GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

VIBRIO CHOLERAЕ AND CHOLERA BIOTYPES

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Summary

Vibrio cholerae, with strains that cause cholera, is a motile, Gram-negative curved rod belonging to the family *Vibrionaceae*. Although approximately 200 recognized O serogroups have been identified, only serogroups O1 and O139 strains are known to cause severe disease and cholera pandemics. Intestinal and/or extra-intestinal infections with non-O1 and non-O139 serogroups or non-toxigenic O1 strains are seldom found and seem to have little impact on public health. Serogroup O1 and O139 pandemic strains exist as natural inhabitants of aquatic ecosystems, making them facultative human pathogens. *V. cholerae* serogroup O1 has two biotypes (classical and El Tor) and three serotypes (Ogawa, Inaba and Hikojima). The most prevalent serotype is Ogawa, whereas Hikojima is very rare and unstable in the environment. Non-O1 and non-O139 *V. cholerae* strains are more frequently isolated from rivers and estuarine areas compared to O1 and O139 strains. There is evidence that the cholera toxin (CT)-encoded phage CTX ϕ can transduce these non-toxigenic environmental strains, converting them into toxigenic strains, an event that has been postulated to also take place in the gastrointestinal environment resulting in the detection of toxigenic strains.

In the past, the permanence of toxigenic *V. cholerae* in the environment was considered short and cholera outbreaks have been mainly driven by fecal-oral transmission. Currently, it has been found that *V. cholerae* could survive for a long time in the water, assuming a viable, but non-culturable state. This finding pointed to a new hypothesis, namely that an environmental reservoir of *V. cholerae* is responsible for endemic cholera, and a corresponding cholera epidemiology framework was formulated that incorporates an environmental *V. cholerae* reservoir. The role of the aquatic environment on maintenance of cholera dynamics depends on the sanitary condition of the community. Endemism in a well-sanitized community requires a permanent environmental reservoir, while endemism in a poor community requires only a transient reservoir.

Onset of cholera as a disease is the result of a multifactorial process, involving several genes encoding virulence factors that aid *V. cholerae* in its colonization, coordinated expression of virulence factors, and toxigenicity. *Vibrio cholerae* also acquire virulence genes in a step-by-step process, in which a pathogenicity island and a temperate bacteriophage play important roles. Globally, cholera represents an estimated burden of 1.4 to 4.3 million cases and 28,000 to 142,000 deaths per year. Even though considerable efforts have been made by many countries to contain the spread of cholera, it is now endemic in many countries and there is growing concern over the increasing number of people living in unsanitary conditions and being at risk of contracting cholera and other epidemic-prone diarrhoeal diseases.

This chapter aims to provide the reader with a broad understanding of recent discoveries of *V. cholerae* and new recombinant *V. cholerae* biotypes and an outlook toward

future developments. Using the very latest international scientific research, this chapter outlines historical perspective and classification, methods (molecular and culture-based) proposed for the detection and isolation in waters and environmental occurrence, persistence and survival of *Vibrio cholerae* and their biotypes, the route of transmission and the global distribution of cholera as well as its control by vaccination and good sanitation and hygiene interventions to reduce the incidence of cholera outbreaks. A comprehensive and multidisciplinary approach to cholera prevention and control is provided to assist communities and policy-makers in averting cholera outbreaks.

Vibrio cholerae and Cholera biotypes

Vibrio cholerae is a normal inhabitant of aquatic environments such as drinking water, freshwater, wastewater, brackish water and sea water. This bacterium consists of a wide variety of strains and biotypes, capable of receiving and transferring genes for toxins (Waldor and Mekalanos, 1996), colonization factors (Brown and Taylor, 1995; Karaolis et al., 1999), antibiotic resistance (Hochhut and Waldor, 1999), capsular polysaccharides that provide resistance to chlorine (Yildiz and Schoolnik, 1999) and surface antigens such as the O139 lipopolysaccharide and O antigen capsule (Bik et al., 1995; Waldor et al., 1994). As a species, *V. cholerae* includes both pathogenic and nonpathogenic strains that generally differ in their virulence gene content (Faruque et al., 1998). Although, *V. cholerae* contains more than 200 serotypes based on its O antigen, only serotypes O1 and O139 are known as the causative agents of epidemic and pandemic cholera outbreaks (Bag et al., 2008). These serotypes are characterized by their ability to produce cholera toxin that is encoded by *ctx* gene (Kaper et al., 1995), which has been used for specific detection of cholera toxinogenic *V. cholerae* in environmental samples (Karunasagar et al., 1997). Certain phenotypic and genetic properties are used to categorise *Vibrio cholerae* O1 into two biotypes: classical and El Tor.

In recent years, the rise and spread of new pathogenic variants of *V. cholerae* has been reported throughout many countries, especially in Asia (Na-Ubol et al., 2011; Tran et al., 2012) and Africa (Ceccarelli et al., 2011; Naha et al., 2013) as well as in Mexico (Alam et al., 2012). These include the Matlab (MT) variant from Bangladesh (Nair et al., 2002) and the Mozambic variants, an atypical El Tor strain harbouring CTX ϕ ^{Cla} (Faruque et al., 2007b; Das et al., 2007). Similar strains carrying tandem copies of CTX ϕ ^{Cla} in the small chromosome or in large chromosome have also been isolated in other parts of world (Nair et al., 2006; Nguyen et al., 2009). The Haitian variant of the El Tor biotype has been reported to contain a unique mutation at the 58th nucleotide of *ctxB* (the gene for the B subunit of cholera toxin) (Nair et al., 2006) that has completely replaced the El Tor biotype on Kolkata, India, since 1995 (Raychoudhuri et al., 2009). Haitian *V. cholerae* strain also

had a novel mutation at the 64th amino acid position of the matured TcpA subunit (Naha et al., 2012; Ghosh et al., 2014).

Serogroups, which exclude cholerae O1 and O139, are categorized as *V. cholerae* non-O1 and non-O139, or non-agglutinating vibrios (NAGs) (Hasan et al. 2012; Dutta et al., 2013). Such non-agglutinating serogroups have less than 200 somatic (O) antigens and mostly lack cholera toxins coding genes (Shin et al. 2011; Marin et al. 2013). While the non-O1 and non-O139 serotypes are rarely associated with human infections, they are able to cause only gastroenteritis or even septicemia but not cholera disease (West, 1989; Powell, 1999). The infection by non-O1 and non-O139 is characterized by diarrhea, abdominal cramps, and fever in sever cases where this disease is self limiting and controlling in most of the cases. *Vibrio* species demonstrate a significant portion of the culturable heterotrophic bacteria of aquatic environments (Stabili and Cavallo, 2004). It is not easy to distinguish O1 from non-O1 by general laboratory methods (Singh et al., 2004).

The ability of *V. cholerae* to persist in the environment plays a role in the intensity of the cholera outbreak. This bacterial species has both a tendency to trigger epidemics with pandemic potential and the ability to stay endemic in all affected areas. In the epidemic setting, the entire population is susceptible to infection at the start of the epidemic due to little or naturally acquired immunity to cholera (Kaper et al., 1995; Faruque et al., 2007a). In contrary in highly endemic areas, the population had prior exposure to *V. cholerae* and at least some immunity to reinfection (Faruque et al., 2007a). In other words, in the endemic settings, there may be slower decay rates for *V. cholerae* in the environment, or positive growth during some seasons of the year. This can enable the long-term persistence of cholera even when conditions are not favourable for the transmission of the disease to human (Epstein, 1993; Colwell and Huq, 1994).

In general, cholera is endemic where socioeconomic conditions are poor, sanitary systems and public hygiene are rudimentary, and safe drinking water is not available, especially during floods. Such situations are even more hazardous to human health in countries in which fuel for boiling water is in scarce supply (Siddique et al., 1992). A broad understanding of recent discoveries of *V. cholerae* and new recombinant *V. cholerae* biotypes, as well as evolution and spread is therefore needed for an outlook toward future developments.

1.0 Epidemiology of the Disease and Pathogen(s)

Epidemiologically, cholera is described as a disease that has its tendency to cause explosive outbreaks and its potential to cause pandemics. It appears to exhibit three major epidemiological patterns: i) heavily endemic or epidemic in areas with poor sanitation and without clean water; ii) neo-epidemic in newly invaded cholera-receptive areas; and iii) occasional limited outbreaks in developed countries with good sanitation. Cholera has both a predisposition to cause epidemics with pandemic potential and an ability to remain endemic in all affected areas. While people of all ages are at risk of contracting the infection in epidemic settings, children older than two years are the target group mostly affected in these areas.

Historically, there have been descriptions of a disease resembling cholera in an ancient Indian medical treatise, the Sushruta Samhita, written in Sanskrit ~500 to 400 B.C. (Bishagratna, 1963). Historical records tracing back 2000 years in both Greek and Sanskrit also point to the description of diseases similar to cholera (Lacey, 1995). This means that cholera existed long before the 1817 pandemic, the first of the seven recorded pandemics (Colwell, 1996). In 1849, Dr John Snow, an English physician, recognized that cholera was spread through contaminated water and he devised methods for the prevention and control of this disease (Keen and Bujalski, 1992; Shears, 1994).

Vibrio as a microorganism was accurately described by Filippo Pacini in 1854 and isolated by Robert Koch and his collaborators in 1883. In 1885, Robert Koch discovered that *V. cholerae* was the causative organism of cholera and that the cholera toxin was found to be the agent leading to severe diarrhoea (Shears, 1994). In 1953, the two bacteriologists, De and Chatterjee in Kolkata, West India, discovered the crude cholera toxin, responsible for stimulating fluid secretion from the small intestine (Maheshwari et al., 2011). During the 19th century and even still today, the pread of cholera epidemics has resulted in many waves of infection. Since 1817, the world has acknowledged seven cholera pandemics. The first six pandemics are assumed to have started in the Ganges River delta. The seventh pandemic, which continues today, started in 1961 in Sulawesi, Indonesia (Shears, 1994). The first five pandemics were caused by the classical biotype, while the classical strains appear to be the causative agent of the sixth pandemic (Pollitzer, 1959). Between the sixth and seventh pandemics, there was an intervening period of 38 years during which cholera only occurred as local outbreaks caused by El Tor strains (Safa et al., 2010). Figure 1 illustrates the seven pandemic cholera events from 1817 to 2010.

Figure 1. The seven pandemic cholera events from 1817 to 2010. Reproduced with the permission of the authors Sara A and King R.Y. (Sara et al., 2010).

1.1 Global burden of disease

Worldwide, cholera still remains a major public health concern, although the risk of infection differs from one country to another. While cholera outbreaks have been reported with minor cases in Australia and America, the most affected continents are mainly Asia and Africa, with

Sub-Saharan Africa being the centre of several cholera epidemics and where the risk associated with cholera infection is high (Figure 2). Detailed accounts of the history of cholera are available, but the 1990s was found to be a crucial decade in the history of cholera as a remarkable increase in the global incidence of this disease was recorded. Thus, this section focuses on the reports of cholera outbreaks between 1995 and 2017.

Figure 2. Map of countries reporting on cholera between 2010 and 2015. [Extracted with the permission of WHO (2016)]

1.1.1 Global distribution

Basically, the majority of cholera cases are not reported

regularly by many countries and the limited factors include not only inadequate capacity of epidemiological surveillance and laboratories, but also the social, political,

and economic hindrances (Ali et al., 2015). The World Health Organization (WHO) maintains a repository of reported cases and deaths, and publishes annual statistics in the Weekly Epidemiological Record (WER). Nevertheless if the number of cholera cases occurring in a country is not reported, this country is automatically classified as a cholera free area.

According to WHO (2014), the cholera cases reported annually range between 5 and 10%. The reports of cholera provided by the WHO from 1995 to 2016 include the cholera outbreaks, deaths and CFR reported in Africa, Asia,

Europe, Americas and in Oceania, annually (WHO, 1996-2017). The numbers of countries in each continent have been determined based at least on one cholera case reported per year during the selected period. Taking into consideration this criterion, there has been at least one cholera case per year in 48 African countries, 38 countries in Asia, 24 countries in Europe, 23 countries in Americas and 7 countries in Oceania (Tables 1A and 1B). Although the European continent had a higher number of reporting countries than America, the majorities of outbreak cases reported in Europe were imported cases and the number of fatalities was very low.

Table 1A. Total number of cholera cases and deaths in the five continents reported by WHO, and case fatality^a rate(CFR), from 1995 to 2005. The total cases, deaths and case fatality rate for 95-2005 was 1,855,542 cases, 56,470 deaths and 3.04 CFR

Year		Continent (number of countries that report in each)					TOTAL
(Number reporting in that year) ^b		Africa (48)	Americas (23)	Asia (38)	Europe (24)	Oceania (7)	
1995	Cases	71,081	85,809	50,921	937	7	208,755
(78)	Deaths	3,024	845	1,145	20	0	5,034
	CFR ^a	4.2	0.98	2.24	2.13	0	2.41
1996	Cases	108,535	24,643	10,142	13	4	143,337
(67)	Deaths	6,216	351	122	0	0	6,689
	CFR	5.7	1.42	1.20	0	0	4.66
1997	Cases	118,349	17,760	11,293	18	5	147,425
(67)	Deaths	5,853	225	196	1	0	6,275
	CFR	4.9	1.26	1.8	7.69	0	4.25
1998	Cases	211,748	57,106	24,212	47	8	293,121
(74)	Deaths	9,856	558	172	0	0	10,586
	CFR	4.6	1.18	0.71	0	0	3.61
1999	Cases	206,746	8,126	39,417	16	5	254,310
(60)	Deaths	8,728	103	344	0	0	9,175
	CFR	4.2	1.26	0.84	0	0	3.60
2000	Cases	118,932	3,101	11,246	35	3,757	137,071
(56)	Deaths	4,610	40	232	0	26	4,908
	CFR	4.87	1.28	2.06	0	0.69	3.58
2001	Cases	173,359	535	10,340	58	19	184,311
(58)	Deaths	2,590	0	138	0	0	2,728
	CFR	1.49	0	1.33	0	0	1.48
2002	Cases	137,866	23	4,409	7	6	142,311
(52)	Deaths	4,551	0	13	0	0	4,564
	CFR	3.3	0	0.291	0	0	3.20
2003	Cases	108,067	33	3,463	12	0	111,575
(45)	Deaths	1,884	0	10	0	0	1,894
	CFR	1.74	0	0.288	0	0	1.69
2004	Cases	95,560	36	5,764	21	2	101,383
(56)	Deaths	2,331	0	14	0	0	2,345
	CFR	2.43	0	0.24	0	0	2.31
2005	Cases	125,082	24	6,824	10	3	131,943
(51)	Deaths	2,230	0	42	0	0	2,272
	CFR	1.78	0	0.62	0	0	1.72

^aCase Fatality Rate; ^bTotal countries reporting that year

Table 1B. Total number of cholera cases and deaths in the five continents reported by WHO, and case fatality^a rate(CFR), from 2006 to 2016.

