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REVIEW

Cholera: an overview with reference to the Yemen epidemic

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Abstract **Cholera is a secretory diarrhoeal disease caused by infection with *Vibrio cholerae*, primarily the *V. cholerae* O1 El Tor biotype. There are approximately 2.9 million cases in 69 endemic countries annually, resulting in 95 000 deaths. Cholera is associated with poor infrastructure and lack of access to sanitation and clean drinking water. The current cholera epidemic in Yemen, linked to spread of *V. cholerae* O1 (Ogawa serotype), is associated with the ongoing war. This has devastated infrastructure and health services. The World Health Organization had estimated that 172 286 suspected cases arose between 27th April and 19th June 2017, including 1170 deaths. While there are three oral cholera vaccines prequalified by the World Health Organization, there are issues surrounding vaccination campaigns in conflict situations, exacerbated by external factors such as a global vaccine shortage. Major movements of people complicates surveillance and administration of double doses of vaccines. Cholera therapy mainly depends on rehydration, with use of antibiotics in more severe infections. Concerns have arisen about the rise of antibiotic resistance in cholera, due to mobile genetic elements. In this review, we give an overview of cholera epidemiology, virulence, antibiotic resistance, therapy and vaccines, in the light of the ongoing epidemic in Yemen.**

Keywords cholera; epidemic; multi-drug resistant; catechin; luteolin; ToxT; CTXΦ

Introduction

Cholera is a secretory diarrheal disease which is caused by infection with *Vibrio cholerae*, and is associated with high mortality if left untreated. Estimates suggest that there are approximately 2.9 million cases in 69 endemic countries every year, resulting in 95 000 deaths, mainly in Sub-Saharan Africa where approximately 60% of cases and 68% of deaths occur annually [1]. However, in 2015 World Health Organization (WHO) was notified of only 172 454 cases from 42 countries, resulting in 1304 deaths [2]. The discrepancy between actual and recorded disease burden is thought to result from low reporting for a variety of reasons, including limitations in environmental, community and public health surveillance systems in endemic countries, and financial and commercial concerns on potential consequences for trade and tourism [1,3–5]. The disease is associated with developing countries and poor infrastructure, especially in relation to sanitation and access to clean drinking water. It has been essentially eradicated in developed countries, with cases arising mainly due to travel to endemic countries [6,7]. For example, between 2010 and 2011 an increase in travel-associated cases observed in the United States was attributed mainly to travel to Hispaniola in Haiti, linked to a major cholera outbreak there in the aftermath of a devastating earthquake [7]. *V. cholerae* strains are natural inhabitants of the marine environment, found attached to multiple surfaces, with water temperature, saline content and seasonality being vital components in distribution and bacterial counts [8]. This explains the importance of sanitation and access to clean drinking water in cholera spread and also the importance of natural disasters and climate change in causing "spillover" into the human population. The disease is transmitted by the faecal-oral route, and contaminated food and water act as disease transmission vehicles.

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Cholera epidemic in Yemen

Yemen, a country with a population of approximately 27 million people at the southern end of the Arabian Peninsula, is currently in the grip of a devastating cholera

epidemic. The outbreak was first announced in October 2016, and a surge in cases has occurred since 27th April 2017. The speed of this surge has been described as "unprecedented" by Dr. Nevio Zagaria, the WHO representative in Yemen. WHO and the United Nations Children's Fund (UNICEF) have estimated that 172 286 suspected cases arose between 27th April and 19th June 2017, associated with 1170 deaths [9]; on 26th October there was a cumulative total of 862 858 suspected cases. Aid efforts are currently hampered by closure of all air, sea and land ports. The most vulnerable citizens are suffering most, with 46% of cases occurring in those age under 15 years and 33% of fatalities in those aged over 60 years. The epidemic has been designated as a WHO Grade 3 emergency. Spread has been facilitated by the ongoing war in Yemen, which has left 18.8 million people in need of humanitarian assistance. The infrastructure of the country has been left in ruins, leaving little or no access to safe drinking-water or sanitation for more than 14 million people [10]. Cholera is a disease of poverty, which flourishes and spreads in such conditions. The war has also destroyed the health system in the country; a WHO survey carried out in November 2016 found that only 45% of the facilities surveyed were fully functional and accessible, while 274 health facilities had been damaged as a result of the war [11]. There are fears that there is a risk of further spread due to the rainy season, as well as high levels of malnutrition and lack of food security for most Yemeni people [10]. While WHO has responded to the crisis by beginning to set up 350 cholera treatment centers and 2000 oral rehydration points, as well as tracing infection hotspots, this epidemic underlines the need to increase our understanding of cholera and in particular to ensure that the best possible options are available in terms of vaccination and therapy in outbreak situations. In this review, we give an overview of cholera epidemiology, virulence, antibiotic resistance, vaccination and therapy.

