *V. cholerae* non-O1 (Ramamurthy et al. 1993; Albert et al. 1993), presently classified as O139 Bengal (Shimada et al. 1993); the death in Africa in 1994 of as many as 14,000 Rwandan refugees due to cholera related to the poor quality of the water supply, sanitation, and treatment facilities (Siddique et al. 1995) and the dramatic cholera epidemic affecting the countries in the Horn of Africa in 1997 (WHO 1996). Clearly, there was a fresh resurgence of this ancient disease.

7.1 Latin America Epidemics of Cholera

The seventh cholera pandemic reached Latin America in January 1991 for the first time in more than a century. Nearly a million cases were reported from the epidemic of cholera in Latin America, and almost 9,000 people died between

January 1991 and December 1993 (Tauxe and Blake 1992; Tauxe et al. 1994). Latin America's cholera epidemic struck first on January 29 at the harbor city of Chimbote, Peru and caused a major outbreak along the Peruvian coast and it also spread inland through the Andes, as well as through the Peruvian Amazon, and by April, 1991 it had reached most of Peru (Tauxe et al. 1994).

The epidemic disseminated rapidly from country to country, and cholera first spread to neighboring Ecuador to the north, and then in Colombia. Later on, Guatemala, Brazil, Mexico, Bolivia, Chile, El Salvador, Venezuela, and Honduras were also affected. Notifications of cholera markedly diminished during the cool season in South America, but with the return of warm weather in December 1991, the incidence of cholera once again rose. By the end of 1993, all countries of Latin America except Uruguay and the Caribbean reported cholera cases. The greatest proportion of cholera cases and the highest incidence rate were in Peru (63.7 % and 26.9/1000, respectively) (Guthmann 1995). Most cholera cases were reported in 1991 and were concentrated in Peru (82.3 %). 45.5 % of all cholera deaths occurred in 1991 (Guthmann 1995). Central America had the highest case fatality rates. Actually, Peru accounted almost half of the cholera cases registered in Latin America (Kaper et al. 1995). The overall case fatality rate was only 0.92 %, mainly due to good oral rehydration treatment provided by local health services and the Pan American Health Organization. In each of these countries, cholera struck underprivileged low socioeconomic populations lacking unpolluted drinking water and proper sanitation (Pan American Health Organization. 1991).

The causative organism was *V. cholerae*, serogroup O1, serotype Inaba (and Ogawa) of the El Tor biotype. The cholera epidemic in Latin America was originally suspected to have come from Asia and to have been facilitated by the discharge of contaminated ballast water into Peruvian ports by international trade ships (Seas et al. 2000). Another view was that as the seventh pandemic spread through Africa during the 1970s, travel from Africa to South America and inadequate sanitation in parts of South America were thought to place the Latin American continent at particular risk (Wachsmuth et al. 1993).

Latin American isolates from Peru, Bolivia, Chile, Colombia, Guatemala, Ecuador, El Salvador, and Mexico were identical when isolates were compared by restriction enzyme *Hind*III digestion and hybridization with probe *ctxA* or by *Bgl*I digestion and a 16S + 23S rRNA probe. When those isolates were compared with isolates from other parts of the world, they were clearly different from the Gulf Coast clone (Wachsmuth et al. 1993). From the result of their study, Wachsmuth et al. (1993) concluded that Latin American isolates are clonal, probably a variant of the seventh pandemic clone. Another study also showed that all *V. cholerae* O1 isolates tested from the Latin American epidemic were indistinguishable by their MEE, ribotype, or PFGE patterns (Cameron et al. 1994). Genetic characterization showed this strain to be unique, and the designation is reserved for the Latin American strain, distinguishing it from the other El Tor isolates from the seventh pandemic.

Recently, Lam et al. 2010 performed genome-wide single-nucleotide polymorphisms (SNPs) to track the evolution and spread of the seventh cholera

pandemic and showed that the isolates from Latin American epidemic were closely related to isolates found in Africa in the 1970s and 1990s. This finding suggested that the strain that caused the epidemic in Latin America came from Africa rather than Asia. The outbreak in Peru occurred in parallel with the upsurge of cholera generally in Africa (Reeves and Lan 1998) and could have been imported at that time. However, the epidemic strain may have reached Latin America well before it caused the epidemic in 1990s, given the ability of the organism to persist in the marine environment for long periods (Seas et al. 2000). Phylogenomic analysis of 136 El Tor isolates clustered the Latin American epidemic strains with an Angolan strain isolated in 1989 (Mutreja et al. 2011). In this period, an increased circulation of people between Africa and Latin America was reported, due to the Cuban Angolan intervention (1975–1991).

At the late epidemic period (1994), in the state of Amapá, Brazil, outbreak was caused by the sucrose non-fermenting *V. cholerae* strain (Ramos et al. 1997; Garza et al. 2012) and Multi Locus Enzyme Electrophoresis (MLEE) later showed that this strain belonged to the same zymovar as the Latin American epidemic lineage, suggesting that this variation was the consequence of a mutation in the sucrose operon (Freitas et al. 2002).