The total cases, deaths and case fatality rate for 2006-2016 was 2,603,189 cases, 46,881 deaths and 1.8 CFR

Year		Continent (number of countries that report in each)					TOTAL
(Number reporting in that year) ^b		Africa (48)	Americas (23)	Asia (38)	Europe (24)	Oceania (7)	
2006	Cases	234,349	10	2,472	62	3	236,896
(52)	Deaths	6,303	0	8	0	0	6,311
	CFR ^a	2.69	0	0.32	0	0	2.66
2007	Cases	166,583	8	11,325	44	3	177,963
(53)	Deaths	3,994	0	37	0	0	4,031
	CFR	2.4	0	0.33	0	0	2.27
2008	Cases	179,323	7	10,778	22	0	190,130
(56)	Deaths	5,074	0	69	0	0	5,143
	CFR	2.83	0	0.64	0	0	2.7
2009	Cases	217,333	17	1,902	17	1957	221,226
(45)	Deaths	4,883	0	18	0	45	4,946
	CFR	2.53	0	0.95	0	2.3	2.24
2010	Cases	115,106	179,594	13,819	15	9000	317,534
(48)	Deaths	3,397	3,990	60	1	95	7,543
	CFR	2.95	2.22	0.434	6.66	1.056	2.37
2011	Cases	188,678	361,266	38,298	71	1541	589,854
(58)	Deaths	4,183	3205	426	0	2	7,816
	CFR	2.22	0.89	1.11	0	0.13	1.33
2012	Cases	117,570	120,433	7,369	18	5	245,395
(48)	Deaths	2,042	965	27	0	0	3,034
	CFR	1.7	0.8	0.4	0	0	1.23
2013	Cases	56,329	61,152	11,576	7	3	129,067
(47)	Deaths	1,366	636	100	0	0	2,102
	CFR	2.43	1.01	0.86	0	0	1.62
2014	Cases	105,287	28,456	56,787	17	2	190,549
(42)	Deaths	1,882	307	42	0	0	2,231
	CFR	1.79	1.08	0.07	0	0	1.17
2015	Cases	71,176	36,664	64,590	22	2	172,454
(42)	Deaths	937	337	30	0	0	1,304
	CFR	1.3	0.9	0.05	0	0	0.75
2016	Cases	71,058	42,595	18,445	22	1	132,121
(38)	Deaths	1,762	474	184	0	0	2,420
	CFR	2.5	1.1	1.0	0	0	1.83

^aCase Fatality Rate ; ^bTotal countries reporting that year

Table 2. Incidence of *V. cholerae* in African countries

Area	Incidence ^a	Reference
Angola	108,319	WHO 1996-2014 ^b
Benin	18,779	WHO 1996-2014
Botswana	23	WHO 1996-2014
Burkina-Faso	5,572	WHO 1996-2014
Burundi	17,542	WHO 1996-2014
Cameroon	59,876	WHO 1996-2014
Cape-Verde	561	WHO 1996-2014
Central African Republic	923	WHO 1996-2014
Chad	57,260	WHO 1996-2014
Côte d'Ivoire	15,635	WHO 1996-2014
Comoros	15,130	WHO 1996-2014
Equatorial Guinea	6,450	WHO 1996-2014
Ethiopia	113,080	WHO 1996-2014
Eritrea	120	WHO 1996-2014
Democratic Republic of the Congo (Previously named Zaire till 1997)	285,335	WHO 1996-2014
Congo	18,472	WHO 1996-2014
Djibouti	7,085	WHO 1996-2014
Gabon	630	WHO 1996-2014
Gambia	249	WHO 1996-2014
Ghana	57,690	WHO 1996-2014
Guinea	34,548	WHO 1996-2014
Guinea-Bissau	74,150	WHO 1996-2014
Kenya	76,203	WHO 1996-2014
Libya	22	WHO 1996-2014
Liberia	63,041	WHO 1996-2014
Madagascar	46,084	WHO 1996-2014
Malawi	82,972	WHO 1996-2014
Mayotte	12	WHO 1996-2014
Mali	10,233	WHO 1996-2014
Mauritania	9,316	WHO 1996-2014
Mozambique ^b (Beira)	NR	Deen et al., 2008
Mozambique	229,660	WHO 1996-2014
Namibia	9,141	WHO 1996-2014
Niger	19,719	WHO 1996-2014
Nigeria	40,000	Oladele et al., 2012
Nigeria	59,478	Oladele et al., 2012
Nigeria	119,556	WHO 1996-2014
Rwanda	7,769	WHO 1996-2014
Sao Tome and Principe	3,101	WHO 1996-2014
Senegal	58,291	WHO 1996-2014
Seycells	178	WHO 1996-2014
Sierra Leone	42,076	WHO 1996-2014
Somalia	223,060	WHO 1996-2014
South Africa	160,509	WHO 1996-2014
Sudan	58,074	WHO 1996-2014
Swaziland	7,112	WHO 1996-2014

Area	Incidence ^a	Reference
Uganda ^c (Kampala city)	6,228 in which 1,091 were children under five years of age	Legros et al., 2000
United Republic of Tanzania	5,028	WHO 1996-2014
Togo	12,097	WHO 1996-2014
Uganda	96,966	WHO 1996-2014
Zambia	55,087	WHO 1996-2014
Zimbabwe	144,982	WHO 1996-2014

^aIncidence: number of cases reported during the specific years of the data collection 1995 to 2013 unless otherwise noted; ^bWHO (1996; WHO,1997;WHO, 1998; WHO, 1999; WHO, 2000; WHO, 2001; WHO, 2002; WHO, 2003; WHO, 2004; WHO, 2005; WHO, 2006; WHO, 2007; WHO, 2008; WHO, 2009; WHO, 2010; WHO, 2011; WHO, 2012; WHO, 2013; WHO, 2014); ^cMicroorganism *V. cholerae* O1 and O139, Prevalence 4.0 E-03, Period of Study December 2003 to January 2004; ^dMicroorganism *V. cholerae* O1 El Tor, serotype Ogawa, Period of Study is December, 1997 to March, 1998.

Table 3. Incidence of *V. cholerae* in Asian countries from 1995 to 2013

Area	Incidence ^a	Reference
Afghanistan	82,853	WHO 1996-2014 ^b
Armenia	25	WHO 1996-2014
Bangladesh	0	WHO 1996-2014
Bhutan	44	WHO 1996-2014
Brunei Darussalam	96	WHO 1996-2014
Cambodia	8,677	WHO 1996-2014
China	20,711	WHO 1996-2014
Democratic Republic of Timor	30	WHO 1996-2014
East Timor	581	WHO 1996-2014
Hong Kong	168	WHO 1996-2014
India ^c (Kolkata)	NR	Deen et al., 2008
India	61,972	WHO 1996-2014
Indonesia ^d (Jakarta)	NR	Deen et al., 2008
Indonesia	2,411	WHO 1996-2014
Iraq	9,639	WHO 1996-2014
Iran	7,630	WHO 1996-2014
Israel	1	WHO 1996-2014
Japan	812	WHO 1996-2014
Kazakhstan	14	WHO 1996-2014
Kuwait	1	WHO 1996-2014
Lao Peoples Democratic Republic	14	WHO 1996-2014
Lebanon	1	WHO 1996-2014
Macoo	8	WHO 1996-2014
Malaysia	6,407	WHO 1996-2014
Magnolia	177	WHO 1996-2014
Myanmar	1,564	WHO 1996-2014
Nepal	4,609	WHO 1996-2014

Area	Incidence ^a	Reference
Oman	12	WHO 1996-2014
Pakistan	1,904	WHO 1996-2014
Philippines	8,321	WHO 1996-2014
Qatar	2	WHO 1996-2014
Republic of Korea	246	WHO 1996-2014
Saudi Arabia	38	WHO 1996-2014
Singapore	132	WHO 1996-2014
Sri Lanka	2,085	WHO 1996-2014
Thailand	4,504	WHO 1996-2014
Turkmenistan	55	WHO 1996-2014
United Arab Emirates	3	WHO 1996-2014
Vietnam	5,624	WHO 1996-2014
Yemen	32,144	WHO 1996-2014

^aIncidence: number of cases reported during the specific years of the data collection; ^bWHO (1996; WHO,1997;WHO, 1998; WHO, 1999; WHO, 2000; WHO, 2001; WHO, 2002; WHO, 2003; WHO, 2004; WHO, 2005; WHO, 2006; WHO, 2007; WHO, 2008; WHO, 2009; WHO, 2010; WHO, 2011; WHO, 2012; WHO, 2013; WHO, 2014); ^cMicroorganism is *V. cholerae* O1 and O139, Prevalence 1.6 E-03, Period of Study May, 2003 to April, 2005; ^dPrevalence 5.0 E-04, Period of Study August, 2001 to July, 2003

An estimated 3,907,290 cases of cholera outbreaks were reported between 1995 and 2013 globally (WHO, 2014). The most affected continent was Africa, with a rate of 68.90% of global outbreaks, followed by America, with 23.50%, and Asia, with 7.05%. The least affected continents were Oceania (0.41%) and Europe (0.04%). Moreover the global number of deaths was 96 030 cases of which 85.12% were reported in Africa, followed by 11.36% in the Americas and then 3.30, 0.17 and 0.02% in Asia, Oceania and Europe, respectively. After several years of steady increase (since 2007), the number of cholera cases reported to WHO, as well as the number of countries, which reported cholera cases, showed an important decrease in 2012 compared to 2011 in which the highest outbreaks was observed especially in Americans continent (WHO, 1996 to 2013).

In 2016, 38 countries reported a total of 132,121 cases of cholera including 2,420 deaths, giving a case fatality rate (CFR) of 1.83%. This represents a decrease of 22% compared to the number of cases (589,854) reported by 58 countries in 2011 and this is the second consecutive year in which there was a decline in reported cholera cases (WHO, 2012; 2017). Overall, Ali and co-workers estimated the global burden of cholera between 1.4 and 4.3 million cases, and between 28,000 and 142000 deaths per year (Ali et al.,

2012).

Using a multivariable spatial regression model, Ali and co-workers (2015) defined the status of a country as endemic, non-endemic or cholera free (Figure 3). This model predicted 1.3 billion people at risk for cholera in the 69 countries classified as cholera-endemic areas and another 99 million persons in the three countries (i.e., Bolivia, Pakistan, and Sri Lanka) at risk for non-endemic areas between 2008 and 2012. These authors pointed out that over ten million persons at risk of cholera resided in 23 endemic countries. The highest number of people at risk for cholera was found in India, Nigeria, China, Ethiopia and Bangladesh. Annually, estimates of more than 100,000 cases countries were found in India, Ethiopia, Nigeria, Haiti, the Democratic Republic of the Congo, Tanzania, Kenya, and Bangladesh. As can be seen in Figure 3, cholera remains a major public health problem in many countries in sub-Saharan Africa compared to the rest of the world. In endemic countries, Ali et al., (2015) highlighted that cholera resulted in approximately 95,000 deaths, figures that translate to approximately 7.50 deaths/100,000 population at risk per year in endemic countries. The countries with more than 1,000 deaths due to cholera annually, are found in the African region with the exception of India, Bangladesh, and Haiti (AMR).

Figure 3. Number of cholera cases reported annually in endemic countries (Source: Ali et al., 2015)

An epidemiological update of cholera outbreaks in selected countries of sub-Saharan Africa between 1994 and 2016

also highlighted the persistence of this disease in the region in spite of the decrease in case numbers that occurred in some countries (Table 4) (UNICEF, 2016).

Table 4. Epidemiological update of cholera outbreaks in selected countries of sub-Sahara African countries between 1994 and 2016 (UNICEF, 2016).

Area		Cases: 1994-2013	Cases in 2014	Cases in 2015	Cases in 2016
Benin	2004 to 2013	5,432	874	00	874
Cameroun	2004 to 2013	46,172	3,355	120	77
Côte d'Ivoire	2002 to 2013	7,573	248	200	16
Democratic Rep Congo	NR ^a	NR	19,305	18,403	28,162
Ghana	1998 to 2013	5,432	28,944	687	600
Guinea Bissau	1996 to 2013	74,031	18	00	00
Lake Chad Basin	2004 to 2013	31,918	41,188	6,084	883
Niger	1994 to 2013	21,538	2,059	51	38
Nigeria	2004 to 2013	105,483	35,996	5,913	768
Togo	2006 to 2013	2,142	329	50	02

^aNR: Not Reported

In the Democratic Republic of Congo, Siddique et al., (1995) reported a deadliest cholera epidemics in a refugee camps located around Goma and Bukavu. This explosive outbreak of cholera resulted in approximatively 70,000 cases and 12,000 deaths. In 2017, this country experienced an impressive outbreak that originated from North Kivu province, affecting mainly Goma, Karisimbi, Nyirango and

Binza. This resulted in a total of 15,456 suspected cases including 441 deaths (UNICEF, 2017). During the week 26 of the same year, 186 suspected cholera cases including 10 deaths were also reported in Nigeria by UNICEF (2017).

1.1.2 Symptomatology [morbidity and case fatality rates (CFRs)]

It is an ancient and devastating acute diarrheal disease caused by toxigenic *V. cholerae*. This bacterium infects only humans and other primates. *Vibrio cholerae* causes a spectrum of infections in both adults and children ranging from asymptomatic colonization to severe and rapidly fatal secretory diarrhoea known as cholera gravis. Although diarrhea is a common symptom for various enteropathogenic microorganisms (e.i viruses, bacteria and protozoan parasites) or the body's reaction to bad food, it results in a dangerous loss of fluids in the case of cholera-related diarrhea. Generally, the cholera-related diarrhea is of a watery consistency, and presents a pale milky appearance, which can occur very suddenly and can lead to

various complications. The infection by non-O1 and non-O139 is also characterized by diarrhea, with acute abdominal cramps, and fever in sever cases where this disease is self limiting and controlling in most of the cases.

As can be seen in Figure 4, at least one cholera case was reported between 1995 and 2016 in all the five continents of the world. The WHO's reports during this period also provide the estimated outbreak cases, number of death cases and (CFRs) for each of these continents, with a rising evidence of the large and growing burden of cholera in Africa (Table 1A). Total cholera outbreaks in over all five continents (Africa, Americas, Asia, Europe and Oceania) during 21 years between 1995 and 2016 resulted in 4,407,445 cases and the total cholera deaths of 62,865 with CFR (1.42%) (Tables 1A and 1B).

Figure 4. Number of cholera cases in Africa, Americas and Asia and global number of countries containing even at least one cholera outbreak case during the period extended from 1995 to 2016 (Adapted from WHO, 1996-2017).

In 2015, 42 countries reported a total of 172,454 cases including 1,304 deaths, resulting in an overall case fatality ratio (CFR) of 0.8%. Cases were reported from all regions, including 16 countries in Africa, 13 in Asia, six (6) in Europe, six (6) in the Americas, and one (1) in Oceania. Afghanistan (no. of cases 58,064), the Democratic Republic of the Congo (DRC) (no. of cases 19,182), Haiti (no. of cases 36,045), Kenya (no. of cases 13,291), and the United Republic of Tanzania (no. of cases 11,563) accounted for 80% of all cases. Of cases reported globally, 41% were from Africa, 37% from Asia and 21% from Hispaniola. Imported cases were reported from 13 countries (WHO, 2016).

In 2016, 38 countries reported a total of 132,121 cases including 2,420 deaths, resulting in an overall case fatality rate (CFR) of 1.8%. Cholera was reported from countries in all regions: 17 countries in Africa, 12 in Asia, four (4) in the Americas, four (4) in Europe and one (1) in Oceania. Five

countries - the Democratic Republic of the Congo (DRC) (no. of cases 28,093), Haiti (no. of cases 41,421), Somalia (no. of cases 15,619), the United Republic of Tanzania (no. of cases 11,360) and Yemen (no. of cases 15,751) - together accounted for 80% of all cases. Of cases reported globally, 54% were from Africa and 13% from Asia (WHO, 2017). In 2017, the most recent cholera outbreaks were observed in Yemen between April and August months. The total suspected cholera cases were 503,484 and the death cases were 1,975 with CFR 0.4% (EWARS, 2017).

Total cholera outbreak cases in Africa between the year 1995 and 2016 were estimated to be 2,941,788 case with a total of 86,330 death cases (CFR 2.93%). The highest outbreak cases of 234,349 (CFR 2.66%) were recorded in 2006, followed by 217,333 cases (CFR 2.24%), 211,748 cases (CFR 3.61%) and 206,746 cases (CFR 3.60%) recorded in 2009, 1998 and 1999, respectively. In the sub-

Saharan Africa, the highest cholera outbreak that was recorded in South Africa in 2001 reached a total of 106,151 cases, followed by Angola in 2006 with a total of 68,257 cases. Nigeria also reported a high cholera outbreak that reached a total of 44,456 cases (CFR 3.85%) (WHO, 1996-2017).