Cholera serology

Serologically, *V. cholerae* has been classified into more than 200 O serogroups according to variations of the *V. cholerae* "O"antigen [12–15]. However, only the O139 and O1 serogroups have been identified as causative agents in epidemics [15,16]. The O1 serogroup can be sub-classified into three serotypes, Ogawa, Inaba and Hikojima, based on expression of antigenic factors A, B and C, and into two biotypes, Classical and El Tor [15]. The O1 serogroup is responsible for more than 99% of global cholera cases [17]. Stool samples from patients in the current outbreak in Yemen have tested positive for the O1 serogroup, Ogawa serotype [18,19]. However, O139 arises from time to time, for example as a major cause of infection among older individuals in Pakistan between 1995 and 2010 [20], as an important pathogenic strain in Thailand [21], and sporadically in Bangladesh [22] and China [23]. Non-O1/non-O139 serogroups occur rarely and are not considered to be of great clinical significance, although they have been associated with cholera cases in Czechoslovakia in 1960, caused by the O5 serogroup [24] and Sudan in 1970, caused by the O37 serogroup [25]. Fig.1 shows an overview of the classification of *V. cholerae* by serogroup and biotype. The development of molecular tools to study *V. cholerae*, including pulsed field gel electrophoresis (PFGE), ribotyping, polymerase chain reaction (PCR)-based methods, and sequencing-based methods such as variable number of tandem repeats (VNTR) analysis, multi-locus sequence typing (MLST) and multi-locus variable tandem repeat analysis (MLVA),

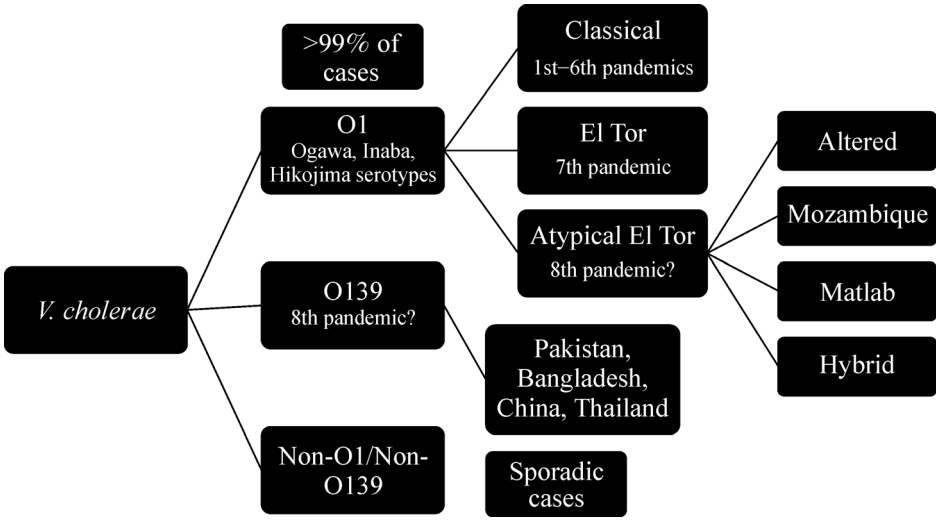

Fig. 1 Overview of *V. cholerae* classification by serogroup and biotype.

means that we now have a better understanding of the evolution and molecular epidemiology of cholera [26].

Historically there have been six pandemics of cholera prior to 1961, caused by the Classical O1 biotype which emerged in the Indian subcontinent and spread worldwide between 1817 and 1923 [27,28]. Currently, we are in the seventh pandemic, which is believed to have originated in Indonesia in 1961 and is caused by the El Tor biotype; it has spread throughout Asia, Africa and Latin America. It has caused large epidemics such as the 2010 epidemic in Haiti, and major outbreaks in several African countries, including epidemics in Mozambique in 1997 – 1999 and 2012 – 2014 and Cameroon in 2010 [4,15,29–31]. Some commentators suggest that there may also be an ongoing eight pandemic, which began in 1992, based on serogroup O139 and "atypical El Tor" [15,26]. These atypical El Tor strains have traits of both classical and El Tor biotypes. Pathogenic *V. cholerae* strains carry a prophage, CTXΦ, which carries the gene encoding cholera toxin, the major cholera virulence factor. Atypical El Tor strains isolated in Matlab in Bangladesh in 1991 and 1994 carried the *ctxB* allele which is characteristic of the classical CTXΦ ($CTX\Phi^{Cla}$) and are designated "MB El Tor" [32]. In fact, all El Tor strains isolated in Bangladesh since 2001 express *ctxB* and have been designated "altered El Tor." Variants isolated in Mozambique in a 2004 outbreak contained two copies of a prophage almost identical to $CTX\Phi^{Cla}$ on the small chromosome, but had the El Tor phenotype and was otherwise genotypically El Tor ("Mozambique El Tor") [33,34]. Meanwhile, it was also shown that strain VC44 isolated in Kolkata, India in 1992 also carried the classical *ctxB* [33]. Further atypical El Tor strains termed "hybrid El Tor" carrying classical *ctxB* and the *rstR* gene, needed for phage gene expression regulation, from both classical and El Tor strains were isolated between 1991 and 2004 in various African and Asian countries. These variants have arisen due to transfer of mobile genetic elements. There is as yet no information on the serogroups or biotypes mainly responsible for the Yemen outbreak.