Since the beginning of the 1990s, Kivu provinces have been identified as one of the most active foci of cholera in the world (Bompangue et al. 2009). In July 1994, 500,000–800,000 Rwandans crossed the border into the North Kivu region of Zaire (now called the Democratic Republic of the Congo, DRC). During the first month after the influx, almost 50,000 refugees died; an average crude mortality rate of 20–35 per 10,000 per day; and cholera was a major contributor (Goma Epidemiology Group 1995; Bhattacharya et al. 2009). The refugee camps located around Goma and Bukavu experienced the deadliest cholera epidemics recorded during the last hundred years. This explosive outbreak of cholera, which affected Rwandan refugees, resulted in about 70,000 cases and 12,000 deaths (Siddique et al. 1995).

7.2 Genesis of *Vibrio cholerae* O139: A Bewildering Event in the History of Cholera

The emergence of *V. cholerae* O139 Bengal as the second etiologic agent of epidemic cholera in 1992 in the south Indian coastal city of Madras has changed the long-held dogma that only *V. cholerae* belonging to serogroup O1 are capable of causing epidemic (and pandemic) cholera. The first report of an epidemic of “cholera-like” diarrhea was from a suburban area of north Madras, India, in September 1992 (Ramamurthy et al. 1993). In December 1992, there was an outbreak of “cholera-like” diarrhea in southern Bangladesh, which over the subsequent several months, spread to the entire country (Nair et al. 1994). Retrospective examination of all the *V. cholerae* isolates collected from 1992 at the Communicable Diseases Hospital in Madras showed that these strains belonged to *V. cholerae* O139

serogroup (Dhamodaran et al. 1995). This serogroup made its first appearance in January 1992 (Sharma et al. 1997a, b) and took almost 10 months for this serogroup to cause an epidemic. More than a year earlier, from April to July 1991 and again in September 1992, there had been an increase in the number of *V. cholerae* non-O1 isolates from patients with cholera at the Christian Medical College Hospital at Vellore, India, indicating an outbreak (John 1996). However, *V. cholerae* non-O1 strains isolated during 1991 were not stored for identification.

These strains of *V. cholerae* which did not agglutinate with antisera from O1 to O138 were included as a new serogroup O139 (Shimada et al. 1994). This serogroup had spread quickly to Bangladesh and to other states of India within a span of about 10 months (Albert et al. 1993; Nair et al. 1994). Since *V. cholerae* O139 was first discovered in the areas surrounding the Bay of Bengal (Tamil Nadu, Andhra Pradesh, West Bengal and Bangladesh), this serogroup was given a synonym "Bengal."

In a span of 1 year, this serogroup has been reported in many Asian countries including Bangladesh, Pakistan, Nepal, and China. The O139 infection produced severe dehydrating diarrhea, which is indistinguishable from clinical cholera and does not appear to confer any cross-protection from the O1 serogroup (Mahalanabis et al. 1994). Based on the clinical symptoms and severity of diarrhea, the disease caused by *V. cholerae* O139 is now considered as cholera (Bhattacharya et al. 1993) and the infections caused by the rest of the non-O1 and non-O139 serogroups are known as "cholera-like diarrhea."

Before its total replacement, the O139 prevailed along with the O1 serogroup in many countries. The affected age group by the O139 infection depends on the period and place of its occurrence. In Delhi the incidence of O139 and O1 cholera was frequent in children below 5 years of age (Singh et al. 1997). In this study, the incidence of both the serogroups was detected among 1.4 % of the children with cholera. A similar epidemiological observation was made in Karachi, Pakistan (Sheikh et al. 1997). The incidence of O139 among children during 2003 in Pakistan was 21 % and the infected patients were more likely to be febrile ($P < 0.001$) (Siddiqui et al. 2006).

After its initial explosive epidemic during late 1992 and early 1993, occurrence of O139 serogroup declined in many cholera-endemic regions (Faruque et al. 1996; Mukhopadhyay et al. 1996). The emergence of *V. cholerae* O139 initially caused a complete displacement of the O1 El Tor biotype strains in India and Bangladesh. However, *V. cholerae* O139 was again displaced in 1994 by a new genetic variant of the O1 strain, and this variant strain dominated until 1996 in India (Fig. 1). In August, 1996, a new variant of the O139 strain emerged, and cholera caused by the new O139 genetic variant dominated for a year, until September, 1997 in Calcutta. Similarly in neighboring Bangladesh, during 1994 and till the middle of 1995, in most northern and central areas of the country, the O139 vibrios were replaced by a new clone of *V. cholerae* O1 of the El Tor biotype, whereas in the southern coastal regions, the O139 vibrios continued to exist (Faruque et al. 1997a, 1999). During late 1995 and through 1996, cases of cholera caused by both *V. cholerae* O1 and O139 were again detected in various