In the American continent, the highest number of cases was recorded in 2011 with a total number of 361,266 cases (CFR 0.89%), followed by 2012 with a total of 120,433 cases (CFR 0.80%). In Haiti, since the beginning of the epidemic in October 2010 and until December 2013, 696,794 cholera cases were reported, of which 389,903 individuals were hospitalized (56%) and 8,531 died (CFR 1.22%). The CFR ranged from 4.4%, in the Department of “Sud Est” to 0.6% in Port-au-Prince. During 2013, 58,809 cases including 593 deaths were reported (CFR 1%), which shows a 47% decrease compared to the cases reported in 2012 (112,076). In 2016, this country struggled with the hurricane Matthew, which resulted in almost 27,000 cholera cases and 240 deaths according to the UN's Central Emergency Response Fund (UN, 2016). Between 2010 and 2016, cholera has plagued at least 770,000 Haitians and caused over 9,200 lives. The cholera epidemic in Haiti alone resulted in an increase of 85% in the number of cholera cases worldwide. The second highest outbreak was recorded in Peru in 1995 and 1998 with a total number of cases of 22,337 and 41,717, respectively. In Peru, the

second highest outbreak was recorded in 1995 and 1998 with total cases of 22,337 and 41,717, respectively (WHO, 2016).

In Asia the highest numbers were recorded in 1995 with a total of 50,921 cases (CFR 2.24%) and in 2010 with a total of 13,819 cases (CFR 0.434%), while these numbers increased to 56,787 cases in 2014 (CFR 0.07%) and 64,590 cases (CFR 0.05%) in 2015 (Table 1; Figure 4) (WHO, 1996-2017).

1.2 Taxonomic classification of *Vibrio cholerae*

Vibrio cholerae is a curved Gram-negative, facultative anaerobic, rod-shaped, non-spore-forming bacterium, which is highly motile with either unipolar or bipolar flagella, that belongs to the family Vibrionaceae and shares common characteristics with the family Enterobacteriaceae (Baumann and Baumann, 1984).

Vibrio cholerae is not homogeneous and can include over 200 serogroups based on its O antigen (Shimada et al., 1994; Yamai et al., 1997). Of all these serogroups only serogroups O1 and O139 are able to cause cholera epidemics or pandemics. As stated above, the non-O1 and O139 serogroups are rarely associated with human infections, causing mild gastroenteritis or septicaemia (West, 1989; Powell, 1999). Figure 5 illustrates *V. cholerae* and its biotypes and serotypes.

Figure 5. Schematic diagram illustrating *V. cholerae*, their biotypes and serotypes

Vibrio cholerae O1 and O139 serogroups are extremely similar in the composition of the lipopolysaccharides (LPS) (Hisatsune et al., 1993; Isshiki et al., 1996). The most notable differences between *V. cholerae* serogroups O1 and O139 are the lengths and composition of the O side chains of the cell-wall LPS. Unlike *V. cholerae* O1, which has long O-polysaccharide side chains, the LPS of *V. cholerae* O139 contains only short O-antigen side chains (Knirel et al.,

1997).

Epidemiologically, biotyping is viewed as an important classification method for *V. cholerae* O1 strains. There are two biotypes of *V. cholerae* serogroup O1, classified on the basis of various phenotypic and genotypic markers, namely classical and El Tor, and three serotypes: Ogawa, Inaba and Hikojima (Kaper et al., 1995; Faruque et al., 2007b). The

most prevalent serotype is Ogawa, while Hikojima is very rare and unstable in the environment. The differences between the classical and El Tor biotypes of *V. cholerae* O1 are as follows:

- El Tor possesses better adaptability to subsist in the environment and in the human host as they are better able to colonize the intestinal epithelium than the classical biotype (Finkelstein, 1996).
- However, the *V. cholerae* O1 classical biotype is suggested to be more toxigenic and virulent than El Tor (Huq et al., 1983; Koelle et al., 2005a).
- In terms of phenotype disparities, El Tor is sensitive to both Mukerjee El Tor phage 5 and 50 IU of polymycin B, while the classical biotype is sensitive to Mukerjee Classical phage IV. In addition to these, the El Tor biotype is agglutinated by chicken erythrocytes and gives a positive Voges-Proskauer test reaction while the classical biotype does not (Mandal et al., 2011).

Vibrio cholerae O1 classical (CL) biotype is assumed to be the main causative agent of the first six cholera pandemics, resulting in both symptomatic and asymptomatic cases. Thereafter it became extinct and the seventh and current cholera pandemics have been associated with the El Tor biotype, which first appeared in 1905 in the village of El Tor, Sinai, Egypt and caused more asymptomatic infections than any other biotype. With the current development, altered *V. cholerae* O1 El Tor isolates produce cholera toxin of the classical biotype, but can be biotyped as El Tor by conventional phenotypic assays. The O139 serogroup (synonym: Bengal) is contained on different genetically-diverse strains, both toxigenic and non-toxigenic; it is genetically closer to El Tor *V. cholerae* (Ansaruzzaman et al., 2004).

The three Ogawa, Inaba and Hikojima serotypes are actually different, depending on their antigenic structures and are classified in terms of their somatic (O) antigen. The Ogawa serotype has A and B antigens, the Inaba serotype has A and C antigens, while Hikojima has unstable antigenic types containing all three antigens (A, B and C). The Ogawa and Inaba serotypes are positive for Ogawa and Inaba antisera, respectively. Hikojima serotype is positive for both Ogawa and Inaba antisera (Shimada et al., 1994; Mandal et al., 2011).

Choleraogenic *V. cholerae* are characterized by their ability to produce cholera toxin (CT) that is encoded by the *ctx* gene (Kaper et al., 1995). The first island, chosen CT, is a 7000–9700 bp region encoding no less than six genes (Waldor and Mekalanos, 1996; Mekalanos et al., 1997). The foremost of these gene products is CT, an oligomeric protein (MW 84,000) comprising five B subunits (*ctxB*) and one A subunit (*ctxA*). The B subunit binds holotoxin to the host cell receptor, while the A subunit is responsible for toxigenic activity intracellularly after proteolytic cleavage into 2 peptides, A1 and A2. The internal activation of the A1 peptide results in ADP-ribosyltransferase activity, which leads to the change of ion transport and hyper-secretion of water and Cl into the lumen of the intestine.

In recent years, there are emergence of new pathogenic variants of *V. cholerae*, which exhibit a combination of phenotypic and genotypic traits from the Classical and El Tor biotypes and were classified as hybrid biotype (Asish et al., 2014). The rise and spread of these variants has been reported throughout many countries, especially in Asia (Na-Ubol et al., 2011; Tran et al., 2012) and Africa (Ceccarelli et al., 2011; Naha et al., 2013) as well as in Mexico (Alam et al., 2012). They include the Matlab (MT) variant from Bangladesh (Nair et al., 2002) and the Mozambic variants, an atypical El Tor strain harbouring CTX ϕ Cla (Faruque et al., 2007b; Das et al., 2007). Similar strains carrying tandem copies of CTX ϕ Cla in the small chromosome or in large chromosome have been also isolated in other parts of world (Nair et al., 2006; Nguyen et al. 2009). The Haitian variant of the El Tor biotype contains a unique mutation at the 58th nucleotide of *ctxB* (the gene for the B subunit of cholera toxin) (Nair et al., 2006). This strain has completely replaced the El Tor biotype on Kolkata, dia since 1995 (Raychoudhuri et al., 2009). The Haitian *V. cholerae* strain also had a novel mutation at the 64th amino acid position of the matured TcpA subunit (Naha et al., 2012; Ghosh et al. 2014). Extensive reviews and studies on numerous aspects of the cholera toxin are currently available (Kaper et al., 1995; Sears and Kaper, 1996; Scott et al., 1996; Reidl and Kiose, 2002; Edwards and March, 2007; Chen et al., 2008; Ghosh-Banerjee et al., 2010; Maheshwari et al., 2011).

1.3 Transmission

Cholera disease is usually transmitted by ingestion of food or water contaminated directly or indirectly by the excretions of infected people, e.g. faeces or vomit (Tauxe and Blake, 1992; Rabbani and Greenough, 1999). In historical treaties on cholera, seaborne transport of cholera provides the fundamental theory of propagation. However, early records show an association with contaminated water, rivers or swampy areas, or marshes where the streams were much reduced (Colwell, 1996). Cholera infects only humans and always transmits by fecal oral route; there is no insect vector or animal reservoir hosts (WHO, 2010a), but *V. cholerae* is able to accumulate in zooplankton, which are fed-upon by oysters (Colwell, 2013).

1.3.1 Routes of transmission

Two main transmission routes of *V. cholerae* have been documented. The first and primary route is via the aquatic environment, and the second route occurs when the infection is transmitted from infected to healthy people by water or food contamination (Ruiz-Moreno et al., 2010). Once the primary transmission has initiated an outbreak, the secondary transmission causes epidemics in the endemic areas.

In endemic areas, the aquatic environment is the main route of transmission for cholera disease. Cholera is usually transmitted by ingestion of drinking water contaminated with sewage (Tauxe and Blake, 1992). Sewage contamination of groundwater in Delhi was responsible for the epidemic of *V. cholerae* (Pathak et al., 1993). In Haiti in

2010, cholera infection was documented along the Artibonite River (Barzilay et al., 2013; Gaudart et al., 2013). Several studies have reported the isolation and detection of *Vibrio* species and *V. cholerae* from a variety of aquatic environments, including drinking water (Blake et al., 1977; Ries et al., 1992; Shapiro et al., 1999; Downs et al., 1999), freshwater (Hughes et al., 1982; Grim et al., 2010; Madoroba and Momba, 2010; Rashid et al., 2013), wastewater (Momba et al., 2006; Igbinosa et al., 2009; Nongogo and Okoh, 2014), brackish water (Reilly and Twiddy, 1992; El-Lathy, 2009), and sea water (Colwell et al., 1977; 1981; Lipp et al., 2003; Gazi et al., 2010; Rashid et al., 2013). There is a relationship between *V. cholerae* and planktonic crustacean copepods, which leads to the contamination of water. Hug and co-workers (1983) have pointed out that several copepods with *V. cholerae* cells attached to the surface and in the gut may contain up to 10^4 cells of *V. cholera*, a concentration, which is the prerequisite infectious dose for clinical cholera. During a plankton bloom, these copepods may be ingested in a glass of water if this water is not subjected to any treatment. This may increase the chances of consuming water contaminated with *V. cholerae*.

Another route of cholera transmission during inter-epidemic periods via water environments is horizontal transfer of virulent genetic matter from non-predominant toxigenic *V. cholerae* to predominant non-toxigenic *V. cholerae*. This may occur in both *V. cholerae* O1 and non-O1, which survive in the same water environments, whereas non-toxigenic *V. cholerae* non-O1 predominates over toxigenic O1 bacteria. In some unfavourable conditions and as a result of lysogeny and horizontal gene transfer, the genetic material such as cholera toxin (CTX) gene and other virulence factors have been reported to be transferred to non-toxigenic *V. cholerae* (Faruque et al., 2004; Valdespino and Garcla-Garcla, 2011).

Cholera diseases are able to spread via food, and thus infection due to *V. cholerae* begins with the ingestion of contaminated water or food. Transmission of cholera in non-endemic areas is more frequently associated with consumption of foods, such as raw or undercooked sea food, imported from cholera-endemic areas (Mandal et al., 2011). In many countries, consuming contaminated uncooked vegetables and seafood is a long-standing source of cholera outbreaks. Vegetables are possibly contaminated during pre- and post-harvest due to irrigation or wash waters contaminated with *V. cholerae* (Wachsmuth et al., 1994; Faruque et al., 1998; Anon, 2000). Fruits, vegetables, sea food, fish, shellfish, crabs and oysters, even poultry, red meat and dairy products may play a vital role in the transmission of cholera in many areas (Maheshwari et al., 2011). Fish samples from fresh and marine waters carry *V. cholerae* and it has been reported that fish intestines contain *V. cholerae* counts of up to 5.0×10^3 CFU/g (Senderovich et al., 2010).

Cholera may also be transmitted by an additional route; it is transported over long distances by human migration. It has been suggested that *V. cholerae* may have re-entered South America in 1991, after a century of absence, from the bilge and ballast water of cargo ships (WHO, 2003a).

1.3.2 Reservoirs

Asymptomatically infected people having sub-clinical doses of *V. cholerae* are some of the main reservoirs of cholera. Once conditions are favourable, those people are able to spread the disease. The aquatic environments can also be a reservoir for *Vibrio cholerae* even in the absence of outbreaks (Akoachere and Mbuntcha, 2014). Since the nineteenth century, the physician Snow recognized that water acts as a reservoir for cholera diseases and that *V. cholerae* is able to spread by infected water (Shears, 1994). Recently many researchers have confirmed that the different water sources are able to serve as a reservoir for *V. cholerae* and responsible for spreading cholera disease (Colwell and Huq, 1994; Shapiro et al., 1999; Blokesch and Schoolnik, 2007). Akoachere and Mbuntcha (2014) investigated well-, tap- and stream-water samples in Bepanda, Cameroon, as reservoirs of *Vibrio cholerae*; they found that 33% of 96 isolates were confirmed as *V. cholerae* O1.

Available literature suggests that fish act as reservoirs of *V. cholerae*. Cholera has been associated with consumption of fish in several countries: India (Pandit and Hora, 1951), Germany (Schürmann et al., 2002), Japan (Kiiyukia et al., 1992), Thailand and Srilanka (Plesnik and Prochazkova, 2006), Tanzania (Acosta et al., 2001), and Peru (Carvajal et al., 1988). Numerous reports (Huq et al., 1983; Dumontet et al., 1996; Bhaskar et al., 2004; Halpern et al., 2004; Jeffs et al., 2004) have pointed out that macroalgae, seafood, and marine crustaceans such as copepods and chironomid egg masses act as environmental reservoirs of *V. cholerae*. Wakeham et al. (1997) suggested that the sediment of the equatorial Pacific Ocean is another environmental reservoir of *V. cholerae*.

1.3.3 Incubation period

The incubation period is usually very short and ranges from 2 h to 5 days. After a typical incubation period of 1 to 2 days, there can be an extended period up to 5 days in some cases; the infected people experience a sudden onset of watery diarrhoea. Vomiting is common and often precedes the onset of diarrhoea with fluid loss often 500 to 1,000 mL/h, leading to severe dehydration. Over a 24 h period, up to 20 litres of watery diarrhoea can be excreted in adults. If left untreated, the mortality rate may reach 40 to 60% (Speck, 1993; Sack et al., 2004; WHO, 2009a). The stool of the infected people became highly infectious containing high counts of cholerae *V. cholerae* up to 10^9 CFU/mL of stool (Dizon et al., 1967). Without antibiotic treatment, organisms are secreted in the stool for an average of 6 days (Lindenbaum et al., 1967). The incubation period for non-cholerae *Vibrio* spp. infection is usually 12 h to 3 days, but can be extended for up to one week (Seas and Gotuzzo, 2009).

1.3.4 Period of communicability

After ingestion, *V. cholerae* colonizes the small intestine for 12 to 3 days asymptotically. Then the symptoms of cholera begin to appear with stomach cramps and vomiting

followed by diarrhoea, which may progress to fluid losses up to 1 L/h. Rice water stools of the infected people can carry *Vibrio cholerae* counts ranging between 10^{10} and 10^{12} CFU/L (Phillips, 1964; Nelson et al., 2009).

Symptomatic patients may shed *V. cholerae* before the onset of illness (Cash et al., 1974) and will continue to shed those organisms for 1 to 2 weeks (Kaper et al., 1995). The minimum infective dose of *V. cholerae* is about 10^3 cells (Bitton, 2005). Asymptomatic patients typically shed *Vibrio* organisms in their stool for only one day, at approximately 10^3 CFU/g of stool (Mosley et al., 1968; Nelson et al., 2009). Therefore, the distribution of symptomatic patients influences the quantity of *V. cholerae* that is shed for subsequent transmission. The infected people who have not received any antibiotics for treatment may be continuously shedding *Vibrio cholerae* for one to two weeks. Less than 1% of patients continue shedding the organisms in their faeces for extended time periods, in some cases reaching up to 10 years and have positive stool cultures for *Vibrio cholerae* after an initial case of cholera (Azurin et al., 1967; Dizon, 1974; Kaper et al., 1995).