Epidemiology of cholera

The seventh cholera pandemic is the latest manifestation of a disease that has been recognized since the time of Hippocrates [35]. As mentioned in Section 2.0, we are currently experiencing the seventh pandemic which began in 1961 when the El Tor biotype, named after the town of El Tor in Egypt where it was first identified in 1905, was the cause of an epidemic in Indonesia. In recent years El Tor and "atypical El Tor" strains have caused several major outbreaks and epidemics throughout Asia, Africa and Latin America. Table 1 shows a timeline of some of the important cholera epidemics and outbreaks within the last decade.

Most cholera cases and deaths occur in Africa, especially in sub-Saharan Africa, including the Great Lakes Region, which has had the highest burden of cholera in the world over the past 20 years. For example, among fishing communities of Uganda, there are recurrent seasonal outbreaks of cholera. Between 2011 and 2015, several outbreaks occurred in these communities with a high case fatality rate, associated with contaminated lake water use, and poor sanitation and hygiene [36]. Cholera outbreaks also occur regularly in Tanzania, with seven outbreaks reported between 2011 and 2016 due to genetically diverse *V. cholerae* O1 isolates [37]. However, both O1 and O139 have been identified in estuaries in Tanzania, although O1 predominates [38]. Other outbreaks in the African Great Lakes Region include 2009 and 2011/2012 outbreaks in the Democratic Republic of Congo (DRC) and a 2012 outbreak in Zambia caused by *V. cholerae* O1 isolates which clustered closely together genotypically according to Multi-locus VNTR (variable number tandem repeat) Analysis (MLVA) [39].

Table 1 Timeline of large cholera outbreaks in last decade

Year	Location	References
2008	Mozambique (9087 cases); Zimbabwe (2008 – 2009; 98 522 cases)	[30, 44]
2009	DRC; Mozambique (19 679 cases); Zimbabwe (continued from 2008); Tanzania (7700 cases); Kenya (11 769 cases)	[30, 39, 44]
2010	Haiti (epidemic begins October; approximately 700 000 cases to date); Zimbabwe (Kadoma City, 127 cases); Cameroon (2010 – 2011; 23 152 cases); Nigeria (41 787 cases); Bangladesh (MDR resistance rising— 93% of isolates from coastal areas 2010 – 2014— approximately 450 000 cases/year); India (El Tor variant; 2152 cases)	[4, 29 – 31, 43, 44, 50, 51, 63]
2011	Philippines (O1 hybrid El Tor; Palawan, 1226 cases); DRC (8038 cases); Uganda (fishing villages— recurrent yearly outbreaks 2011 – 2015, 5059 cases); Cameroon (continued from 2010)	[36 – 39, 48]
2012	Guinea (2009 – 2012, >15 500 cases) ; DRC (Betou, 355 cases); Tanzania; India (El Tor *ctxB*7 allele)	[37, 39, 47]
2013	India (MDR O1 Ogawa, Bagalkot, 49 cases); Tanzania (Dar Es Salaam, approximately 3400 cases)	[37, 52]
2014	Ghana (Accra region, continued into 2015, more than 20 500 cases)	[37]
2015	Tanzania (approximately 9900 cases); Southern Sudan (began 2014; insufficient vaccines; 2260 cases)	[37, 40]
2016	Yemen (epidemic begins October; ongoing); Tanzania	[9, 37]
2017	Surge in cases in Yemen since April; 862 858 suspected cases (26/10/17 WHO update)	[9]

An International Coordination Group under the WHO Secretariat manages the global cholera vaccine stockpile, and countries or agencies who need to access the stockpile, for example, in the event of an outbreak, must submit a detailed request to this group. While this system has been designed to ensure that scarce vaccine stocks are targeted to areas where they are most needed, the obstacles that can arise were highlighted in another study from the African Great Lakes Region, this time from Southern Sudan [40]. The study concerned an outbreak that occurred in the city of Juba in June 2015. Like Yemen, this city was suffering from civil strife, which began in December 2013 and resulted in major movement of people in and out of the city, making Juba a potential submission hub for the whole country. Global shortages of cholera vaccine meant that there were insufficient doses to vaccinate the entire at-risk population, which numbered approximately 1 million in Juba alone, with the usual two-dose regime [40]. After internal consultations, a request was made to the International Coordination Group two weeks after the epidemic start was declared. Three weeks after the International Coordination Group made a decision to release 270 340 doses, a targeted vaccination campaign was eventually agreed among stakeholders, in which a single dose strategy was used and 140 249 doses were targeted to areas of Juba in which transmission was sustained or increasing, as well as to high-risk groups. Remaining doses were used in a program combining sanitation and hygiene promotion, and for use among internally displaced people outside Juba in places where fighting was ongoing and where there had been recent large population movement [40]. This study has parallels to the current situation in Yemen, and highlights the issues arising from civil unrest and internal displacement of people. It makes clear the challenges to planning effective vaccination campaigns in the face of outbreaks in areas of civil unrest, the lack of data-based guidance on when, where and how vaccines should be used reactively, the time that can be lost when a country's surveillance and laboratory capacity is undermined, in applications to the International Coordination Group, and consultations with stakeholders, and the difficulties inherent in prioritising competing needs in the context of a global vaccine shortage [40]. Another study focused on the issue of cross-border cholera outbreaks in the African Great Lakes Region, for example, on the borders between Malawi and Mozambique and between Uganda and DRC [41]. These cross-border outbreaks accounted for 603 cases and five deaths in Malawi and Uganda in 2015. Contributing factors included poor sanitation and hygiene, contaminated water and environmental factors such as flooding. Children of school age or aged under five years were the most commonly affected age groups. Typically, there were only unilateral efforts by one affected country to control such outbreaks and they were increased by frequent cross-border movements between countries [41]. This type of cross-border outbreak could be a risk in the current situation in Yemen, if affected individuals crossed borders into neighboring countries such as Saudi Arabia or Oman. Other studies from the Middle East have shown how travel between neighboring countries can contribute to cholera spread. For example, in a study on cases arising in Iran in 2013, 83% of cases were associated with individuals traveling from Afghanistan or Pakistan, and were dominated by the *V. cholerae* O1 Inaba serotype, with evidence of increasing antibiotic resistance compared to previous years [42].