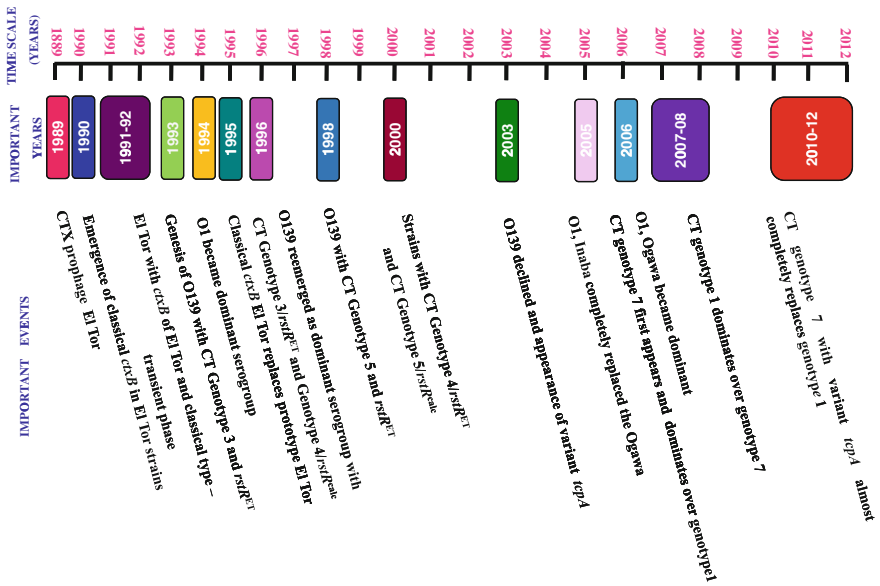


Fig. 1 Major events in the evolution of *Vibrio cholerae* O1 El Tor and O139 CTX prophages in Kolkata for the last two decades

regions of Bangladesh. However, since 1996, cholera in Bangladesh was caused mostly by *V. cholerae* O1 of the El Tor biotype, whereas only a few cases were caused by strains of the O139 serogroup. Between 1997 and 2000, incidence of cholera due to O139 serogroup decreased to 3.8 % in rural Bangladesh (Sack et al. 2003). This changing epidemiology of cholera shifted further, and a large outbreak of cholera caused predominantly by *V. cholerae* O139 occurred in the capital city of Dhaka and adjoining areas during the first half of 2002 (Faruque et al. 2003a). Resurgence of O139 cholera was also reported in other Asian countries including Pakistan (2000–2001), and India (1997, 2001), mostly affecting the older age groups (Jabeen and Hasan 2003; Mitra et al. 1996; Sundaram et al. 2002; Agarwal et al. 2003). From 1999 to 2000, most of the cholera outbreaks in India were caused by the O139 serogroup (Sinha et al. 2002). Investigations conducted in Indonesia revealed that the O139 serogroup had not invaded into this country till 1999 (Simanjuntak et al. 2001). The first incidence of O139 was recorded in Baghdad, Iraq, in 1999, though the numbers of cases were low (Al-Abbassi et al. 2005). In 1993, the first outbreak caused by serogroup O139 strains occurred in Xinjiang, China, where 200 cases were reported. In 1994, outbreaks of *V. cholerae* O139 were reported in six Chinese provinces. Imported cases of O139 cholera were reported soon after its emergence in Asia in California (CDC 1993) and other parts of the USA (Mahon et al. 1996), Japan (Kurazono et al. 1994), and Denmark (Dalsgaards et al. 1995).

The recurrent infection caused alternatively by the O1 and O139 serogroups in cholera-endemic regions emphasize the fact that acquired immunity plays an important role in the emergence and dissemination of specific serogroups in a population. In addition, rapid genetic reassortment in *V. cholerae* O1 and O139 serogroups might play a role in the changing epidemiology of cholera (Faruque et al. 2003a). It is still a mystery why the so-called highly infectious O139 serogroup has not spread to the other cholera-endemic regions such as Africa.

O139 strains have undergone various alterations in both phenotypic and genetic characteristics such as changing pattern of antimicrobial resistance, restriction fragment length polymorphisms in conserved rRNA genes (ribotype), rearrangement of the CTX prophage, and acquisition of new CTX prophages (Basu et al. 1998; Faruque et al. 2000; Mitra et al. 1996; Mukhopadhyay et al. 1998; Sharma et al. 1997a, b) (Fig. 1). Molecular evolutionary studies have also recorded temporal variations in prevalence of O139 and O1 serogroups over the years in India along with the emergence of new clones within the O139 serogroup. Subsequent studies depicted that the O1 serogroup which replaced the O139 serogroup was a new clone of O1 El Tor biotype (Faruque et al. 1997b; Yamasaki et al. 1997; Sharma et al. 1997a, b). A quiescent period followed in the history of *V. cholerae* O139, and it was thought that the appearance of O139 was a one-time event. But a resurgence of serogroup O139 occurred in 1996 in Kolkata (Mitra et al. 1996). Between December 1999 and December 2000, escalating events of O139 were recorded in several outbreaks occurring throughout India, including Kolkata (Sinha et al. 2002). After this period, *V. cholerae* O1 continued to be a dominant serogroup in Kolkata and the incidence of O139 gradually decreased over the years. The factor(s) contributing to the diminished isolation of O139 vibrios and the re-emergence of O1 El Tor vibrios are not understood. The vibrios might have undergone changes that would have affected their ability to survive and compete in the environment. One study from Kolkata reported the appearance of new genotype of *ctxB* in 1996 with the re-emerged *V. cholerae* O139 strains that had CTX Calcutta phage and was designated as genotype 4 and in 1998, another new genotype designated as genotype 5 was detected that prevailed mostly in CTX phages with El Tor *rstR* (Raychoudhuri et al. 2010). During the course of appearance and disappearance over a decade, five types of O139 strains have been detected based on CT genotypes (Raychoudhuri et al. 2010). Frequent mutations thus acquired by *V. cholerae* O139 strains since its genesis may have an impact in their declining prevalence in cholera-endemic areas like Kolkata (Fig. 1).