1.3.5 Population susceptibility

Historically, *V. cholerae* infection has had a profound effect on human populations, including their evolution and cultural development. It is important to note that the susceptibility to cholera depends on a number of factors, which include local intestinal immunity from previous exposure or vaccination, bacterial load and intrinsic host factors such as stomach pH (gastric acid providing a barrier) and blood group (Sack et al., 2004).

In general, exposure to *V. cholerae* through contaminated drinking water or food products such as undercooked seafood can lead to cholera infection in any population category, while immuno-compromised individuals, elderly people and children at the highest risk. Risk factors for the disease include poor sanitation, not enough clean drinking water, and poverty. Sporadic outbreaks can be due to contamination of drinking water and food in highly populated areas harbouring endemic toxigenic *V. cholerae*. Water sources contaminated with *V. cholerae* are probably the main origin of epidemics, followed to a lesser extent by contaminated food, especially seafood products like oysters, crabs, and shellfish (Kaysner and Hill, 1994). Drinking unboiled or untreated water is a risk factor for cholera, while the use of soap is typically associated with a lower likelihood of infection (O'Connor et al., 2011). In food-borne outbreaks, the risk factors may include consumption of specific contaminated foods, such as rice products, vegetables or fruits (Rabbani and Greenough, 1999). In regions where cholera is sporadic, for example in the United States, most cases are linked to consumption of undercooked shellfish including oysters.

Displacing large populations and the collapse of infrastructure, associated with natural disasters and intensified by climate change, provide an ideal ground for cholera (HolNAer et al., 2010). Climate variability has been assumed to have the ability to directly influence the outbreaks of infectious diseases, such as cholera. A long-

term study on El Tor cholera in Matlab, Bangladesh that spanned a period of four decades has established such a correlation (Koelle et al., 2005b).

Recently, Bavishi and Dupont (2011), found that the other risk factors for cholera infection are related to specific biological interactions between host and pathogen. For example, individuals with hypochlorhydria (reduced gastric acid) are much more prone to develop cholera. This may be due to the fact that gastric acid is a key barrier that protects individuals against infection.

Cholera and other waterborne diseases pose an growing threat to people already living at the margins of survival, where populations with a high predominance of blood type O are especially at risk. People with blood type O are more expected to develop severe cholera (cholera gravis) than individuals having other blood types. This has been demonstrated in various case-control studies which showed that people with blood type O were at increased risk of hospitalization due to *V. cholerae* O1 biotype El Tor as well as to *V. cholera* O139 (Glass et al., 1985; Swerdlow et al., 1994; Faruque et al., 1994; Tacket et al., 1995; Harris et al., 2005; Harris et al., 2008). However, no such association has been found for cholera infection with classical *V. cholera* strains (Clemens et al., 1989). A recent study by Heggelund and co-workers (2012) has revealed that the cholera toxin is assumed to be the main culprit of this blood type dependence as they found that both El Tor and classical cholera toxin B-pentamers bind blood type determinants (with equal affinities). It is therefore confirmed that blood-type dependence is strain specific. The shorter blood type H-determinant characteristic of blood type O individuals binds with similar binding affinity compared to the A-determinant, however, with different kinetics (Heggelund et al., 2012).

Although many factors are involved in *V. cholerae* virulence, the key agent responsible for the severe diarrhoea is the cholera toxin (CT), which is secreted from vibrios co-expressing a bacteriophage (Davis et al., 2000; Davis and Waldor, 2003). The most recent study in cholera endemic areas in Bangladesh has revealed a direct relationship between susceptibility of cholera infection and specific genes in some people. A number of genetic regions, including genes related to inherent immunity and potassium channels, are found to be associated with cholera (Karlsson et al., 2013). A study by Harris and co-workers (2008) also demonstrated that IgA antibodies, a type of antibody associated with the mucosal immune system, such as the mucosal surface of the intestinal tract, that target several components of the bacteria, are associated with immunity to *V. cholerae* infection.

Natural infection with *V. cholera* O1 has been reported to induce adaptive immune responses that provide protection against subsequent reinfection with the same pathogen. Studies conducted with volunteers in non-endemic settings have shown that infection with classical biotype *V. cholera* O1 provides 100% protection from subsequent challenges with a classical biotype strain. However, infection caused by the El Tor biotype of *V. cholera* O1 results in 90% protection from subsequent

challenges with an El Tor strain. It was found that individual volunteers were protected for a period of 3 years (Levine et al., 1981). In an endemic area of Bangladesh, Glass and co-workers (1982) reported an initial episode of El Tor cholera, which reduced the risk of a second cholera infection by 90% over the next several years.

The nutritional status of households has been identified as another risk factor for cholera infection. Evidence has shown that increasing levels of retinol (Vitamin A) deficiency are associated with both a higher risk of infection with *V. cholerae* O1 and a higher likelihood of developing symptomatic disease if infected (Harris et al., 2008).

Although all age groups seem to be susceptible to cholera infection, still the majority of children are at high risk (Glass and Black, 1992; Sack et al., 2003; Harris et al., 2008). For example, in endemic areas of rural Bangladesh, especially at the Ganges River Delta, children are more likely to be hospitalized with severe illness (Glass et al., 1982). Hartley et al. (2006) reported that direct person-to-person transmission of cholera is still thought to be uncommon. In individual volunteer studies, the infective dose was determined to be 10^2 to 10^3 cells. However, the size of the inoculum needed to cause severe infection is based on the health status of the individual. Generally some 10^5 to 10^8 bacterial cells are required to produce disease in healthy people, but a very small inoculum can trigger the disease in individuals with low levels of gastric acid (Sack et al., 2004). Only a small inoculum is required to transmit the disease directly via the faeces of an infected individual, (Pascual et al., 2006).

1.4 Population and Individual Control Measures

In both endemic and epidemic cholera settings, the most efficient control measures are the early detection by well-established surveillance systems, announcement of the infection by the governments and health authorities and international organizations such as WHO and CDC, prompt treatment of infected people by administering appropriate medication, providing health education and proper disposal of human waste. In some instances, vaccination is required to protect the other people from cholera infections. Also, in some cases, restrictions on trade and import of mainly fish, seafood, and others foodstuffs from endemic areas in addition to travel restrictions offer some advantages to control the disease. But travellers to epidemic areas should be very careful about their eat and drink and scrupulous about personal hygiene. Surveillance by molecular epidemiology and antimicrobial susceptibility in addition to strain-tracking are important tools for cholera control at both national and international levels (WHO, 2014).

Although control measures should be put in place, the cheapest and most effective way of avoiding a cholera outbreak is by providing safe clean drinking water and improved sanitation infrastructure to all. Unfortunately, these basic requirements are still lacking in many parts of developing countries. Statistics mentioned in Table 1 reveal that hundreds of thousands of people have died of cholera in Africa and in Asia between 1995 and 2013. These

statistics are based only on reported cases while underreporting and/or underestimating cases remain common in the developing world.

1.4.1 Vaccines

1.4.1.1 Types of vaccine

Two types of cholera vaccines are available depending on the route of administration - via injection and oral. For the injectable version, killed whole-cell (WC) cholera vaccines were used during the nineteenth century and still up until 1970. Since then, no injectable vaccines have been used due to their low levels of efficacy, short duration of protection, and their known side-effects and unfavourable safety profile (Mosley et al., 1972). The second type is oral cholera vaccines, which are now widely used because they are easier to administer, more acceptable to recipients, and have a reduced risk of transmitting blood-borne infections (Holmgren and Czerkinsky, 2005; Shin et al., 2011).

The current internationally-licensed cholera vaccine contains, in addition to the killed whole cell vibrios of both classical and El Tor biotypes, the cholera toxin B subunit. This additive boosts short-term protection against *V. cholerae* infection (Clemens et al., 1989), acting as a potent mucosal immunogen and as an immunoadjuvant (Rask et al., 2000). The two major types of oral vaccines against cholera are the killed whole cell-based and the genetically attenuated live vaccines (Shin et al., 2011). Currently, there are two types of WHO-prequalified oral cholera vaccines, namely Dukoral® and Shanchol®, both of which are licensed in many countries (WHO, 2014).

1.4.1.1.1 Dukoral®

This vaccine is manufactured by Crucell Sweden AB, located in Stockholm, Sweden, and consists of killed whole cell *V. cholerae* O1 with purified recombinant B subunit (WC/rBS) of cholera toxin. Each dose must be diluted in 150 mL of bicarbonate buffer and in 75 mL for children. The immunization schedule varies with different age groups. It is administered to adults and children aged >6 years in 2 doses; and to children aged >2 years and <6 years in 3 doses. For both regimens, the recommended minimum time between the two doses is 7 days, whereas the maximum time is 6 weeks. Protection can be predictable after the last dose by one week. It was found that, Dukoral® also generates some cross-protection against enterotoxigenic *Escherichia coli* (ETEC). Field trials in Bangladesh and Peru have demonstrated that this vaccine is harmless and results in 85% protection for 4-6 months throughout various group ages. Although the protection degenerated sharply in young children after 6 months, it persisted at about 60% after 2 years in older children and adults (WHO, 2014). A re-analysis of the data obtained from the Bangladesh study when using a stochastic model of cholera transmission advocated that the effectiveness of WC/rBS is prominently boosted by herd protection (Faruque et al., 2007a). This vaccine is not approved for use in children aged less than 2 years.

1.4.1.1.2 Shanchol®

The Shanchol® vaccine is bivalent and contains killed whole-cell *V. cholerae* serogroups O1 and O139 and is manufactured by Shantha Biotechnics, located in Hyderabad, India; it does not contain a recombinant B subunit, and therefore does not need to be reconstituted in a buffer solution. The immunization schedule is 2 doses given at an interval of 2 weeks for those aged >1 year. Shanchol® has provided longer term protection than Dukoral® in children aged <5 years, and therefore does not require a booster dose after 6 months in this age group, unlike Dukoral®. Shanchol® provided 67% protection against clinically significant *V. cholerae* O1 cholera in an endemic area for at least 2 years after vaccination. A field trial in Kolkata, India showed a sustained protective efficacy (65%) of the vaccine up to 5 years (Bhattacharya et al., 2013). Annexure 2 illustrates a comparison of the currently available oral cholera vaccines.

1.4.1.1.3 Fecal shedding of live vaccine strains

A case study about the safety, immunogenicity and shedding level of a live oral *V. cholerae* O1 El Tor vaccine candidate, named Peru-15, was conducted in Bangladesh. It was found that *V. cholerae*, genetically identical with the vaccine strain, was isolated from the stool of only one volunteer (Qadri et al., 2005). Also, the rate of fecal shedding for the same oral *Vibrio* vaccine Peru-15 was found to be 0.7% in adults and 7.5% in children (Bhutta et al., 2009). Another live attenuated cholera vaccine candidate called *V. cholerae* 638 (Cuba) has been studied on volunteers in Havana, Cuba and Maputo, Mozambique during 2005 and 2007. It was found that the fecal shedding rates reached up to 46% in Mozambique. Moreover the fecal shedding rate was 0% in the study which was carried out in Kolkata, India during 1999-2004 on the live attenuated V 1.3 vaccine; this means that this vaccine is very safe (Bhutta et al., 2009).

1.4.1.2 Future aspects

Oral cholera vaccines offer short-term protection against cholera infection. However, access to treated and safe water, food hygiene and adequate sanitation are still important and are the main issues to be taken into consideration in control measures. The limitation of global production and supply of the licensed WHO-prequalified cholera vaccines is one of the most important challenges in the future. For example, since its inception in 2013, 1.4 million doses of oral cholera vaccines have been deployed from the stockpile. Thus, a rigorous system of short- and longer-term monitoring and evaluation embedded within the oral cholera vaccines stockpile deployment mechanism is required (WHO, 2014). The design of future vaccine evaluations and efficacy studies are needed to consider the role of herd protection. The hypothetical existence of significant herd protection will have implications for the choice of target populations for cholera vaccination. It is likely that access to the vaccine might be enhanced for groups who do not usually have access to or seek treatment. It remains to be determined how these factors will influence the development of strategies that focus on reaching a particular threshold level of vaccination in order to achieve an acceptable level of protection in a community

(WHO, 2006). Furthermore, there is a call for the development of improved vaccines that confer protection, especially for blood type O individuals (HolNAer et al., (2010). New vaccination strategies are needed to consider information on the blood-group dependence of the disease for this particular group of individuals.

1.4.2 Hygiene measures

Recently, countries have made great strides to prevent and respond to cholera outbreaks; nonetheless, many concerns remain about the high proportion of people living under unsanitary conditions. WHO/UNICEF (2012) estimated that 2.5 billion people still live without improved sanitation and these people are at risk of contracting cholera and other diarrheal diseases worldwide. There is a need to extend prevention measures in order to stop cholera and other waterborne diseases by intensifying access to improved sources of drinking water and improved sanitation, and by working hand in hand with communities to encourage the change of their behaviour in order to reduce the risks of infection (WHO/UNICEF, 2012). For example in Darfur, Sudan has been offered valuable indications of the cost, impact, and challenges of water and sanitation projects in complex emergencies and of the role of such projects in preventing cholera outbreaks. In May 2004, only 20% people of living in areas reachable by the United Nations agencies had access to adequate water, and only about 5% to proper sanitation; by September 2005, these ratios had risen to 52% and 76%, respectively. Clearly, despite the enormous effort provided by all humanitarian bodies active in the field for more than a year, a significant number of people still lacked access to minimum water supply and sanitation facilities (WHO, 2006).

2.0 Environmental Occurrence, Persistence and Survival

V. cholerae has been found not only in freshwater environments, but also in marine water, colonizing the water biota, particularly plankton and attached primarily to crustacean copepods in the zooplankton. Thus the cholera disease appears not to be eradicable due to the fact that *V. cholerae* is a natural inhabitant of the aquatic environment (Valdespino and García-García, 2011). De Magny and co-workers (2011) examined the incidence of cholera and occurrence of pathogenic *V. cholerae* including both serogroups O1 and O139 within zooplankton. They found that two dominant zooplankton groups were consistently associated with the detection of *V. cholerae* and/or occurrence of cholera cases, namely rotifers and cladocerans, in addition to copepods. Local differences indicate that there are subtle ecological factors that can influence interactions between *V. cholerae*, its plankton hosts, and the incidence of cholera.

2.1 Detection Methods

2.1.1 Culture-based methods

Detection, enumeration and identification of *V. cholerae*

from the environmental and medical samples by culture-based methods followed by biochemical and serological tests have been established in many standard methods (ISO, 2007; APHA, 2012; CDC, 2015). The procedures for detection of *V. cholerae* by culture-based methods includes growth in enrichment by using salt-free non-selective enrichment media such as alkaline peptone water (APW), buffered peptone water (BPW), brain heart infusion (BHI), marine broth or Luria broth (LB) at 37°C for 18-24 h. This step is very important for recovering the stressed or injured *V. cholerae* cells, therefore avoiding the false-negative results (Reissbrodt et al., 2003). Thereafter, selective isolation of *Vibrio* species is achieved by plating on a selective agar culture plate, namely thiosulphate citrate bile salts sucrose (TCBS) agar, for 24 to 48 h at 37°C, which allows colonies of *V. cholerae* to ferment sucrose and produce yellow colonies. The TCBS agar is still a golden standard culture medium. It is widely used and extremely useful for isolating *V. cholerae* from different water and food samples and as well as from human clinical specimens (Colwell, 1984; Farmer et al., 2003; 2005). These yellow colonies are subjected to further biochemical and serological tests for the identification of *V. cholerae* (Cash et al., 1974; Nair et al., 1987).