Outbreaks of cholera in West Africa and in Zimbabwe have highlighted the importance of seasonality and climate [43,44]. In a study on cholera outbreaks which occurred in Cameroon between 2000 and 2012, seasonal patterns of the three waves of outbreaks which occurred in this time period differed significantly depending on climate subzone, emphasizing the need to understand local climate and environmental conditions when considering cholera transmission patterns [43]. Other studies in Cameroon also identified the importance of poor food preservation methods as a risk factor in contracting cholera, as well as the necessity for improvements in hygiene and sanitation and in water infrastructure in tackling current and preventing future epidemics and outbreaks [31]. This further highlights the difficulties currently faced in Yemen, where the ongoing war is undermining the water infrastructure and leaving citizens without reliable food sources or food preservation resources.

The issue of contamination of water sources is further underlined in studies in Southern Asia, especially in Bangladesh [45,46] and India [47], and in South-East Asia, for example, in the Philippines [48]. In Bangladesh, cholera is endemic and epidemic, with approximately 450 000 cholera cases every year [1,45]. Both O1 and O139 biotypes are found in ponds, river water and irrigation canals [46], with outbreaks displaying a robust seasonality [49]. As in other areas, the emergence of increased multi-drug resistant (MDR) strains is causing concern. In a study on *V. cholerae* O1 isolates from remote coastal areas of Bangladesh, 93% of isolates were MDR, with evidence of reduced susceptibility to ciprofloxacin and azithromycin among other antibiotics [50]. Outbreaks and epidemics in India have been associated with both El Tor and atypical El Tor O1 isolates. A tetracycline-resistant O1 El Tor variant was associated with a 2010 epidemic in the tribal area of Odisha in India [51], and a hybrid O1 El Tor carrying the *ctxB*7 allele was associated with two outbreaks that occurred in South-West India in 2012, originating with faecal contamination of the potable water supply leading to the first outbreak, which then spread to other areas and caused the second outbreak [47]. Another hybrid El Tor variant was responsible for a large outbreak

in the Philippines in 2011, which was found to be indigenous and present locally in the aquatic ecosystem [48]. The Philippine isolates were unique and differed from O1 isolates found in other countries in Asia, or in Africa or Haiti. Again, in India outbreaks tend to be seasonally driven, such as the clusters that occur during the monsoon season every year in Karnataka. During the outbreaks of 2013, an MDR variant of *V. cholerae* O1 (Ogawa serotype) was identified in cases in the remote Bagalkot area of Karnataka [52]. In Mumbai, a waxing and waning of MDR among *V. cholerae* O1 El Tor (most commonly Ogawa serotype) has been observed among isolates gathered between 2004 and 2013 [53]. These studies highlight the need to be vigilant about seasonal changes in Yemen potentially affecting cholera transmission and to be aware of possible MDR arising, making it essential that antibiotic sensitivity testing be facilitated as much as possible. At present it is unclear what the predominant variants are in the Yemen outbreak.

In October 2010, one of the largest ever recorded cholera outbreaks began in Haiti in the aftermath of a devastating 7.0 magnitude earthquake which struck on January 12th, 2010. The cholera epidemic has since caused approximately 700 000 cases and 10 000 deaths to date. The experience of Haiti highlights how this disease flourishes in conditions where infrastructure is damaged, sanitation and hygiene breaks down and the healthcare system is compromised. The Haiti outbreak originated with the introduction in 2010 of the hybrid O1 El Tor carrying the *ctxB*7 allele, which also caused outbreaks in India, including the two outbreaks in South-West India in 2012 [47], as well as outbreaks in Cameroon and Zimbabwe in 2009, and Nepal in 2010 [54,55]. Prior to this outbreak, there had been no cases of cholera in Haiti for a century. It was established by genomic epidemiology studies that the epidemic originated due to a single introduction from Nepal, followed by rapid clonal spread [29]. It has since been widely accepted that the original source of the infection was Nepalese soldiers who arrived in October 2010 as part of the United Nations Stabilization Mission in Haiti (MINUSTAH), although there is still some debate on the matter [56,57]. The Artibonite watershed was contaminated with infected sewage, leading to downstream cases within days [57,58]. Two years on, toxigenic *V. cholerae* of the outbreak strain was still detectable in surface waters [59], while between April 2013 to March 2014, the number of surface water samples containing culturable *V. cholerae* O1 increased more than 5-fold from the previous year, with seasonal water temperatures and precipitation playing a significant role [60].