8 Cholera Outbreaks in the Twenty-first Century

Among the 39 African countries that reported cases of cholera in any year from 2000 through 2005, 18 (46 %) countries reported cases in all 6 years: Benin, Burundi, Cameroon, Democratic Republic of the Congo, Ghana, Guinea, Liberia, Malawi, Mozambique, Niger, Nigeria, South Africa, Swaziland, Togo, Uganda,

United Republic of Tanzania, Zambia, and Zimbabwe. Countries with such high endemicity are found in East, Southern, Central, and West Africa (Gaffga et al. 2007). A total of 67,738 cases and 3,666 deaths (CFR 5.4 %) were reported between 2000 and 2005 in eastern provinces of DR Congo (Bompangue et al. 2008). During the period between 2000 and 2005, four countries in Africa had a cholera density greater than 200 cases per 1,000,000 people: Mozambique (793/million), Liberia (594/million), Somalia (441/million), and the Democratic Republic of the Congo (242/million) (Gaffga et al. 2007). In 2005, 31 (78 %) of the 40 countries that reported indigenous cases of cholera to WHO were in sub-Saharan Africa (Gaffga et al. 2007). The reported incidence of indigenous cholera in sub-Saharan Africa in 2005 (166 cases/million population) was 95 times higher than the reported incidence in Asia (1.74 cases/million population) and 16,600 times higher than the reported incidence in Latin America (0.01 cases/million population).

In 2004–2005, as part of a significant series of cholera outbreaks in West Africa, an epidemic took place in Senegal, resulting in 31,719 cases, i.e., 293 cases/100,000 habitants, with 458 deaths (case fatality rate (CFR) of 1.44 %) (de Magny et al. 2012; WHO 2008a). This epidemic was the largest recorded by the World Health Organization (WHO) for Africa during that time (WHO 2006). A large outbreak occurred in Cameroon during 2004, when 8,000 cases were reported in Littoral and West regions. The outbreak which started in Bepanda, an area located in the northwest of Douala, spread rapidly to other areas (New Bell and Nylon), and soon reached the entire town of Douala (WHO 2012). In 2005, Cameroon reported 2,847 cases including 110 deaths (CFR 3.86 %) with 70 % of the cases from the Littoral region (WHO 2012). In 2006, Cameroon reported 922 cases including 35 deaths (CFR 3.8 %). A first outbreak occurred from April to June in Bafoussam (Ouest province) and a second one occurred in the Far North region in November (WHO 2012). Over 25,000 cases of cholera have been reported in Sierra Leone and Guinea including 392 deaths since February 2012, when the epidemic was reported. It is the country's worst outbreak of cholera in 15 years.

A retrospective study on cholera outbreaks in Mali reported 12,176 recorded cholera cases, including 1,406 deaths, between 1995 and 2004 (Dao et al. 2009). South Africa experienced a cholera epidemic between the years 1997 and 2005 and the worst cholera epidemics in the country's recent history reached its peak in 2001 (Keddy et al. 2007). Initial reports of the cholera outbreak came from the largely rural and impoverished communities on the outskirts of the Ngwelezane Township, near the Empangeni town. The source of the epidemic was traced to the Mhlathuze River, also in the northern part of the KwaZulu-Natal Province. However by the end of the year 2000, the northern KwaZulu-Natal cholera outbreak had replicated itself in eight of South Africa's nine provinces and cases were identified in the Eastern Cape, Mpumalanga, Limpopo, and Gauteng. Over 100,000 cases were notified based on clinical diagnosis between 2000 and 2002 (Department of Health; <http://www.doh.gov.za/facts/stat-notes-f.html>). Initially, the causative organism was identified as *V. cholerae* O1, Ogawa. But in mid-2001,

O1, Inaba emerged in KwaZulu and subsequently, further isolates of *V. cholerae* O1 Inaba were identified from other provinces. It is noteworthy that during the 2001/2002 epidemic the death rate was less than 1 % (Keddy et al. 2007).

In August 2008, a new cholera epidemic was reported in Zimbabwe, which affected all 10 provinces and 56 of the 62 districts. This is the largest ever recorded outbreak of cholera in Zimbabwe. Over 7 months, more than 90,000 suspected cholera cases were reported, with more than 4,000 of these patients died. Nelson opined that these values were likely underestimates, because during the crisis reporting clinics were largely on strike, communication was severed by stolen telephone lines, and deaths in the bush devalue as fast as the currency (Nelson 2009). This epidemic brought rates of mortality similar to those witnessed as a consequence of cholera infections a hundred years ago and the Zimbabwean Government declared the outbreak a national emergency.