During a cholera outbreak, there is no need to do a large number of biochemical tests to confirm a culture as *V. cholerae*. Agglutination in *V. cholerae* O1 or O139 serum is a diagnostic method that can be followed by biochemical testing for the first few isolates to confirm the presence of this pathogen (APHA, 2012). Haemolysis of sheep blood cells has been traditionally used as one of several tests to distinguish the two biotypes of *V. cholerae* O1. The classic biotype is non-haemolytic, while the El Tor biotype is haemolytic; non-O1 *V. cholerae* strains are usually haemolytic (Karaolis et al., 2001). Commercial immunoassays for testing the enterotoxin of *V. cholerae* are currently available and they allow growing the culture in special media (APHA, 2012). Recently the chromogenic and fluorogenic media such as chromID™ *Vibrio* Agar, HiCrome *Vibrio* agar, *Vibrio* chromogenic agar, and chromogenic *Vibrio cholerae* agar for the detection of *Vibrio cholerae* and other *Vibrio* species have been used (Duan and Su, 2005; Richards et al., 2005; Eddabra et al., 2011).

Madoroba and Momba (2010) collected 594 water samples aseptically once a month over a 4-month period from 32 sampling sites on major rivers in the Mpumalanga Province of South Africa. They used three different approaches for collecting samples from the above-mentioned rivers. Two of the sampling methods involved placing either sterile Moore gauzes or tampons (tied at the centre to strong twine) in duplicate in flowing water at various river sites. Weights were also tied to the gauzes and tampons to ensure their submergence in the river water. The gauzes and tampons were left in the various rivers for 5 - 6 days in order to maximize trapping of *V. cholerae*. The third method of sampling involved the collection of 5 L of water in sterile containers, followed by sealing of the containers and appropriate labelling. They found that almost similar numbers of *V. cholerae* were obtained from water samples that were collected in sterile containers directly (7 isolates) and from gauzes that were

submerged in the river water for 5 to 6 days (8 isolates). No *V. cholerae* were isolated from water samples collected using tampons. They concluded that both methods are suitable for isolating these bacteria from environmental samples. Tampons were found to be unsuitable for obtaining *V. cholerae* from water sources as no isolates were recovered with this method. This may be due to some inhibiting substances present in tampons.

2.1.2 Molecular-based methods

Several molecular-based methods have been used for *V. cholerae* O1 and O139 detection in environmental samples. These methods have high sensitivity, specificity, and speed. The methods used for *V. cholerae* detection by conventional PCR and/or real-time PCR are well established (Wang et al., 2007; Tebbs et al., 2011; Teklehaimanot et al., 2014). Many research studies have identified *Vibrio cholerae* strains using 16S rRNA sequencing, pulsed-field gel electrophoresis (PFGE), nested and multiplex PCR (Chakraborty et al., 1999; Kong et al., 2002; Tarr et al., 2007; Mendes et al., 2008; Keshav et al., 2010). Moreover, molecular detection and characterization of virulence factors and cholera toxin genes (*ctxs*) of *Vibrio cholerae* have been investigated in many studies (Lipp et al., 2003; Blackstone et al., 2007; Son et al., 2011).

Other rapid methods have been developed for environmental monitoring of *V. cholerae*, including the use of monoclonal fluorescent antibodies and immunochromatographic dipsticks for LPS detection (Nato et al., 2003; Goel et al., 2005). Chen et al. (2014) developed a new monoclonal antibody (McAb) pair, named IXiao3G6 and IXiao1D9, which is specifically against *V. cholerae* O1 serotype Ogawa. In addition, they developed immunochromatographic lateral flow device (LFD) using this McAb pair for the highly specific and rapid (within 5 min) detection of Ogawa.

Wang et al. (2010) developed an immunofluorescent-aggregation (IFAG) assay to detect *V. cholerae* O1 and O139 in estuarine water samples. They compared the results of IFAG with the conventional culture method and real-time PCR. Their results showed that the percentage positive by the IFAG assay method was 19.9%, which was significantly higher compared to the conventional culture method (10.3%), but lower than that of real-time PCR (29.5%). They concluded that the IFAG method, with a high specificity and a relatively high sensitivity, may be used for the detection and isolation of *V. cholerae* in environmental water samples.

Recently, Yu et al. (2015) developed a nucleic acid-sensing platform for detection of toxigenic *Vibrio cholerae* serogroups O1 and O139. The sensitivity and specificity (100%) of the platform showed excellent diagnostic capabilities when tested with 168 spiked stool samples. Their study highlighted the potential of combining a dry-reagent-based nucleic acid amplification assay with an electrochemical genosensor in a more convenient, sensitive, and sequence-specific detection strategy for multiple target nucleic acids.

2.1.3 Immune-diagnosis/detection of the pathogen infection

Challenges still remain vis-à-vis the diagnosis and detection of *Vibrio cholerae* infections, due to limited resources especially in developing countries. The most commonly performed methods for serodiagnosis of *Vibrio cholerae* O1 infection comprise the measurement of antibacterial antibodies by agglutination or vibriocidal antibody essays (Barret et al., 1994). The vibriocidal antibody test has been used widely for serologic surveys as it is a friendly method based on the titration of large numbers of sera by microtiter procedure. According to Barret et al., (1994), the acquisition of antibody through natural exposure or vaccination has demonstrated obvious correlation to immunity on a population basis and in volunteers challenged with the organism.

More procedures such as the indirect hemagglutination test and enzyme-linked immunosorbent assays (ELISAs) and cell culture models for detecting cholera toxin (CT) have been also reported to provide marked improvement in the ability to measure antitoxic antibodies (Barret et al., 1994). Rapid diagnostic tests such as reverse passive latex agglutination test (RP LA) have also been proved effective for the detection of CT (Yamasaki et al., 2013). Recently, various tests have been developed and they mostly focus on the detection of the lipopolysaccharide of *V. cholerae* O1 and O139 by monoclonal antibodies, using the vertical-flow immunochromatography principle (Keddy et al, 2013). These are commercial membrane-based rapid diagnostic tests that have been used to detect the presence of cholera infection under laboratory and field conditions with variable sensitivity and specificity. The immunochromatographic Test Strip developed by Yamasaki et al., (2013) demonstrated the capability to detect the CT in culture supernatant of all 15 toxigenic *V. cholerae* isolates examined, whereas no false-positive signal was detected in all 5 nontoxigenic *V. cholerae* isolates examined. A combination of different target analytes for example, immunoassay which detects the existence of CT can result in the surveillance of toxigenic *V. cholerae*.

2.2 Data on occurrence

2.2.1 Excreta in the environment (fecal waste, night soil and dry latrines)

The occurrence of toxigenic *V. cholerae* in water is typically associated with fecal pollution. Several research studies have detected *V. cholerae* in human, domestic and farm animal faeces (Kay et al., 1994; Visser et al., 1999; Cox et al., 2005; Cabral, 2010). The person infected with cholera is able to excrete 10^6 *V. cholerae* cells per gram faeces (Viraraghavan et al., 2007). Keshav et al. (2010) investigated the occurrence of *V. cholerae* in animal faecal samples collected in rural areas in the Vhembe region of the Limpopo Province in South Africa. In their work, *V. cholerae* O1 was detected in 17 out of 74 cow and (3/74) chicken faeces samples, of which (9/17) cow faeces samples and (3/3) chicken faeces samples tested positive for toxigenic *V. cholerae* O1.

Although much research has been carried out concerning the occurrences of *V. cholerae* in different aquatic environments, fish, shellfish and food stuffs, recent data and statistics are still not available about the occurrence of *V. cholerae* in night-soil and dry latrines. In a case study by Bart et al. (1970) *V. cholerae* from night-soil and latrines was investigated during epidemics of classical and El Tor cholera in East Pakistan. Findings of this study revealed only 2 classical Inaba isolates that were isolated from 9 906 individual latrine and pooled communal night-soil samples, whereas in the Chittagong area, from 62 588 similar samples, 2 classical Inaba and 52 El Tor Ogawa isolates were detected. In areas where cases due to both biotypes were occurring simultaneously, El Tor Ogawa vibrios were isolated 10 times more frequently than the classical Inaba.

The occurrence of *V. cholerae* was found to be higher in human faeces (2.2×10^8 CFU/g), cow dung (1.1×10^7 CFU/g) and poultry manure samples (5.9×10^7 CFU/g), whereas a lower load of *V. cholerae* was observed in compost samples (2.2×10^3 CFU/g) (Uddin et al., 2012). Table 5 summarises the occurrence of *V. cholera* in various environmental samples (Table 5).

Table 5. Occurrence of *V. cholerae* in environmental sources including fecal waste, night soil, dry latrines

Area	Period of Study	Matrix	Detection Method	Sample Volume	Percent Positive (# of Samples)	Concentration Average CFU ^a /L, MPN ^b /L, or CFU/g	Reference
Bangladesh	NR ^c	Patient Faeces	Culture	1.0 mg	NR	2.2 E+05	Uddin et al., 2012
Bangladesh	NR	Compost	Culture	1.0 mg	NR	2.2	Uddin et al., 2012
Bangladesh	NR	Cow Faeces	Culture	1.0 mg	NR	1.1 E+04	Uddin et al., 2012
Bangladesh	NR	Poultry Faeces	Culture	1.0 mg	NR	5.9 E+04	Uddin et al., 2012
East Pakistan ^d	1968 to 1969	Night soil	Culture	NR	0.12%	NR	Bart et al., 1970
East Pakistan ^e	1968 to 1969	Night soil	Culture	NR	0.006%	NR	Bart et al., 1970

Area	Period of Study	Matrix	Detection Method	Sample Volume	Percent Positive (# of Samples)	Concentration Average CFU ^a /L, MPN ^b /L, or CFU/g	Reference
East Pakistan	1965 to 1966	Asymptomatic cholera patients stool	Culture	1.0 g	NR	1.0 E+03 CFU	Nelson et al., 2009; Mosley et al., 1968
Philippines	1964 to 1966	Patient faeces (watery)	Serological examinations	1.0 ml	21.7%	1.0 E+04 to 1.0 E+06	Dizon et al., 1967
South Africa	2008	Cow Faeces	PCR	0.15g	74.3% (55/74)	NR	Keshav et al., 2010
South Africa ^f	2008	Cow Faeces	PCR	0.15g	22.9% (17/74)	NR	Keshav et al., 2010
South Africa ^g	2008	Cow Faeces	PCR	0.15g	12% (9/74)	NR	Keshav et al., 2010
South Africa	2008	Chicken Faeces	PCR	0.15g	10.8% (8/74)	NR	Keshav et al., 2010
South Africa ^h	2008	Chicken Faeces	PCR	0.15g	4% (3/74)	NR	Keshav et al., 2010
South Africa	2008	Goats Faeces	PCR	0.15g	2.7% (2/74)	NR	Keshav et al., 2010
South Africa	2008	Donkeys Faeces	PCR	0.15g	5.4% (4/74)	NR	Keshav et al., 2010
South Africa	2008	Pig Faeces	PCR	0.15mg	4% (3/74)	NR	Keshav et al., 2010
South Africa	2008	Pigeons Faeces	PCR	0.15 mg	1.3% (2/74)	NR	Keshav et al., 2010
NR	NR	Faeces	Culture	1.0 mg	NR	1.0 E+03 to 1.0 E+06	WHO, 2011a
NR	NR	Human Faeces	Culture	1.0 g	NR	1.0 E+06	Viraraghavan et al., 2007

^aCFU: Colony Forming Unit; ^bMPN: Most Probable Number; ^cNR: Not Reported; Microorganism ^dEl Tor Ogawa; ^eClassical Inaba; ^fO1; ^{g,h}Toxigenic O1

2.2.2 Sewage

In many developing countries, sewage is discharged directly into the rivers, it leaks into groundwater and is used for irrigation purposes without any prior treatment or with inadequate treatment. It was found that 80% of the globally produced wastewater does not receive any kind of treatment, whereas only 20% receives suitable treatment (UNESCO, 2012). The pollution of rivers and groundwater by sewage causes the spread of diseases and environmental deterioration. Control of sewage discharges and proper water treatment practices have dramatically reduced widespread epidemics in the United States. However, one outbreak of cholera occurred in 1981, caused by wastewater contamination of an oil rig's potable water system, resulting in 17 cases of severe diarrhoea (CDC, 1982). It is only necessary to remove cholera from sewage if failure to do so will result in the transmission of the disease. Typically, this occurs if the treated wastewater contaminates food that is eaten raw. For example, cabbage used for preparing coleslaw was implicated in case-control studies in the Peruvian outbreak (Swerdlow *et al.*, 1992); the cabbage may have been irrigated with water contaminated with sewage. Tamplin and Parodi (1991)

worked in Peru at the height of the epidemic period, and they reported 10⁵ and 10² *V. cholerae* cells/100mL in municipal sewage in Lima and Puno, respectively.

Explosive epidemics of cholera have been documented in Peru and Chile and have been associated with the consumption of sewage-contaminated vegetables (Shuval, 1991). *V. cholerae* El Tor was found in sewage (oxidation pond), and levels of 10 to 10⁴ organisms/100mL during a cholera epidemic have been reported (Kott and Betzer, 1972). In a case study to determine the efficiency of 14 sewage treatment plants in the Mpumalanga Province in South Africa, among all the other pathogenic bacteria detected, it was found that *Vibrio* spp. were the most encountered in the raw and the treated sewage effluent. This is an indication of the predisposition of the community to cholera (Samie *et al.*, 2009). Also in the same country, toxigenic *V. cholerae* were detected in the final effluents of wastewater treatment plants as follows: Baviaanspoort (35.3%), Refilwe (21.9%) and Rayton (9.4%) (Dungeni *et al.*, 2010). Moreover, in a study conducted by Teklehaimanot *et al.* (2014; 2015) on the effluents of activated sludge treatment plants, the PCR results revealed that between 20 and 60% of samples tested positive for *Vibrio cholerae*. Table 6 illustrates the occurrence of *V. cholerae* in sewage in some countries of the world.

Table 6. Occurrence of *V. cholerae* in sewage

Area	Period of Study	Microorganism	Matrix	Detection Method	Sample volume	Percent Positive (# of Samples)	Concentration Average CFU ^a /L, MPN ^b /L, or CFU/g	Reference
Brazil, Peru, Mexico, India	1977 to 1991	<i>V. cholerae</i> O1	Waste water	PCR	NR ^c	96.7%	NR ^c	Rivera et al., 1995
Brazil, Peru, Mexico, India	1977 to 1991	<i>V. cholerae</i> O139	Waste water	PCR	NR	100%	NR	Rivera et al., 1995
Egypt	2005 to 2007	<i>Vibrio</i> spp.	Raw wastewater	Culture	100mL	100%	100 to 1.0 E+04	El-Lathy et al., 2009
Egypt	NR	<i>V. cholerae</i>	Treated wastewater	Culture	100mL	NR	10 to 100	Hassanin et al., 2013
India	NR	Toxigenic <i>V. cholerae</i>	Sewage	PCR	mL	10%	NR	Yadava et al., 2013
India	NR	<i>V. cholerae</i> non-O1	Sewage	PCR	mL	70%	NR	Yadava et al., 2013
Peru	NR	<i>V. cholerae</i>	Sewage water	Culture	100 mL	NR	10 to 1.0 E+04	Tamplin and Parodi, 1991
South Africa	2007 to 2008	Toxigenic <i>V. cholerae</i>	Effluent of Refiwe Wastewater plants	PCR	NR	21.9%	NR	Dungeni et al., 2010
South Africa	2007 to 2008	Toxigenic <i>V. cholerae</i>	Effluent of Rayton Wastewater plants	PCR	NR	9.4%	NR	Dungeni et al., 2010
South Africa	2007 to 2008	Toxigenic <i>V. cholerae</i>	Effluent of Baviaanspoort Wastewater plants	PCR	NR	35.3%	NR	Dungeni et al., 2010
South Africa	2012 to 2013	<i>Vibrio</i> spp.	Treated wastewater	Culture	100mL	NR	0.1 to 1.0 E+03	Nongogo and Okoh, 2014
South Africa	2011 to 2012	Toxigenic <i>V. cholerae</i>	Treated wastewater	PCR	NR	20 to 60%	NR	Teklehaimanot et al., 2014; 2015
South Africa	2011 to 2012	<i>V. cholerae</i>	Effluent wastewater	Culture	NR	93%	NR	Teklehaimanot et al., 2015
South Africa	2011 to 2012	<i>V. cholerae</i>	Effluent wastewater	PCR	NR	20 to 60%	NR	Teklehaimanot et al., 2015
NR	NR	<i>V. cholerae</i>	Raw sewage	Culture	100mL	NR	1.0 E+06	AWWA, 2006
NR	NR	<i>V. cholerae</i>	Untreated wastewater	Culture	L	NR	100 to 1.0 E+06	WHO, 2011a

^aCFU: Colony Forming Unit; ^bMPN: Most Probable Number; ^cNR: Not Reported

2.2.3 Manure

The contamination of vegetables, fruits and other edible foodstuffs with *V. cholerae* is mainly due to the use of sewage or manure directly as a fertilizer. Therefore proper animal manure treatment and extensive management is highly recommended before it is used as a fertilizer

(Abakpa et al., 2013). The same authors examined 336 environmental samples comprising soil, water, manure and vegetables for the presence of *V. cholerae*. The samples were collected from some selected households in Zaria, Nigeria. Results revealed 20% *Vibrio cholerae* O1 from totally confirmed *Vibrio* isolates (5) from manure samples. Mossel et al. (1992) suggested a strategy applicable to the

acceptance of imported foods for avoiding the use of manure and/or sewage for fertilization and faecally contaminated water for irrigation or processing and

maintaining worker hygiene and sanitation practices during harvesting, processing and packaging. Table 7 illustrates the occurrence of *V. cholera* and/or biotypes.