The nature of the way in which cholera was introduced to Haiti has resulted in an UN-commissioned independent report with recommendations on pre-deployment interventions to reduce risk of transmission of diseases such as cholera from endemic to non-endemic regions by peacekeepers [61,62]. A recent computational modeling analysis suggested that of the three options suggested — screening for *V. cholerae* carriage, immunisation with oral cholera vaccines (OCV) or administering prophylactic antibiotic chemotherapies — the latter would be the most efficient and cost-effective, although all would be effective to some extent [62]. The administration of prophylactic antibiotics would be somewhat controversial in the current era of increased antimicrobial resistance among pathogens.

The altered El Tor variant strain responsible for the Haiti epidemic is part of Wave 3 of the spread of El Tor strains that have evolved since their emergence in 1961. When compared to strains from Wave 1, the Haiti strain is hypervirulent, with greater cholera toxin (CT) and hemolysin production [63]. It has caused a substantial mortality rate [4]. Risk factors for transmission in intra-peak periods in Haiti have been identified as close contact with cholera patients, eating food bought from street vendors or drinking/washing dishes with untreated tap water, while protection is associated with prevention messages such as hygiene promotion [64]. Living in remote areas poorly accessible by road was identified as a mortality risk factor [65]. This is relevant in the Yemen situation, where the damage to the infrastructure leaves greater numbers of people remote from help.

Cholera virulence genes and CTXΦ phage infection process

As mentioned above, pathogenic *V. cholerae* strains carry a prophage CTXΦ, which expresses the CT-encoding *ctxAB* genes. Production of CT is central to *V. cholerae* pathogenicity and its ability to cause outbreaks and epidemics, and is responsible for the disease symptoms. CT causes the watery diarrhea associated with cholera by binding to the GM1 ganglioside receptor on erythrocytes, which leads to its internalization and consequent cAMP increases, which then leads to catastrophic water and electrolyte loss in the form of diarrhea [66,67]. The non-toxic CT subunit, CTB, from both classical and El Tor biotypes also complexes with blood group determinants when binding to its primary GM1 receptor. This mediates blood-group-dependence of cholera infection; individuals with blood group O suffer the most severe symptoms, probably because blood group H determinant, characteristic of blood group O, binds CT with higher affinity than blood group A-determinant [68]. CTB can also bind to other mediators beyond as well as GM1 on immune cells such as monocytes, including the Toll-like receptor TLR4, and the immunoglobulin (Ig) superfamily members, TREM2 (triggering receptor expressed on myeloid cells 2) and LMIR5/CD300b (leukocyte mono-immunoglobulin (Ig)-like receptor 5) [69]. This immunological targeting is

implicated in the inflammatory responses induced by *V. cholerae* infection.

The CTXΦ consists of core and RS2 gene clusters [15,70]. The *ctxAB* genes are carried on the core cluster, along with other virulence and phage morphogenesis-associated genes including *psh*, core-encoded pilin (*cep*), $pIII^{CTX}$, accessory cholera enterotoxin (*ace*) and zonula occludens (*zot*). The RS2 cluster contains the genes *rstR*, *rstA* and *rstB*, which are associated with the regulation of CTXΦ gene expression, phage replication and phage integration respectively [15]. In O1 El Tor strains, the CTXΦ RS2 and core elements are flanked by satellite phages called RS1, which resemble RS2 but have an extra gene, *rstC*, encoding an anti-repressor protein that enhances CTXΦ gene transcription [15].

CTXΦ infection and virulence also requires the host-encoded toxin co-regulated pilus (TCP), which is encoded on the VPI-1 pathogenic island and which mediates small intestine colonization. The CTXΦ interacts with TCP and TolQRA division proteins for delivery to the host cell cytoplasm, then undergoes rolling circle replication (RCR) as the host replication machinery converts its single-stranded DNA genome into double-stranded [70,71]. This allows RCR to proceed, with production of new CTXΦ particles [72]. CTXΦ is then irreversibly integrated into *V. cholerae* at integration sites called *dif*, via the action of host tyrosine recombinases XerC and XerD, part of the chromosomally encoded site-specific recombination machinery [73]. While integration of non-replicative forms of CTXΦ tends to be inefficient, the integration of replicative forms is highly efficient and usually results in multiple tandem integrations. RCR is dependent on the CTXΦ HUH endonuclease protein RstA, which is in turn controlled by the CTXΦ repressor protein RstR and the host cell SOS machinery [74]. RstA creates a nick at the CTXΦ *ori*(+) to give a 5′ phosphotyrosine intermediate and free 3′-OH in replicative CTXΦ, and primes the remainder of the replication process to be carried out by host proteins [74,75]. Various host proteins have been identified as essential in CTXΦ replication including histone-like protein HU, which is needed by RstA to introduce the nick at CTXΦ *ori*(+), and UvrD, a DNA helicase usually involved in DNA repair [76]. HU and UvrD are therefore potential candidates for targeting of live attenuated cholera vaccines.