Nigeria is in one of the three major current cholera foci in the world (Piarroux and Faucher 2012). In 2009, outbreaks began in Nigeria and other countries at the Lake Chad basin (Quilici et al. 2010) with the first reports coming from Maiduguri, a city in the far northeast of the country. Subsequently, outbreaks were reported from distant locales in Northern and Western Nigeria, and in 2010 a severe outbreak, which started in the Northern Nigeria spreading through the country, was projected as the worst outbreak in Nigeria since 1991. This outbreak was marked with highest case fatality (Adagbada et al. 2012), what could be in part due to changes in *V. cholerae* infectivity even though the organism remains largely unknown. It can be hypothesized that an index strain has been disseminated cross-country by human travel. Marin et al. (2013) performed a comprehensive characterization of representative *V. cholerae* strains from sequential outbreaks in Nigeria and reported that recent cholera outbreaks in Nigeria are driven by atypical El Tor strains. The occurrence of cholera outbreaks in the African continent is dealt with in greater detail in another chapter in this book.

During the 2002 cholera outbreak in Chandigarh, India, *V. cholerae* O1 Ogawa was isolated from 18 % of the hand-pump water samples (Kaistha et al. 2005). Remote areas such as Andaman and Nicobar Islands were free from cholera for many years. The first cholera outbreak was recorded during early 2000 due to the spread of *V. cholerae* O1 from the main land (Shah et al. 2002). In 2002, cholera was identified due to El Tor vibrios among Nicobarese tribe in 16 villages with an attack rate of 12.8 % and a case fatality ratio of 1.3 % (Sugunan et al. 2004). Concomitant infections by *V. cholerae* O1 and O139 serogroups were reported in 2000 from a large cholera outbreak in Ahmedabad, India (Chakraborty et al. 2001).

During 2004–2005, cholera caused by the Inaba serotype was recorded in 15 states of India, mostly associated in the form of outbreaks (Dutta et al. 2006). In Delhi, the serotype switchover from Ogawa to Inaba has started in 2004 and 88 % of the strains were identified as Inaba during 2005 (Sharma et al. 2007). Among children below 5 years of age, the incidence of cholera in Delhi was 33 %. Cholera caused by the Inaba serotype was also reported from other parts of India such as

Kolkata, Orissa, Andaman and Nicobar Islands (Dutta et al. 2006; Raychoudhuri et al. 2007; Pal et al. 2006; Sugunan et al. 2007).

These Inaba strains had unique PFGE (pulsotype H1) and ribotype (RIV) profiles that were not recorded before. After its first appearance in July 2004, the Inaba serotype completely replaced the dominant Ogawa serotype from May 2005 in Kolkata (Raychoudhuri et al. 2007). These Kolkata Inaba strains belonged to a new ribotype as well as PFGE clone, identical to the Delhi strains and had a CTX prophage with two RS elements. Similar results were obtained with Inaba strains isolated in Trivandrum, southern India, except for ribotyping, which showed that the Inaba and Ogawa strains were similar (Mohapatra et al. 2007).

Based on the spatial patterns and exploratory spatial data analysis, the risk factors for cholera were associated with environmental niches (Ali et al. 2001). Environmental studies conducted during 2004 in Mathbaria and Matlab, Bangladesh, revealed that both *V. cholerae* O1 and O139 serogroups occurred predominantly as viable but non-culturable state (Alam et al. 2006). However, culturable cells were also detected in the biofilms, which were considered as additional reservoirs of toxigenic *V. cholerae* in the aquatic environments during interepidemic seasons. Isolation of *V. cholerae* O1 from the aquatic environments of Bangladesh through selective enrichment using antibiotics has re-emphasized the hypothesis that the humans act as reservoirs of this pathogen during interepidemic periods and spreading occurs through contaminated water (Faruque et al. 2006).

The cholera toxin producing *V. cholerae* non-O1 strains were isolated from seafood in Taiwan (Wong et al. 1992). Turtles and their breeding environment are the major reservoirs of *V. cholerae* and responsible for many outbreaks of cholera in Sichuan Province and Guangzhou area, China during 2003–2005 (Liu et al. 2006; Zhang et al. 2007). In an investigation it was shown that turtles and other seafood harbored toxigenic *V. cholerae* O139 (Chang et al. 2007). In Zhejiang Province, the incidence of O1 serogroup of *V. cholerae* was found to be high (9 %) in turtles and cholera epidemics in this region might be associated with consumption of contaminated turtles (Lü et al. 2006).

Resurgence of *V. cholerae* O139 in the beginning of twenty-first century was reported in many Asian countries including Pakistan (2000–2001), India (1997, 2001), and Bangladesh (2002), mostly affecting the older age groups (Jabeen and Hasan 2003; Sundaram et al. 2002; Agarwal et al. 2003; Faruque et al. 2003b; Sinha et al. 2002). *Vibrio cholerae* O1 and O139 consecutively appeared during cholera outbreaks (2002–2003) near Karachi (Siddiqui et al. 2006). This study has also revealed that children less than 2 years of age were the most affected age group with O1 (49 %) than O139 (21 %).

Following the Iraq war, the communicable disease control program was disturbed, resulting in cholera epidemics in several districts of Basrah, Iraq, in 2003 (Valenciano et al. 2003). Cholera outbreak struck Kabul, Afghanistan, in 2005 and spread nationwide. The health authorities gave importance to the disease control program that included proper management and treatment supported by partner agencies that kept the mortality rate well below 0.1 % (Kakar et al. 2008). A limited cholera outbreak occurred in Iran in the summer of 2005 and out of 1,150

patients, 11 people died (Ataei et al. 2005; Azizi and Azizi 2010). In 2007 there was an epidemic of cholera in Iraq with 4,667 cases and bacteriological testing confirmed that the outbreak was caused by *V. cholerae* O1, biotype El Tor, serotype Inaba (Khwaif et al. 2010). Another study reported the isolation of Ogawa serotype from this outbreak (Saleh et al. 2011).