Table 7. Occurrence in manure

Area	Period of Study	Matrix	Microorganism	Detection Method	Sample Volume	Percent Positive (# of Samples)	Concentration Average CFU ^a /L, MPN ^b /L, or CFU/g	Reference
Bangladesh	NR ^c	Compost	<i>V. cholerae</i>	Culture	1g	62.5% (15/24)	2.2 E+03	Uddin et al., 2012
Nigeria ^d	2006	Manure	O1 biotype	Culture	100g	20%	NR	Abakpa et al., 2013

^aCFU: Colony Forming Unit; ^bMPN: Most Probable Number; ^cNR: Not Reported; ^dMatrix is O1 biotype, 1 isolate out of 5. The test was based on presence/absence.

2.2.4 Surface water

Surface water is a natural habitat for *V. cholerae* where it reproduces and survives in relationship with other organisms. *Vibrio. cholerae* has been detected during both endemic and non-endemic periods of cholera in several types of surface water including rivers, lakes and estuaries (Shukla et al., 1995; Jackson and Beney, 2000; El-Lathy, 2009; Wang et al., 2007, 2010; Karlsson et al., 2013).

In 2010 and during the cholera outbreak in Haiti, Hill et al. (2011) collected surface water samples from eight freshwater bodies from different sites on the Artibonite River, and irrigation canals and six marine water samples. They found *V. cholerae* O1, serotype Ogawa, *ctxA*-positive strains in two irrigation canals. Both of these canals were used for drinking water by the local population, and communities near the canals were heavily affected by the outbreak. Furthermore, by the *tcpA* sequencing analyses of the freshwater and human isolates they found that isolates from both types of samples matched that of CIRS 101, an altered El Tor strain from Bangladesh, and the *ctxAB* sequences matched those of the sequences from strains isolated in 2007 during an outbreak in Orissa, India (Talkington et al., 2011). In South Africa, Momba et al. (2006) collected surface water samples from the Lenge Dam, the Tyume River, the Sityi River and the NAikina River. Their results showed the presence of *V. cholerae* in

all surface water samples by culture methods and 25% of the isolated organisms were found to be potentially toxigenic *V. cholerae*. Also in another study, Madoroba and Momba (2010) reported *V. cholerae* in the Gutshwa, Komati and Crocodile Rivers in Mpumalanga Province. In South Africa in August 2000, the first cases of a cholera outbreak were reported from the outskirts of Empangeni in northern KwaZulu-Natal that then spread to seven of South Africa's nine provinces. There were more than 114 000 cases and 260 reported deaths reported by the end of January 2002, nearly all from KwaZulu-Natal. The outbreak developed into the most serious epidemic yet experienced in South Africa.

In Argentina, Aulet et al., (2007) investigated 18 water samplings collected from the Salí River (in Canal Norte and Banda) and the Lules River. They found that all isolated *V. cholerae* corresponded to *V. cholerae* non-O1 and non-O139 (Lules 26%, Canal Norte 33% and Banda 41%). Du Preez et al. (2010) collected 99 estuarine water samples at six sites in Beira, Mozambique. An additional 54 samples were collected from rural areas around Beira, which included three freshwater lake samples, 15 river samples, five pond samples, and four estuarine water samples. The samples were analysed for the presence of *V. cholerae* O1 and O139. Their results showed the presence of *V. cholerae* O1 only, in nine (32.1%) of the total of 28 water samples which comprised river, estuary, lake and pond water. Table 8 illustrates the occurrence of *V. cholerae* in surface water sources in various countries.

Table 8. Occurrence in surface water

Area	Period of Study	Matrix	Detection Method	Microorganism	Sample volume	Percent Positive (# of Samples)	Concentration Average CFU ^a /L, MPN ^b /L, or CFU/g	Reference
Argentina	2003 to 2005	Lules river	Direct Immuno-fluorescence	Non-O1 & O139	NR ^c	26%	NR	Aulet et al., 2007
Argentina	2003 to 2005	Canal Norte	Direct Immuno-fluorescence	Non-O1 & O139	NR	33%	NR	Aulet et al., 2007

Area	Period of Study	Matrix	Detection Method	Microorganism	Sample volume	Percent Positive (# of Samples)	Concentration Average CFU ^a /L, MPN ^b /L, or CFU/g	Reference
Argentina	2003 to 2005	Banda river	Direct Immuno-fluorescence	Non-O1 and O139	NR	41%	NR	Aulet et al., 2007
Azerbaijan	NR	Fresh water	Culture/ PCR	Non toxigenic <i>V. cholerae</i> O1	NR	57%	NR	Rashid et al., 2013
Bangladesh	1986	River and pond	Fluorescence staining method	<i>V. cholerae</i>	mL	NR	1	Brayton et al., 1987
Bangladesh	1997 to 2000	Surface water	Culture and colony blots	<i>V. cholerae</i> O1	NR	0.45%	NR	Huq et al., 2005
Burkina Faso	NR	Channel water	Culture	<i>V. cholerae</i>	NR	10%	NR	Traoré et al., 2014
Burkina Faso	NR	Reservoir water	Culture	<i>V. cholerae</i>	NR	10%	NR	Traoré et al., 2014
China	NR	Pearl River	Immuno-fluorescent-Aggregation Assay	<i>V. cholerae</i>	NR	19.9%	NR	Wang et al., 2010
China	NR	Pearl River	Real Time PCR	<i>V. cholerae</i>	NR	29.5%	NR	Wang et al., 2010
Egypt	2005 to 2007	River Nile (Rossita branch)	Culture	<i>V. cholerae</i>	NR	66.6%	NR	El-Lathy, 2009
Egypt	2005 to 2007	Qarun Lake	Culture	<i>V. cholerae</i>	NR	6%	NR	El-Lathy, 2009
Egypt	NR	Agriculture drain	Culture	<i>V. cholerae</i>	100ml	NR	10 to 1.0 E+03	Hassanin et al., 2013
India	NR	Surface water	PCR	<i>V. cholerae</i> non-O1	NR	40%	NR	Yadava et al., 2013
Iran	2001	Gorgan river	Culture	<i>V. cholerae</i> non-O1	NR	84.2%	NR	Amirmozafari et al., 2005
Iran	2001	Gorgan river	Culture	<i>V. cholerae</i> O1	NR	13%	NR	Amirmozafari et al., 2005
Iran	2001	Gonbad surface water	Culture	<i>V. cholerae</i> non-O1	NR	75%	NR	Amirmozafari et al., 2005
Italy	1995	River	Hybridization, Restriction Fragment Length Polymorphism	<i>V. cholerae</i> non-O1	200mL	82.7% (124/150)	NR	Caldini et al., 1997
Italian Adriatic coast	1995	Metauro river	Culture	<i>V. cholerae</i> Non-O1	100mL	7% (3/43)	NR	Barbieri et al., 1999
Italian Adriatic coast	1995	Foglia river	Culture	<i>V. cholerae</i> Non-O1	100mL	10% (6/60)	NR	Barbieri et al., 1999
Mozambique	NR	Surface water	PCR and DFA	<i>V. cholerae</i> O1	NR	32.1%	NR	Du Preez et al., 2010
South Africa	NR	River	PCR	<i>V. cholerae</i>	NR	33%	NR	Du Preez et al., 2003
South Africa	2003 to 2004	Surface water	Culture	<i>V. cholerae</i>	NR	100%	NR	Momba et al., 2006
South Africa	2003 to 2004	Surface water	PCR	Toxigenic <i>V. cholerae</i>	NR	25%	NR	Momba et al., 2006
South Africa	NR	Crocodile, komati and Gutshwa rivers	Culture and PCR	<i>V. cholerae</i>	NR	17%	NR	Madoroba and Momba, 2010
USA	1998 to 2000	Estuarine water	DFA (Direct fluorescent-antibody Assay)	<i>V. cholerae</i> O1	250mL	23.8% (98/412)	NR	Louis et al., 2003

^aCFU: Colony Forming Unit; ^bMPN: Most Probable Number; ^cNR: Not Reported

2.2.5 Groundwater

In India during the endemic cholera period, groundwater contaminated with sewage in Delhi was found to be responsible for the epidemic of *V. cholerae* (Pathak et al., 1993). An investigation of public water supply systems in south India in mid-1994 and after the endemic period in late 1992 and early 1993 resulted in the isolation of *V. cholerae* O139 from one of the wells supplying the town, the central overhead tank, and domestic taps connected to the public supply (Ramakrishna et al., 1996). There was a need for adequate treatment of the water supply to control the cholera outbreak. Moreover, it was reported in 1994 that the cholera outbreak may have been associated with bottled water taken from a contaminated well. *Vibrio cholerae* is normally sensitive to chlorine, but may

aggregate and assume a “rugose” form that is much more resistant to this disinfectant (Rice et al., 1993).

In South Africa, Momba et al. (2006) collected underground water samples from a borehole using a rotary hand pump, which is connected to a standpipe, and also from the Ngqele, Njwaxa and Ngwenya communities who receive their drinking water directly from standpipes, which are connected to the boreholes. Although all the boreholes are covered, they are surrounded by animal excreta and all of them are located close to pit latrines (with the exception of the Dyamala borehole). Results of this study revealed the presence of *V. cholerae* in all groundwater samples and 25% of all isolates had toxigenic genes. The authors concluded that groundwater could be a vehicle for the transmission of cholera. Table 9 depicts the occurrence of *Vibrio* spp. and/or biotypes in groundwater.

Table 9. Occurrence in ground water

Area	Microorganism	Matrix	Detection Method	Percent Positive (# of Samples)	Reference
India	<i>V. cholerae</i> O139	Wells	Culture	54%	Ramakrishna et al., 1996
India	<i>V. cholerae</i> non-O1	Hand pump water	PCR	20%	Yadava et al., 2013
South Africa	<i>V. cholerae</i>	Ground water	Culture	100%	Momba et al., 2006
South Africa	Toxigenic <i>V. cholerae</i>	Ground water	PCR	25%	Momba et al., 2006

^aPeriod of study was 1994, Sample Volume 5L

2.2.6 Drinking water

For many decades, access to safe water supply and improved sanitation has received considerable attention in policies of the United Nations (UN) and collaborative programs with member states; raising awareness about water, stimulating debate and focusing on the dangers that arise from access to an unimproved water supply. While treated drinking water supply to each house may be a normal situation in developed countries, in contrast, the concept of access to both clean water and improved sanitation is not yet the rule in developing countries. Communities, mainly those in rural areas are prone to use any available water sources for multiple purposes including drinking and cooking. As a result, waterborne infections are

found to be common in drinking water sources, causing more than 1.5 million children a year to die from diarrheal diseases (Fenwick, 2006). The mortality of water-associated diseases exceeds 5 million people per year. Of these, more than 50% of infections are linked to microbial intestinal infections, with cholera standing out in the first place (WHO, 2008a). Hence the isolation of *V. cholerae* from drinking water has alarming implications in the developing world. Also, the use of unsafe drinking water will continue to escalate the number of the cholera outbreaks in many developing countries. Possibly protective for cholera and other gastrointestinal infections, is drinking water quality monitored for *Escherichia coli*, but not when other total or thermotolerant coliforms are used (Gruber et al. 2014). Overall, Table 10 provides examples of *Vibrio* spp. and/or cholera biotype occurrence in drinking water.

Table 10. Occurrence in drinking water

Area	Matrix	Percent Positive (# of Samples)	Detection Method	Reference
Bangladesh ^a	Tap water	12.5%	NR ^b	Haque et al., 2013
India ^c	Potable water	37%	Immuno-fluorescence assay	Goel et al., 2005
Iran	Tap water	2%	PCR	Momtaz et al., 2013
Pakistan	Drinking water	28%	Culture	Shar, 2010
Sudan ^d	Drinking water	0.25%	NR	Payment et al., 1997

^aMicroorganism O1(Ogawa); ^bNR: Not Reported; ^cMicroorganism *V. cholerae* O1; ^dPeriod of study is 1993 to 1994.

2.2.7 Seawater

Pollution of marine waters by *V. cholerae* is primarily due to the disposal of wastewater or inadequately treated wastewater effluents into estuarine waters, to offshore disposal via sewage outfalls, and to rivers contaminated with inadequately treated wastewater effluents. Microbiological examination of coastal waters located near

sewage outfalls showed the presence of *V. cholerae* (Grimes et al., 1984). The coastal waters have been considered as an important reservoir of *V. cholerae* (Colwell et al., 1981). Many authors also detected *V. cholerae* in seawater and other environmental sources around the world, both in cholera-endemic and in cholera-free areas (Kaysner et al., 1987; Huq and Colwell, 1996; Jesudason et al., 2000; Huq et al., 2001) (Table 11).

Table 11. Occurrence in seawater

Area	Microorganism	Matrix	Detection Method	Percent Positive (# of Samples)	Reference
Azerbaijan	Non toxigenic <i>V. cholerae</i> O1	Caspian sea	Culture/ PCR	50%	Rashid et al., 2013
Brazil	Toxigenic <i>V. cholerae</i> non O1 and non O139	Sea water	PCR	11%	Rivera et al., 2001
India	<i>V. cholerae</i>	Coastal water	Culture	73%	Ouseph et al., 2009
Hong Kong	<i>V. cholerae</i>	Marine water	m-PCR	89%	Kong et al., 2002
Multiple Countries ^a	<i>V. cholerae</i> non O1 and non O139	Sea water	Culture/ PCR	NR ^b	Rivera et al., 1995
Peru	<i>V. cholerae</i>	Coastal water	PCR	50%	Lipp et al., 2003
Peru	<i>V. cholerae</i> O1	Coastal water	PCR	36%	Lipp et al., 2003
Peru	Toxigenic O1	Coastal water	PCR	55.5%	Lipp et al., 2003
USA	<i>V. cholerae</i> non O1/O139	Recreational seawater	Culture	51%	Kwon, 2010

^aBrazil, Peru, Mexico, India with a period of study of 1977 to 1991; ^bNR: Not Reported

2.2.8 Sewage sludge

Although no data have reported reported the

occurrence of *V. cholerae* in sewage sludge, this environment may have a similar health hazard effect as the municipal sewage effluents, as this pathogen could remain

undetected in untreated sewage sludge. Land application of untreated sewage sludge can lead to the transport of pathogens and is thus a potential health and environmental hazard as sludge may be harbouring harmful microorganisms. Further research about the occurrence, persistence and presence of *V. cholerae* and their biotypes in sewage sludge is still required, especially in the most cholera-endemic areas.