Transcriptional activation of CT and TCP depends on the ToxT protein, a member of the AraC/XylS transcription factor family [77,78]. Activation of the *toxT* gene depends in turn on synergistic coupling of the membrane-located heterodimers ToxR/ToxS and TcpP/TcpH [79]. These virulence transcriptional regulator proteins, in particular ToxT itself, are potentially effective targets for cholera therapies. Targeting of the bacterial pathogenesis rather than its survival would have the advantage that resistance would be unlikely to develop.

Cholera therapy

Antibiotic resistance and mobile genetic elements

Therapy for cholera generally relies on rehydration, supplemented by antimicrobial treatment in the case of severe infection and septicaemia, as recommended by WHO guidelines [80]. A Cochrane systematic review confirmed the improvements that result from antimicrobial treatment, both clinically and microbiologically, with azithromycin and tetracycline emerging as having possibly increased benefits compared to other antibiotics [81]. There is, however, an increasing problem of emergence of antibiotic-resistant strains of *V. cholerae*, for example strains that are resistant to the fluoroquinolones such as norfloxacin, which had previously had consistently high activity against *V. cholerae,* and β-lactam-resistant strains which have arisen due to acquisition of extended-spectrum β-lactamases (ESBLs) [52,81–85]. Comprehensive information on antibiotic resistance genes associated with cholera can be sources from the DBDiaSNP database, an open-source facility containing information on mutations and antibiotic resistance genes for a range of diarrhea-causing pathogens including *V. cholerae* [86]. This rise in resistance to previously effective antibiotics has further increased interest in use of other antibiotic classes, such as the macrolide antibiotic azithromycin. A randomized control trial carried out on 120 adult cholera patients in India revealed that a single dose of azithromycin was as efficacious in treatment of watery diarrhea and dehydration as three days of twice-daily doses of norfloxacin [87]. However, reduced susceptibility to azithromycin has been observed in strains isolated, for example during outbreaks in Bangladesh [50] and Thailand [21]. Currently, the tetracycline antibiotic doxycycline is recommended as the first line antimicrobial treatment for adult patients with either *V. cholerae* O1 or O139 infection, with erythromycin or azithromycin recommended for children or pregnant women [84,88]. However, the rise in antibiotic resistance highlights the importance of reliably establishing antibiotic susceptibility patterns, which is difficult in situations such as the epidemic in Yemen where healthcare services are seriously undermined. There are multiple reasons for the rise in antibiotic resistance in *V. cholerae*, of which the most important is horizontal gene transfer via mobile genetic elements such as integrative and conjugative elements (ICEs) of the SXT mobile integron family and plasmids such as the IncA/C plasmid family [84,89–94].

SXT family ICE linear DNA sequences have been particularly important in conferring antibiotic resistance on *V. cholerae* strains. Phylogenetic analyses have estimated that between the first and second waves of the seventh pandemic, an SXT ICE of the SXT-R391 family was acquired, coincidental with the dating of the most recent common ancestor (MRCA) of the O1 and O139 biotypes

between 1978 and 1984 [91,95,96]. The SXT SXT-R391 family share a common 52 core-gene backbone, which facilitates integration/excision and conjugation, as well as variable regions which include elements such as antibiotic resistance genes [91]. SXT is named for its conferring of sulfamethoxazole and trimethoprim resistance. Since the first *V. cholerae*-associated SXT was identified in O139 MO10 in India (SXT^{MO10}) [97], several other SXT elements have been identified in *V. cholerae* O1 or O139 strains [91]. Examples include the hybrid SXT element ICEV*ch*CHN1307, which was isolated from the ICDC-1307 O1 strain from a patient in China in 1998 [93]. Along with an IncA/C plasmid, pVC1307 and a chromosomal integron, ICEV*ch*CHN1307 confers intermediate or complete resistance to 13 antibiotics. Other El-Tor O1-expressed SXT elements include the ICEV*ch*Ind5 and ICEV*ch*Moz10, both of which have rearrangements in their variable regions when compared to basic SXT [96,98]. Genetic analyses indicate that there has been multiple acquisitions as well as homologous recombination between ICE elements, contributing to the diversity in pathogenic *V. cholerae* strains [91]. There is also evidence of mobile genetic elements carrying antibiotic resistance in both environmental *V. cholerae* strains and non-O1/non-O139 clinical isolates. For example, in Haiti a mobilizable genomic island (MGI) called MGIV*ch*Hai6 which confers resistance to multiple antibiotics was identified in a non-O1/non-O139 MDR strain HC-36A1, and subsequently all non-O1/non-O139 MDR clinical strains isolated in Haiti [83]. MGIV*ch*Hai6 can be mobilized by IncA/C plasmids and could therefore be transferred to other *V. cholerae* strains in epidemic regions. It has already been identified in strains from India and North and South America [83]. Meanwhile, in environmental strains class 1 integrons conferring resistance to several antibiotics have been found to persist in environmental non-O1/nonO-139 strains from the Amazon region of Brazil, while class 2 integrons conferring resistance to a different range of antibiotics have been found in non-O1/nonO-139 environmental strains from India, Bangladesh and Ghana, and one strain of the *V. cholerae* O1 Amazonia lineage, suggesting that these elements can be transferred between strains and therefore act as a potential reservoir for further antibiotic resistance in cholera [99].