8.1 Haitian Outbreak

Haiti has recently battled the world's largest cholera epidemic in half a century. On, January 12, 2010, a powerful earthquake devastated the capital city of Haiti along with southern towns as the epicenter was 16 miles west of the capital city of Port-au-Prince. Ten months after the devastating earthquake, the first case of cholera was reported from the town of Mirebalais, about 62 miles north of Port-au-Prince, Haiti for the first time in nearly a century. The first hospitalized cases of cholera occurred in Mirebalais on October 17, 2010 (Ivers and Walton 2012). This deadly outbreak has killed around 8,000 Haitians, and infected over 600,000 to date. By the first 10 weeks of the epidemic, cholera spread to all of Haiti's 10 departments or provinces.

Suspected cases of cholera have since been reported in Bolivia, Brazil, Chile, Colombia, Nicaragua, Panama, Peru, and Venezuela. Confirmed imported cases have been reported in Florida. Compared with Haiti's experience, the epidemic has been less severe in Dominican Republic. Transmission was limited, but sustained and continued at low levels. One large outbreak affected guests at a wedding in January 2011, including some visitors from Venezuela and the United States. From October 21, 2010, through July 30, 2011, a total of 14,598 suspected cases of cholera were reported; 256 persons died (of these, cases in 92 patients *V. cholerae* O1 was laboratory confirmed) (Tappero and Tauxe 2011). In late June 2012, Cuba confirmed three deaths and 53 cases of cholera in Manzanillo, in 2013 with 51 cases in Havana. The case fatality rate (CFR) was initially high in some locales (4.6 %), but within three months of the start of the epidemic CFR declined to the WHO target of <1.0 %. The CDC notes that 23 cases occurred in the U.S.; 22 were associated with travel to Haiti, one with consumption of food products from that country.

To understand the evolutionary origin of *V. cholerae* isolated in Haiti, several research groups in different parts of the world studied the Haitian cholera outbreak independently. The Harvard Cholera Group compared the entire genome sequences of the Haitian strain with two strains from Bangladesh and one isolated in South America including sequences from 23 different strains of *V. cholerae* available online in the public domain (Chin et al. 2011). A nearly identical relationship was observed between the Haitian isolates and the variant seventh pandemic El Tor O1 strains that are predominant in South Asia. No relationship was observed between the South American isolates (indicating that this strain is not related to the early-1990s cholera epidemic in South America) or with the African

strains isolated between 1970 and 1998. The study of Mutreja et al. (2011) indicated that the Haitian strains were all identical and most closely related to strains of *V. cholerae* from the Indian subcontinent and distinct from strains of *V. cholerae* isolated in Africa, Bahrain, Germany, Indonesia, Vietnam, Malaysia, and South America. Another study considering the genetic diversity of a total of 187 individual isolates of *V. cholerae* O1 picked from the 16 stool samples from St. Marc hospital at Haiti showed minimal diversity, consistent with a single point source for the 2010 Haiti epidemic (Ali et al. 2011). Separate study on population genetics of *V. cholerae* strains from Nepal in 2010 suggested strong epidemiological link with the Haitian outbreak (Hendriksen et al. 2011). Another comparative genomics of *V. cholerae* strains from Haiti, Asia and Africa using phylogenies for whole genome sequences and core genome single-nucleotide polymorphisms showed that the Haiti outbreak strain is genetically related to strains originating in India and Cameroon (Reimer et al. 2011). However, this study concluded that a definitive genetic origin for the outbreak in Haiti remains speculative due to the lack of identical genetic match among sequenced contemporary isolates (Reimer et al. 2011).

Interestingly, the nucleotide sequence of the *ctxB* (the gene for the B subunit of cholera toxin) of the Haitian strains was found to have a unique mutation at the 58th nucleotide position indicating three coding mutations of *ctxB* gene as opposed to only two seen in typical classical strains of *V. cholerae* O1 or in the El Tor variants (Nair et al. 2006). Retrospective analysis of *V. cholerae* strains from Kolkata, India showed that the Haitian type *ctxB* first appeared in Kolkata during April, 2006 and 93.3 % strains during 2011 carried the new allele (Naha et al. 2012). This genetic polymorphism of the *ctxB* gene was also observed in strains of *V. cholerae* O1 isolated from Orissa, India, and from the West African countries of Nigeria and Cameroon (Choi et al. 2010; Kumar et al. 2009; Quilici et al. 2010). However, the core genome of the Haitian strains resembled more closely the one found in the South Asian strains and to a lesser extent the one found in the African strains, which once again points to a South Asian origin of the Haitian strains. Whole genome sequence analysis of *V. cholerae* strain isolated from the Haitian cholera outbreak revealed a novel single-nucleotide polymorphism (SNP) at nucleotide position 266 (amino acid 89) of the *tcpA* gene uniquely associated with this variant (Chin et al. 2011; Grim et al. 2010; Talkington et al. 2011). A newly developed PCR study showed that Haitian *tcpA* first appeared in Kolkata during October, 2003, and interestingly soon after its appearance; this new variant *tcpA* displaced the canonical El Tor *tcpA* completely in the following years (Ghosh et al. 2014). The bioinformatical analysis showed that among the three different mutations present in 89th position at different alleles of TcpA, only Asparagine to Serine which is present in the Haitian *tcpA* allele is positively selected. Moreover, this particular mutation is the result of a purine–purine transition, which is evolutionarily preferred. It should be noted, however, that acquisition of Haitian *ctxB* and *tcpA* do not always occur in tandem and Haitian variant strain may be result of the sequential event in the evolution of Indian subcontinent strain (Ghosh et al. 2014). Furthermore, Haitian strains showed (i) change in satellite phage RS1