2.2.9 Soils and sediments

No data on occurrence, persistence or presence of *V. cholerae* and biotypes in soil was identified. Several research studies have been focused on the detection of *V. cholerae* in sediment samples (Rivera et al., 1995, 2001; Du Preez et al., 2010). Du Preez et al. (2010) collected sediment samples in Beira/Mozambique. The samples were analysed for the presence of *V. cholerae* O1 and O139 and the former was only in three (11.1%) of the 27 sediment samples (Table 12).

Table 12. Occurrence in soil

Area	Microorganism	Matrix	Detection Method	Percent Positive (# of Samples)	Reference
Brazil	<i>V. cholerae</i> non O1/non O139	Sediments	PCR	NR ^a	Rivera et al., 1995
Brazil	Toxigenic <i>V. cholerae</i> non O1/non O139	Sediments	PCR	100%	Rivera et al., 2001
Mozambique	<i>V. cholerae</i> O1	Sediments	PCR	11.1%	Du Preez et al., 2010

^aNR: Not Reported

2.2.10 Irrigation water and crops

Water contaminated by fecal matter has been used for irrigation purposes in many countries and leads to waterborne diseases. Cholera is no exception, and of particular concern when vegetable, salad and other crops are eaten raw and directly irrigated with raw wastewater. For example, the cholera outbreaks in Jerusalem in the 1970s were reported to have occurred as a result of the irrigation of salad vegetables directly with untreated wastewater (Shuval et al., 1986). Other case studies, which were carried out in Peru pointed out that cabbage irrigated with *V. cholerae* contaminated water acted as a vehicle for the spread of that pandemic cholera outbreak (Swerdlow et al., 1992).

Recently, after the cholera outbreaks in Haiti in 2010, a report was compiled by Cravioto and co-workers (2010) on the predisposing factors that led to the spread of the cholera outbreaks. It was found that the cholera-contaminated water in the Meye Tributary System reached the Artibonite River junction in less than 8 h and flowed downstream in another 1 to 2 days to a dam and a canal system widely used for irrigation throughout the Artibonite River Delta. The cholera outbreak was mainly attributed to the contaminated river water used for irrigation. The infection was mainly noted among young age groups between 20 and 24 years old. An increased risk of cholera transmission was reported in agricultural workers exposed to Artibonite River irrigation water in the rice paddies and fields.

After an outbreak of cholera in Bangladesh, high counts of *V. cholerae* were recorded on aquatic plants. The results of the experimental study showed that the numbers of *V. cholerae* suspended in the water had decreased, but the pathogens attached chiefly to the surface of the aquatic plants. After 5 days of experiment a 300-fold higher concentration of *V. cholerae* was found in the root system of these plants than in the surrounding water (Feikin et al., 2010). Spira et al. (1981) reported that the free-floating aquatic plants or parts of the plants act as a vehicle for the spread of *V. cholerae* or as a reservoir in inter-epidemic phases of the disease.

2.2.11 In fish and shellfish

The significance of fish and shellfish as a vehicle of cholera infection has long been recognized. Fishes are usually contaminated with *V. cholerae* when the surrounding water is contaminated by the sewage or other environmental water sources containing toxigenic strains. For example in Japan as early as 1886 and in the Philippines in 1908, raw fish consumption was associated with cholera outbreaks (Donitz, 1892; Heiser, 1908; Maheshwari et al., 2011).

Also, salted, frozen or inadequately cooked seafood has been highly implicated in the spread of cholera (Merson et al., 1977; Maheshwari et al., 2011). *V. cholerae* isolated from salted raw fish was found to be responsible for cholera outbreaks on Guam during 1974 (Kuberski et al., 1979). *V. cholerae* O1 was found in 0.2% of raw fishery products, whereas *V. cholerae* non-O1 was detected in 26.3% of raw

and 12.14% of frozen fish products on Kerala and Tamil Nadu coasts during 1986 to 1987 (Varma et al., 1989). Feldhusen (2000) reported that raw fish was primarily implicated epidemiologically as a source of transmission of *V. cholerae* in the South American epidemic in 1991. Fish samples from freshwater as well as marine water carry *V. cholerae* and it has been reported that fish intestines contained *V. cholerae* counts of 5×10^3 CFU/g (Senderovich et al., 2010). In fact, it has been shown that toxigenic *V. cholerae* O1 can survive refrigeration and freezing in food supplies shipped internationally, therefore an epidemic strain may travel far from its original endemic location (CDC, 1991).

Recently, during the Haiti cholera outbreak in 2010, Hill et al. (2011) collected nine seafood samples - five along the coast between Saint-Marc port and Grand Saline (1 site) and four from Port-au-Prince port (3 sites). They isolated *V. cholerae* O1 Ogawa *ctxA*-positive strains from one mixed seafood sample containing multiple vertebrate fish and one crab, in addition to a bivalve sample containing multiple species. Moreover by proceeding to sequencing analyses, they found the isolated toxigenic *V. cholerae* strains matching the isolated strains from infected people during the endemic period. Table 13 summarises the occurrence of *V. cholerae* in fish and shellfish.

Table 13. Occurrence in fish and shellfish

Area	Microorganism	Matrix	Detection Method	Percent Positive (# of Samples)	Concentration Average CFU ^a /g	Reference
Brazil	<i>V. cholerae</i> non O1/non O139	Mussels	Culture/PCR	NR ^b	NR	Matte et al., 1994; Rivera et al., 1995
Brazil	Toxigenic <i>V. cholerae</i> non O1/non O139	Mussels	PCR	100%	NR	Rivera et al., 2001
Haiti ^c	Toxigenic <i>V. cholerae</i> O1 Ogawa	Sea food	PCR	NR	NR	Hill et al., 2011
NR	<i>V. cholerae</i> O1	Raw fish	NR	0.2%	NR	Verma et al., 1989
NR	<i>V. cholerae</i> non O1	Raw fish	NR	26.3%	NR	Verma et al., 1989
NR	<i>V. cholerae</i> non O1	Frozen fish	NR	12.1%	NR	Verma et al., 1989
NR	<i>V. cholerae</i>	Fish intestine	NR	NR	10 to 1.0 E+05	Senderovich et al., 2010

^aCFU: Colony Forming Unit; ^bNR: Not Reported; ^cPeriod of study 2010

2.2.12 Air

No data on the occurrence, persistence or presence of *V. cholerae* was directly identified for air. Nonetheless, several researchers have isolated *Vibrio* spp. and/or *V. cholerae* from flying insects that spent a part of their life cycle in water (Broza and Halpern, 2001; Halpern et al., 2003; Hassanain et al., 2013).

An examination of the geographic distribution of three cholera outbreaks showing their association with the wind direction was done by the Paz and Broza (2007). These authors found the following: a) the progress of *V. cholerae* O1 biotype El Tor in Africa during 1970–1971; b) again in 2005–2006; and c) the rapid spread of *V. cholerae* O139 over India during 1992–1993. In another study on the possible influence of the wind direction on windborne dissemination by flying insects, which may serve as vectors, Paz and Broza (2007) established that winds supported the progress of *V. cholerae* throughout continents. This analysis supports the theory stipulating that aeroplankton (the tiny life forms that float in the air and that may be caught and

carried upward by the wind, landing far from their origin) carry the cholera bacteria from one waterbody to the neighbouring one. In addition, the authors pointed out that these findings may improve the understanding of how climatic factors are involved in the rapid distribution of new strains throughout a vast continental area. Awareness of the aerial transfer of *V. cholerae* may, therefore, assist health authorities in improving the prediction of the disease's geographic dissemination (Paz and Broza (2007).

2.3 Persistence and survival data

Vibrio cholerae are able to survive in seawater and in some aquatic environments for months to years, and are thought to be part of the autochthonous microbiota in association with zooplankton (copepods). Seasonal fluctuations are often associated with changing nutrient concentrations; for example, the rainfall runoff is generally higher during spring/autumn and in coastal and estuarine areas. This can lead to high zooplankton blooms (Huq et al., 2005), and as their exoskeleton contains chitin, *V. cholerae* ability to produce chitinases enables them to decompose

zooplankton and easily infect animals that feed on this zooplankton (Alam et al., 2006). Depaola (1981) indicated that infected seafoods, especially clams and oysters, harbour *V. cholerae* for weeks, even if refrigerated. Moreover, Huq et al. (1983) found that *V. cholerae* can rapidly multiply in crabs at ambient temperature, and some cells may maintain infectivity even after immersion in boiling temperature for less than 10 minutes or at steaming temperatures for less than 30 minutes.

Also, *V. cholerae* is able to infect and survive within free-living protozoa, so providing a vehicle for spreading this pathogen through the aquatic environment (Thom et al., 1992; Brown and Barker, 1999). Environmental strains of *V. cholerae* can persist intracellularly in a range of amoebae (Abd et al., 2004, 2005; Jain et al., 2006). Several studies have established that the growth of *V. cholerae* is boosted when it is linked with free-living amoeba, and this explains the role of amoeba as environmental reservoirs of *V. cholerae* (Thom et al., 1992; Sandström et al., 2010; Valeru et al., 2012). In addition to its survival within amoebic trophozoites, *V. cholerae* cells have been found in the stress-resistant cysts formed by amoeba, providing protection from environmental degradation (Thom et al., 1992; Abd et al., 2004). Consequently, amoeba cysts could possibly facilitate the spread of cholera (Winiecka-Krusnell and Linder, 2001).

Vibrio cholerae can survive for hours or even weeks in water contaminated with organic material and at a pH ranging 6 to 9, but is susceptible to desiccation, boiling, chloride, disinfection, and antibiotics (Valdespino and García-García, 2011). For example, *V. cholerae* O1 can survive in dechlorinated drinking water for 10 days with iron oxide and organic material (Joseph and Bhat, 2000). Djaouda et al. (2013) studied the ability of *V. cholerae* to survive or grow in unfiltered-autoclaved, filtered-non-autoclaved, and filtered-autoclaved well waters and they found that *V. cholerae* cells decreased over time, but surviving cells persisted for a longer period in filtered-non-autoclaved water. Vital et al. (2007) studied the growth of *V. cholerae* O1 El Tor Ogawa in autoclaved and filtered freshwaters and reported growth in river water, lake water and effluent of a wastewater treatment plant. Moreover they suggested that assimilable organic carbon (AOC) was a key parameter governing its growth. In an experimental

system, Rajkowski et al. (1996) demonstrated *V. cholerae* grow in reconditioned wastewater containing sufficient nutrients but free from competing microbiota at temperatures ranging from 5 to 42°C.

The association between *V. cholerae* occurrence and salinity seems to be variable. While some studies reported a significant correlation (Singleton et al., 1982; Johnson et al., 2010), others revealed a lack of correlation between the occurrence of *V. cholerae* and salinity (Johnson et al., 2012). Hence, growth is likely impacted by other (biotic) factors.

In nutrient limited environments, *V. cholerae* can enter a state of starvation and this may result in the cells non-growing, but being culturable (Lutz et al., 2013). In a recent laboratory study by Jubair et al. (2012), the long-term starvation survival of *V. cholerae* was demonstrated for 700 days. The authors advocate for the term “persister phenotype” to differentiate starved cells from the viable but non- culturable (VBNC) state. They observed that the presence of phosphate and chitin enhanced the growth of persister cells. Furthermore, they stipulated that both nutrients are important and highlight their prominence for *V. cholerae* survival. In another study on the behaviour of *V. cholerae* starving for 40 days, it was reported that chitin attachment ligands were maintained (Pruzzo et al., 2003). Aulet et al. (2007) described the sporadic isolation of *Vibrio cholerae* from rivers in Tucumán, Argentina, since the outbreak in 1991. In this study, *V. cholerae* was isolated and detected using conventional culture methods and direct immunofluorescence (DFA-VNC) to detect VBNC *V. cholerae* O1. Results revealed the detection of *V. cholerae* O1 in 38 of the 54 water samples analyzed.

Survival of toxigenic *V. cholerae* in the environment during epidemics is not often detected by culture-based methods (Islam et al., 1994), presumably due to its ability to survive in a VBNC state (Alam et al., 2006). It is important to note that toxigenic *V. cholerae* cells may still be viable and able to induce disease but is not easy to recover on the specific culture media (Colwell, 1996). *Vibrio cholerae* have been detected using PCR, in both surface water and groundwater (Momba et al., 2006; Madoroba and Momba, 2010). Realizing that most studies do not report on the presence of VBNC cells, Table 14 depicts the survival of *V. cholerae* in various environments.

Table 14. Persistence/Survival

Matrix	Survival Days	Experimental Conditions	Reference
Dairy Products	More than two weeks	NR ^a	Felsenfeld, 1967
Clams and oysters	Several weeks	At both room temperature and in refrigerator	Depaola, 1981
Cooked chicken	More than 16 hours	NR	Kolvin and Roberts, 1982
Crabs	Rapid multiplication	At ambient temperature	Huq et al., 1983
Crabs	Not completely killed	By 10 min boiling or 30 min steaming	Huq et al., 1983

Matrix	Survival Days	Experimental Conditions	Reference
Aquatic algae	More than 15 months	Inside the mucilaginous sheath of the <i>Anabaena variabilis</i>	Islam et al., 1990
Surface and Drinking water	1 h to 13 days	NR	Pesigan, 1965
Wastewater	Well grown	At temperatures from 5 to 42°C.	Rajkowski et al., 1996
Fresh and wastewater ^b	< 30 but usually	At 20 to 30°C	EPA, 1999
Crops	< 5 but usually < 2 days	At 20 to 30°C	EPA, 1999
Soil	< 120 but usually < 50 days	At 20 to 30°C	EPA, 1999
Dechlorinated drinking water ^c	10 days	With iron oxide and organic material	Joseph and Bhat, 2000
Fresh water	30 days	NR	Carr, 2001
Salt water	More than 285 days	NR	Carr, 2001
Soil	<20 days	NR	Carr, 2001
Crops	<5	NR	Carr, 2001
Carriers People shedding in their stool ^d	4 to 15 months	NR	Nevondo and Cloete, 2001
Surface water	Few hours to 14 days	NR	Nevondo and Cloete, 2001
Industrially polluted water ^e	39 days	pH 8 and Fe conc. 0.5 to 1.0mg/L	Patel et al., 2004
Industrially polluted water ^f	35 days	pH 8 and Fe conc. 0.5	Patel et al., 2004
Industrially polluted water ^g	26 days	pH 8 and Fe conc. 0.5	Patel et al., 2004
Contaminated water with organic matter	Few hours to weeks	pH between 6 and 9	Valdespino and García-García, 2011
In low nutrient environment	700 days	NR	Jubair et al., 2012

^aNR: Not Reported; ^bPersistence of <10 days; Microorganism are ^c*V. cholerae* 01; ^dToxigenic *V. cholerae* ; ^e*V. cholerae* non-O1; ^f*V. cholerae* O1 El Tor; ^g*V. cholerae* O1 Classical

3.0 Reduction by sanitation management

Human excreta and the lack of adequate personal and domestic hygiene have been a great concern in the spread of many communicable diseases, including cholera. Suitable excreta disposal practices and minimum levels of personal and domestic hygiene are essential for preventing the transmission of diseases and protecting the public health. Safe excreta disposal and handling are considered as the first line of defence to prevent enteric pathogens from entering the environment (Carr, 2001). As described in other chapters, the safe use of human excreta means implementing adequate excreta management technologies in a way that reduces the exposure dose of pathogens to very low numbers. This section discusses various ways of managing sanitation for the reduction of *V. cholerae* and surrogates to demonstrate efficacy.