Potential cholera therapies

The increase in antimicrobial resistance has also led to increased interest in development of potential new cholera therapies to supplement or replace antibiotic treatment. A summary of some of the pathogen-directed and host-directed agents which have been shown to have potential utility in cholera treatment within the last three years is shown in Table 2. These include use of natural products such as seaweeds and herbal sources, quorum sensing, host-directed therapeutic agents, re-purposing of existing drugs such as ribavirin, use of bacteriophages, and use of toxin inhibitors such as linoleic acid, bithianol, AraC inhibitors, virstatin or dietary minerals [99–118].

Use of natural compounds from sources such as seaweed

Table 2 Overview of potential cholera therapies

Target	Therapeutic agent	Therapeutic target/mechanism	References
Virulence/toxin mediators	Seaweed polysaccharide (*in vivo*- mice)	CT; GM1 receptor	[100]
	Anethole (*in vitro, in vivo*-rabbits)	Reduced CT and TCP expression via inhibition of ToxT	[101]
	Catechin and luteolin (*in silico*)	Inhibition of ToxT	[102]
	Conjugated linoleic acid (CLA) (*in vitro, in vivo*-rabbits)	Reduced CT and TCP expression via inhibition of ToxT	[111]
	Virstatin (*in vitro, in vivo*- mice)	Reduced CT expression via inhibition of ToxT	[113]
	Model bicyclic compounds (*in vitro*)	Reduced *in vitro tcp* expression via inhibition of ToxT	[110]
	Ribavirin (*in vitro*)	Inhibition of AphB	[106]
	Dietary minerals (Zn, Mg, Se) (*in vitro, ex vivo*)	Reduction of transcription virulence genes *ctxAB, fliA*, *toxR*	[114]
Lytic bacteriophages	ØVC8 (wastewater, Mexico)	Lytic activity against *V. cholerae* O1 strain	[108]
	VPUSM 8 (sewage water, Malaysia)	Lytic activity against *V. cholerae* O1 El Tor Inaba serotype	[109]
	ICP 1, 2 and 3 (*in vitro, in vivo*-mice)	Lytic activity against *V. cholerae* O1	[107]
Host-directed	CFTR inhibitor ®-BPO-27 (*in vitro, in vivo*- mice)	Inhibition of CFTR conductance	[104]
	Bithionol (caspase inhibition) (*in vitro*)	Reduction of CT effects via inhibition of human caspases-1, –3, –6, –7, –9	[112]
	Entinostat (*in vitro, in vivo*-rabbits)	Restoration of antimicrobial peptide CAP-18 levels	[105]
	Quorum sensing	Manipulation of the gut microbiota	[103]

or herbs presents attractive potential source of therapy. Polysaccharides from the seaweed *Gracilaria caudate* have been shown to reduce secretory diarrhea, loss of chloride ions and fluid secretion in response to CT in *in vivo* studies in mice [100]. There was evidence that these polysaccharides interacted directly with both CT and its GM1 receptor. In *in vitro* studies, the sweet fennel component anethole reduced CT and TCP expression in a *toxT*-dependent, *toxR*/*toxS*-independent manner, via *tcpP*/*tcpH* repression [101]. This was mediated by over-expression of the *cyaA* and *crp* genes of the cAMP-cAMP receptor protein (CRP) system. Anethole also reduced fluid accumulation mediated by *V. cholerae* in rabbit ileum, suggesting its possible utility as an anti-cholera agent [101]. Meanwhile, *in silico* screening, absorption, digestion, metabolism, and excretion (ADME) of a range of herbal herbal compounds, with docking on ToxT, indicated that catechin and luteolin were potentially inhibitory of ToxT and had good ADME properties, suggesting that they are potential lead molecules for therapeutic development [102]. Another compound that has potential utility against ToxT include the unsaturated fatty acid (UFA) linoleic acid, including conjugated linoleic acid (CLA), which is currently commercially available as an over-the-counter weight loss supplement. In *in vitro* studies, CLA reduced CT and TCP expression via inhibition of ToxT [111]. Furthermore, *in vivo* studies in an adult rabbit ileal loop model indicated that CT production was reduced in response to CLA after infection with *V. cholerae* El Tor strain C6706 and that fluid accumulation was also significantly reduced [111]. Another ToxT-targeting agent is the synthetic isoquinolone alkaloid virstatin [113]. It reduces CT expression via ToxT inhibition and reduces intestinal colonisation of infant mice after *V. cholerae* infection [113]. A recent study designed to design, synthesize and characterize a new set of potent ToxT inhibitors used a structure-based approach with the folded conformation of UFAs such as CLA to design model bicyclic compounds [110]. The resulting "pre-folded" small molecule inhibitors bound more tightly to ToxT than UFAs or virstatin and reduced *in vitro tcp* expression; they have potential to be developed as anti-diarrhea therapies both in cholera and in disease caused by other enteric pathogens [110]. An advantage of all of these ToxT-directed inhibitors is that they target *V. cholerae* virulence rather than survival, therefore resistance is less likely to arise. Ribavirin, which is an FDA-approved drug, was recently shown to inhibit the *V. cholerae* protein AphB, which is a LysR-type transcriptional regulator (LTTR) involved in *tcpP* and *tcpH* expression [106]. Thus, this represents an opportunity to consider an already-approved, safe drug for repurposing in cholera treatment. Dietary minerals including zinc (Zn), manganese (Mn) and selenium (Se) have also been shown to reduce transcription of *V. cholerae* virulence genes including *ctxAB*, *fliA* and *tox R* in an *ex vivo* mouse intestine model, as well as reducing bacterial motility, Caco-2 cell adhesion and CT expression *in vitro* [114].