(*rstB*); (ii) mutation in *rtxA* (Repeat in toxin) involved in cytotoxin activity; (iii) mutation in *gyrase A*, and (iv) change in the number of repeat regions at the protomer region of CT. Among these, two variations seem to be unique for the Haitian strains (P. Ghosh and AK Mukhopadhyay, Unpublished).

8.2 Evolution of the El Tor Variants and Its Impact

Classical and El Tor strains of *V. cholerae* are closely related but are not directly derived from each other (Karaolis et al. 2001) and are believed to have evolved from separate lineages (Kaper et al. 1982, 1995). Although the classical and El Tor biotypes have different lineages, hybrids between the classical and El Tor biotypes resulting from genetic exchange between different bacterial lineages also exist in nature (Chakraborty et al. 2000; Mukhopadhyay et al. 2001). Since the beginning of the seventh pandemic, El Tor strains have gradually displaced the classical strains as the cause of cholera and both the biotypes had coexisted for at least over a decade following the emergence of the El Tor biotype in 1961. In Bangladesh, the classical biotype apparently disappeared in 1973, but re-emerged in 1982 (Samadi et al. 1983a, b; Siddique et al. 1991) and co-circulated with the El Tor biotype for at least a decade (the last isolation was reported in 1992) (Siddique et al. 1991). Curiously, the transient reappearance of O1 classical strains was observed only in Bangladesh. Classical strains are now believed to be extinct; hence, the source of the *rstR*^{Cla} and *ctxB1* alleles and their mode of transfer to El Tor strains in Bangladesh remains a mystery. The existence of classical strains in aquatic environments of Bangladesh was reported during the early 1990s, thereby supporting the notion that they were not completely replaced in that country (Siddique et al. 1991; Faruque et al. 1993).

Study from Matlab, Bangladesh first reported the existence of naturally occurring atypical El Tor variants among clinical strains of *V. cholerae* O1 isolated between 1991 and 1994 (Nair et al. 2002). Certain strains could not be biotyped as either classical or El Tor, and were designated as Matlab (MT) variants (Nair et al. 2002). Mozambique, a cholera-endemic country in East Africa, reported a large and extended cholera outbreak in 2004. The involved *V. cholerae* strains displayed phenotypic characteristics of the El Tor biotype. Moreover, the results of *rstR* and *ctxB* genotyping were consistent with the classical biotype (Ansaruzzaman et al. 2004; Das et al. 2007). The Mozambique variants contained, in the small chromosome, two tandem copies of the prophage whose sequence was almost identical to that of the typical CTX Φ ^{Cla} (Lee et al. 2006; Faruque et al. 2007; Das et al. 2007). Notably, this was the first report of atypical El Tor strains harboring CTX Φ ^{Cla} in Africa. This is against the background that the classical strains of *V. cholerae* O1 never entered into the African continent during the seventh pandemic of cholera.

Retrospective analysis from Kolkata led to the detection of variant *V. cholerae* O1 strains isolated in 1992 from clinical cases with identical traits to 2004

Mozambique variant O1 strains (Chatterjee et al. 2009). It was proposed that some of the 1992 Kolkata O1 strains might have acted as progenitors for Mozambique variant O1 strains. Similar strains carrying tandem copies of CTX Φ^{Cla} in the small chromosome have also been isolated in samples taken between 1995 and 2004 in Vietnam (Nguyen et al. 2009). Unlike the Mozambique variants, some of these strains have an additional CTX Φ^{Cla} in the large chromosome (Nguyen et al. 2009).

Studies attempting to examine the chronology of appearance of variants of El Tor strains in Kolkata indicated that classical type *ctxB* emerged in 1990, although El Tor type *ctxB* was still present in almost equal numbers during that year. During 1991, a unique event took place when the classical type became predominant, along with strains having both classical and El Tor type *ctxB*. In 1994, isolation of strains with El Tor *ctxB* became rare, and the major *ctxB* allele was of the classical type. *V. cholerae* O1 strains from 1995 onward were found to carry classical type *ctxB*, which totally replaced the El Tor type *ctxB* allele (Raychoudhuri et al. 2009). These types of El Tor variant strains producing classical toxin were isolated in Bangladesh after 2001 (Nair et al. 2006). Later on, these El Tor variant strains were isolated from different outbreaks throughout Asia and Africa including Zanzibar Island (Safa et al. 2008; Naha et al. 2013). Comparative analysis among different group of strains showed that *V. cholerae* O1 El Tor variant strains produced much more cholera toxin than did prototype El Tor strains. The amount of cholera toxin produced by El Tor variant strains both in vitro and in vivo was more or less equivalent to that produced by classical strains (Ghosh-Banerjee et al. 2010; Naha et al. 2013). Fig. 1 shows the chronology of appearance of variants of El Tor strains over two decades in Kolkata.