3.1 Excreta and Wastewater Treatment

3.1.1 Waterless sanitation management

Globally, 2.5 billion people do not have access to improved sanitation facilities. Southern Asia and sub-Saharan Africa continue to have the lowest levels of

coverage. In contrast to Southern Asia, in sub-Saharan Africa the sanitation coverage of 30% reflects only a 5 percentage point increase since 1990. The number of people practising open defecation is still increasing in 26 of 44 countries in sub-Saharan Africa. Hence, application of any type of toilet is strongly related to hygiene behaviour change rather than acquisition of a physical toilet, be it a dry toilet or pour flush.

Experience with cholera cases suggests that developing region epidemics are closely related to poor environment status and lack of basic sanitation infrastructure. Cholera cases tend to increase with the onset of the rainy season, and conditions that permit the persistence of waterborne transmission. During the rainy season, where there is either no sewage disposal or working sewer system, rainfall runoff follows the slopes into the lower parts of towns where shallow wells could easily become contaminated by excreta (Fernandez et al., 2012). Improving water and sanitation infrastructure is critical to achieving the profound health gains brought by these systems elsewhere.

3.1.1.1 Pit latrines, vault toilets, dry toilets

Momba et al. (2006) reported *V. cholerae* and toxigenic

V. cholerae at 100% and 25% respectively in underground water samples linked to pit latrines (with the exception of the Dyamala borehole). Although no specific studies have demonstrated the reduction of *V. cholerae* by pit latrine, evidence has shown that children using pit latrines in a shanty town in Brazil had 1.5 times fewer cases of diarrhoea compared to those practicing open defaecation (Gross et al., 1989). In Lesotho, children < 5 years old using a latrine had 24 per cent fewer episodes of diarrhoea than those without a VIP latrine in their dwellings (Daniels et al., 1990). And in East Africa, 22 per cent of VIP latrine users were less likely to develop diarrhoea compared to those without toilet facilities (Thompson et al., 2001). Ecological sanitation such as composting latrines or dehydrating latrines with urine separation has been identified as a more economic and environmentally friendly alternative to traditional latrines or sewerage (Esrey et al., 1998). Nevertheless, little is known on their effectiveness in the reduction of *V. cholerae* and the incidence of cholerae in communities using these improved sanitation systems.

3.1.2 Water-based sanitation

3.1.2.1 On-site systems

Septic tanks have long been known to be an ineffective barrier for *V. cholerae*. For example, Flu (1921) conducted a study in Indonesia using five different septic tanks that were challenged with cultures containing an unspecified number of *V. cholerae* and detecting it in the effluent of one tank 24 hours after the challenge. In other studies the removal of *V. cholerae* O1 and non-O1 in Oxfam Sanitation Units was examined. The examined units were in a double-chambered septic tank consisting of two flexible butyl rubber containers with a total mean hydraulic retention time of 12 to 15 days. The study on the removal of *V. cholerae* O1 employed the effluent from the wards of the Cholera Research Laboratory in Dacca, Bangladesh. In view of the long retention time, removal was relatively poor, with only 1.7 log₁₀ being removed in the first tank and 1.05 log₁₀ being removed in the second tank (overall removal 2.7 log₁₀). The unit was monitored for two months (November and December); during this time the temperature of the wastewater fell by 10°C without affecting the removal of the *V. cholerae*. The conditions in the tanks were relatively inhospitable (anaerobic, with pH values of 6.3 to 6.7 and 40 to 90 mg/L of ammonia). The poor removal may have been attributable to short-circuiting. The removal of *V. cholerae* non-O1 was also studied in units in refugee camps. The removal of the *V. cholerae* non-O1 was comparable with the results of the other studies, with geometric mean removals of 2.7 log₁₀ and 1.48 log₁₀ in the two systems (Howard et al., 1975; Daniel and Lloyd, 1979; Daniel and Lloyd, 1980).

3.1.3 Coupled environmental and engineered systems

3.1.3.1 Waste stabilization ponds

In a case study of waste stabilization ponds in Northeast Brazil, Arridge (1995) found one log removal of bacterial indicators including faecal coliforms, faecal streptococci

and *Clostridium perfringens* from 1300 to 700 MPN/L, while *V. cholerae* O1 was reduced from 40 to 10 MPN/L. Although the anaerobic ponds appear to be essential for high levels of *V. cholerae* removal, complete removal of this pathogen was not achieved. In another case study, the average percentage removal reached up to 2.1 log₁₀ for the final effluents of oxidation ponds of the wastewater treatment plant in El-Sadat City, Egypt (El-Lathy et al., 2009).

3.1.3.2 Wetlands

Generally little has been reported on the performance of wetlands in removing *V. cholerae*. Gutiérrez-Sarabia et al. (2004) evaluated the performance of a full-scale system with wetlands for slaughterhouse (abattoir) effluent treatment in the State of Hidalgo, Mexico. The treatment system consisted of a primary sedimentation tank, an anaerobic lagoon, and a constructed subsurface-flow wetland in series. In general, the treatment system achieved satisfactory pollutant removals, but the final effluent failed to meet the Mexican environmental regulations for faecal coliform counts, five-day biochemical oxygen demand (BOD₅) and total suspended solids (TSS). Coliform reductions in the overall system were high (of the order of 5 log₁₀ on average), whereas the coliform removal in the wetland was between 2 to 3.5 log₁₀. The treatment system was also effective for removing *V. cholerae*. Further laboratory tests with the wetland effluent suggested that post-treatment in a sand filter stage followed by disinfection with sodium hypochlorite (NaOCl) could help meet the Mexican discharge regulations, particularly the criteria for coliforms and BOD₅.

3.1.3.3 Aerated lagoons and oxidation ditch

Oxidation ditches are alternative processes to more expensive activated sludge treatment, being single-sludge wastewater treatment systems. They are capable of achieving carbon oxidation, nitrification and denitrification with a single biomass slurry. Although both aerated lagoons and oxidation ditches have been used for treating sewage, to date no data have been reported for their performance in removing *V. cholerae*.

3.1.4 Wastewater treatment facilities

A wastewater treatment system is a combination of physical, chemical and biological processes. Generally, conventional wastewater treatment consists of: (i) preliminary treatment (which includes screening and grit removal); (ii) primary treatment (which involves the removal of 0.15 log₁₀ to 0.3 log₁₀ of the suspended solids in a primary settling tank); (iii) secondary treatment also called biological treatment (which is usually a trickling filter or an activated sludge reactor); and finally, (iv) advanced treatment that can be found in some conventional wastewater treatments.

While in most studies, indicator bacteria (such as total coliforms, faecal coliforms and *Escherichia coli*) have been tested to ascertain the performance of these technologies

for microbial quality of the effluent, little has been reported on the effectiveness of each process in removing *V. cholerae*. The discussion in this section mainly focuses on combined sewer overflows, trickling filter, activated sludge and disinfection processes.

3.1.4.1 Combined sewer overflow lagoons

Mariita and Okemo (2009) examined the presence of *V. cholerae* O1 in sewage lagoons around Kenyatta University. The sewage treatment plant was composed of two facultative ponds (primary and secondary) followed by two maturation ponds. Findings of this study revealed no log reduction for *V. cholerae* O1 after facultative ponds, and only one log reduction was observed after maturation ponds. Sewage effluent poses a great risk to farmers using it for irrigation downstream as well as to consumers of vegetables, such as cabbage, who use the fresh produce to prepare salads.

3.1.4.2 Trickling filters

Ngari et al. (2011) investigated the levels of *V. cholerae* at different stages of wastewater treatment at the Nakuru Town Sewage Treatment Plant, Kenya. The plant had two treatment lines, namely a trickling filter line (a combination of conventional/mechanical treatment units) and an anaerobic pond line (wastewater stabilization ponds only). The reduction rate of *V. cholerae* in the trickling filter line was 0.5 log₁₀ after facultative ponds, 4 log₁₀ after maturation ponds, 4 log₁₀ after passing through the rock filter, and 2.52 log₁₀ after application to the grass plot. In the anaerobic line, reduction of *V. cholerae* was as follows: 0.64 log₁₀ after anaerobic pond, 4 log₁₀ after facultative pond, 0.86 log₁₀ after maturation ponds, 0.58 log₁₀ after passing through the rock filter, and an apparent >3 log₁₀ after application to the grass plot. Results showed that the facultative pond of the anaerobic pond line and the final maturation pond of the trickling filter line achieved the highest percentage removal of *V. cholerae*.

3.1.4.3 Activated sludge system

In South Africa a number of studies have been conducted to ascertain the effectiveness of activated sludge systems to remove *V. cholerae* from wastewater. A case study by Samie et al. (2009) determined the efficiency of 14 sewage treatment plants in the Mpumalanga Province. The treatment plant types included ponds, activated sludge and trickling filters followed by a chlorine disinfection step. The final effluent was used for irrigation and recycling purposes in four plants; all the other treatment plants discharged the effluent into the river or to the environment. The authors found that the most encountered potential bacterial pathogens belonged to *Vibrio* spp. and concluded the potential risk for a cholera outbreak in the province. The general reduction of *Vibrio* spp. by each plant was calculated based on the total number of isolates in the influent samples as well as in the effluent samples. Overall, the reduction of *Vibrio* spp. was found to be less than 60%.

Dungeni et al. (2010) detected toxigenic *V. cholerae* in the final effluents of three of the four activated sludge

treatment plants investigated: 35.3, 21.9 and 9.4% of the Baviaanspoort, Refilwe and Rayton effluent samples tested positive for *V. cholerae*, respectively. However, complete removal of *V. cholerae* occurred in the effluent of the Zeekoegat Wastewater Treatment Plant and this was attributed to the presence of a filtration process and suitable retention time. The introduction of a secondary treatment process such as filtration, or a much more efficient disinfection process, was therefore recommended to improve the performance of the wastewater treatment plants that fail to produce the effluent quality required (Dungeni et al., 2010). In their studies, Teklehaimanot et al. (2014, 2015) found that between 20 and 60% of samples tested positive for *V. cholerae*.

In Egypt, Abdel-Wahaab (1995) examined the efficiency of activated sludge treatment plants and found that up to 99.99% removal of heterotrophic bacteria was achieved. In addition, faecal indicator bacteria, *Staphylococcus* spp., *Salmonella enterica* and *V. cholerae*, were completely removed, versus only 33% removal of enteric viruses. Moreover, all helminth eggs were removed except hookworm eggs which were still present even after disinfection with chlorine. No significant removal was recorded for the protozoan parasites following treatment. It was then recommended that a slight to moderate restricted irrigation be applicable for the effluent reuse in irrigation, taking into consideration that sprinkler irrigation should not be applied. In another study in Egypt, El-Lathy et al. (2009) determined *Vibrio* spp. in the influent and effluent of activated sludge from the Zenin Wastewater Treatment Plant and oxidation pond in El-Sadat City in 2006 and 2007. They found that the average removal percentage reached between 2.7 log₁₀ and 2.1 log₁₀ for the activated sludge and oxidation pond, respectively.

3.2 Disinfection as a tertiary or post-treatment of wastewater effluent

Discharge of wastewater treatment plant effluents that contain any type of microorganism including *V. cholerae* may result in the pollution of water supply resource. Indicative levels of *Vibrio* spp. in secondary treated wastewater may range between 10⁴ and 10⁶ organisms/100mL, depending on the treatment process (USEPA, 1992; Rose et al., 1995; Toze, 1997). The ultimate goal of wastewater disinfection is therefore to produce an effluent of such microbiological quality that minimal additional controls are required to manage public health, agricultural or environmental risks. It is important to mention that the quality of effluent depends on upon final use.

3.2.1 Chlorine, combined, etc.

Chlorine has been shown to be effective in killing *V. cholerae*. However, the effectiveness of chlorine is related to the bioavailability of the dose. Many studies have evaluated the effectiveness of chlorine to eliminate *V. cholerae* O1. When the level of chlorine at 7 mg/L is applied for a specific time, viable cells were recovered. However, at 8 mg/L for that specific time, total culturability of *V.*

cholerae was lost. Noting that chlorine efficacy is significantly reduced when organic matter is present or pH is < 6 of greater than 9.

In response to the disastrous outbreak of cholera in Peru during the 1990's, the Commission of the European Community recommended that all Peruvian factories producing fruit and vegetables for export should chlorinate their wastewater (EEC, 1991). In those countries where cholera persists, only a limited amount of money is available for the provision of sanitation. Therefore, it is important to consider the circumstances under which the removal of *V. cholerae* is warranted. To spend money to remove the *V. cholerae* when there is no need to do so may divert funds and administrative expertise from more pressing problems (Drasar and Forrest, 1996). It was found that *V. cholerae* was completely removed in the chlorinated final effluents of 14 sewage treatment plants in the Mpumalanga Province, South Africa (Samie et al., 2009). However, in many studies *V. cholerae* was detected in the chlorinated effluent of wastewater treatment plants (Dungeni et al., 2010; Teklehaimanot et al., 2014, 2015). Hence, it is critical to apply a satisfactory concentration x time disinfection (Refer to related chapter on treatment).

Dungeni et al. (2010) studied the effectiveness of four wastewater treatment plants that use chlorine for disinfection located in the vicinity of Pretoria, Gauteng Province, South Africa, in the removal of pathogenic bacteria including *V. cholerae*. They detected toxigenic *V. cholerae* in 75% of the investigated wastewater treatment plants (three out of four plants) despite high free chlorine residual concentrations in treated effluents. The residual chlorine level ranged between 0.03 and 1.60 mg/L. Thus they suggested an upgrading of these wastewater

treatment plants by including processes such as rapid sand filtration and UV disinfection, which have proved to be effective in the removal and inactivation of pathogenic bacteria.

Rice et al. (1993) studied the effect of chlorine on *V. cholerae* O1 and they found that *V. cholerae* O1 is able to shift between smooth and rugose colonial morphologies. Cultures of smooth *V. cholerae* strains were inactivated in less than 20 s at a concentration of 1.0 mg/L free chlorine in chlorine-demand free water. In contrast, with the cultures of rugose variants exposed to this concentration of chlorine, there was an initial rapid drop in viable counts, followed by persistence of a protected sub-population of cells. The authors stated that viable *V. cholerae* could still be recovered from rugose cultures even after exposure to 2.0 mg/L free chlorine for 30 min. Preliminary studies suggest that resistance to killing by chlorine was due to formation of cell aggregates enclosed in a gelatinous mucoid material. Moreover, the *V. cholerae* rugose phenotype represents a fully virulent survival form of the organism that can persist in the presence of free chlorine.

3.2.2 Ultraviolet

Typical UV doses required for a 4-log inactivation of *V. cholerae* have been reported to be 0.65 mJ/cm² (Malley, 2000; USEPA, 2003). Some studies have shown that environmental populations of bacteria are less susceptible to UV than cultured organisms (Hijnen et al., 2006; Smeets et al., 2006). Hijnen et al. (2006) observed that the UV dosages required for 1, 2, 3 and 4 log inactivation of environmental *V. cholerae* were 2, 4, 7 and 9 mJ/cm², respectively. Table 15 depicts some treatment reduction of *V. cholerae*.

Table 15. Treatment Reductions of *V. cholerae*

Area	Treatment	Treatment Conditions	Treatment reduction	Initial concentration & Subsequent Concentration at treatment time t	Methods	Reference
Northeast Brazil	Stabilization pond	NR ^a	0.12 log ₁₀	40 to 10 MPN/100mL	Culture	Arridge, 1995
Egypt (El-Sadat City)	Oxidation pond	NR	2.1 log ₁₀	2.6 E+05 to 1.9 E+03 CFU/100mL	Culture	El-Lathy et al., 2009
Kenya	Maturation pond	Low pH Low temperature	0.7 log ₁₀	1.1 E+03 to 2.2 E+02 CFU/100mL	Culture and biochemical tests	Mariita and Okemo, 2009
Kenya	Facultative pond	10.3 to 28.4oC	0.54 log ₁₀	NR	Culture	Ngari et al., 2011
Kenya	Maturation pond	10.3 to 28.4oC	4 log ₁₀	NR	Culture	Ngari et al., 2011
Kenya	Anaerobic pond	10.3 to 28.4oC	0.64 log ₁₀	NR	Culture	Ngari et al., 2011

^aNR: Not Reported

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