Vibrio cholerae strains of the recent devastating cholera outbreak in Haiti contained a unique mutation at the 58th nucleotide of *ctxB* gene. Analysis of the Kolkata strains showed that the Haitian *ctxB* first appeared in Kolkata during April, 2006 and 93.3 % strains isolated in Kolkata during 2011 carried the new allele (Naha et al. 2012). Haitian *V. cholerae* strains also contained a novel mutation at the 64th amino acid position of the matured TcpA subunit. Analysis of the Kolkata strains showed that Haitian variant *tcpA* first appeared in Kolkata during 2003 and after that all the El Tor *tcpA* was replaced by this new allele of *tcpA* (Ghosh et al. 2014). These findings indicated that Haitian *ctxB* and *tcpA* alleles might have originated in Kolkata and then spread to the neighboring regions. It must be emphasized, however, that the Haitian strains have certain minor traits not found in collections from other parts of the world, which is consistent with the microevolution that takes place continuously within the El Tor biotype as it moves from continent to continent and even country to country.

Using comparative genomic studies of different variants of *V. cholerae* strains isolated in recent time, Grim et al. (2010) concluded that the unique combination of characteristics of the genome of newly variant *V. cholerae* provides the bacterium with a competitive ecological edge and greater infectivity over that of other pathogenic clones of *V. cholerae*. Historically, El Tor strains of *V. cholerae* are considered to have an improved environmental fitness based on the observation that they have displaced classical strains. In turn, classical strains of *V. cholerae*

are believed to produce a more severe form of the disease, cholera (Kaper et al. 1995; Faruque et al. 1998). However, it is unknown how the classical CT affects the pathogenicity of atypical El Tor strains. Given the fact that CT is directly responsible for the major clinical signs of the disease, genetic changes in the CT genes could alter the clinical manifestation of cholera (Safa et al. 2010). Comparative genomic studies also provided evidence of an amalgamation of environmental fitness of El Tor strains and greater infectivity of classical strains, i.e., a “mixing and matching,” through recombination and lateral gene transfer, resulting in the genesis of new variants of *V. cholerae* with expanded ecological persistence, infectivity, and dispersion. The success of a clone is a combination of its ability to adapt to changing environmental conditions as a stable inhabitant, evidenced by conservation of the El Tor genomic backbone, and its ability to transmit progeny through human populations (Grim et al. 2010). WHO reported that *V. cholerae* El Tor variant caused more severe episodes of cholera with higher case fatality rates (WHO 2008b). From a study in Bakerganj, Bangladesh, it was reported that higher proportion of severe dehydration was observed in 2006 after the appearance of El Tor variant strains (Siddique et al. 2010). These new *V. cholerae* O1 El Tor variant strains not only replaced the *V. cholerae* O1 El Tor prototype strains, but also turned out to be genetically stable and spread rapidly even to remote islands in the east African continent. Moreover, the severity of the disease appears to be intensifying, and recent cholera outbreaks in various places, including Zimbabwe and Haiti, have followed a protracted pattern (Kanungo et al. 2010; Piarroux et al. 2011). An active comprehensive surveillance system should be in place in order to track the dissemination of the *V. cholerae* O1 El Tor variant strains in the population using latest molecular diagnostic assays, as these strains possess the potential and foundation for a new pandemic.

8.3 *Lessons from the Haitian Epidemic*

Cholera’s unexpected emergence in the Americas and the Caribbean islands after 100 years of absence are tragic reminders about the speed at which infectious diseases can be transmitted globally even to other nonendemic countries. The bacteria becomes more hostile when it reaches an immunologically naïve population as happened in Haiti. Although we are passing through the twenty-first century, cholera still remains an epidemic or endemic disease in much of the developing world. New epidemic strains are likely to develop, evolve, and spread. *V. cholerae* cannot be eradicated as it is a part of the normal flora and ecology of the surface water of our planet (Sack et al. 2004). Cholera always teaches us the hard lessons that no one should lack access to basic clean water and sanitation (Guerrant 2006). The Haitian epidemic shows that as long as cholera exists anywhere in the world, many who drink untreated water and live in areas of poor sanitation are at risk. The epidemic also shows how cholera can emerge where it is least expected. An understanding of the ecology of the organism should help to

limit the times that human beings come into contact with this super-pathogen. Therefore, the ability to detect and confirm cholera needs to be broadly available. Recently developed molecular tools should be used for tracking the emergence and dissemination of the new variants of *V. cholerae* isolates. Implementing a coordinated, integrated, multidisciplinary approach is the only effective way to prevent and contain outbreaks among vulnerable populations living in high-risk areas. Prevention, preparedness, and response depend upon an effective and holistic surveillance system and are linked and interdependent. Although the real worldwide burden of cholera is still unknown, and will not be known until the notification of suspected and confirmed cases becomes mandatory. Denial of cholera incidence is so rampant that in this modern age of information most authorities guess that WHO figures include less than 10 % of cholera cases—probably as low as 1 % (Christopher 2009).

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