Another possible strategy for cholera therapy is use of *V. cholerae* lytic phages. They are thought to be important in modulation of *V. cholerae* O1 and O139 populations in aquatic environments, with effects on seasonality of cholera outbreaks depending on their levels [115]. In a study from Mexico, phages with lytic and lysogenic activity were isolated from *V. cholerae* O1 strains found in wastewater samples [108]. Of the 16 phages isolated, only the ØVC8 phage, a member of the VP2-like phage subfamily, had specific lytic activity against *V. cholerae* O1 strains. Bioinformatics studies revealed conserved domains within the ØVC8 phage such as immunoglobulin domains that could enable it to bind to mucus substrates, for example in human intestine. Another lytic environmental bacteriophage active against the O1 El Tor Inaba serotype, VPUSM 8, was recently isolated from sewage water in Malaysia, and its complete genome sequence was reported [109]. It has potential as a therapeutic or biocontrol agent. The potential of such bacteriophages was strikingly demonstrated in a recent *in vivo* study using both infant mouse and rabbit models of cholera showed that administration of a cocktail of three virulent bacteriophages, ICP 1, 2 and 3, up to 24 h before *V. cholerae* infection reduced both intestinal tract colonization and diarrhea [107]. None of the *V. cholerae* colonies that survived were resistant to all of the three bacteriophages, while any resistance was mainly due to mutations in genes encoding bacteriophage receptors. This suggests that prophylactic administration of ICP bacteriophages could be an effective method preventing cholera infection, for example in the context of a household of an infected individual [107]. Bacteriophages have also shown promising results in other animal model studies from India. For example, in a removable intestinal tie–adult rabbit diarrhea (RITARD) model, a mixture of O1 biotype El Tor typing phages reduced diarrhea grade in *V. cholerae* MAK 757-infected animals [116]. Results from another study in rabbits suggested that efficacy of a bacteriophage cocktail in reducing bacterial shedding in a model of *V. cholerae* O1 infection depended on oral administration of the phage cocktail after rather than before infection [117]. Treatment with a phage cocktail subsequent to *V. cholerae* MAK 757 infection of mice reduced bacterial colony numbers and inflammatory cytokine levels [118].

Host-directed therapies are another potential anti-cholera strategy. One example is possible use of cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel inhibitors [104]. These channels are inappropriately activated in secretory diarrhea associated with cholera. The benzopyrimido-pyrrolo-oxazinedione (R)-BPO-27 inhibits CFTR conductance in epithelial cell

cultures and intestine after treatment with CT and also prevented CT-induced fluid accumulation in small intestinal loops in an *in vivo* mouse model, with high bioavailability for more than 4 h at non-toxic(R)-BPO-27 concentrations [104]. Thus re-purposing of CFTR inhibitors is a possible strategy for treatment of secretory diarrhea in cholera. Another approved drug, Bithionol, which was originally approved for helminthic infection treatment, has been found to directly inhibit human caspases-1, -3, -6, -7, and -9, which are involved in apoptosis [112]. It thereby reduced the *in vitro* detrimental effects of CT, as well as a number of other toxins including diphtheria toxin, *Pseudomonas aeroginosa* exotoxin A, anthrax lethal toxin, ricin, *Botulinum* neurotoxin A and Zika virus [112]. Entinostat, an aroylated phenylenediamine, was also an effective host-directed therapy used *in vivo* in a recent study on *V. cholerae*-infected rabbits [105]. *V. cholerae* infection reduced the intestinal expression of the antimicrobial peptide CAP-18. Entinostat treatment restored CAP-18 levels and increased the levels of serum zonulin, a gut permeability marker, as well as mediating recovery from cholera and reduction in *V. cholerae* stool counts [105]. Thus etinostat is a potential host-directed therapeutic agent for cholera treatment which could increase CAP-18 and regulate intestinal permeability. Another possible host-directed "therapy" would be manipulation of the gut microbiota in order to exploit the fact that commensal gut microorganisms can apparently antagonise *V. cholerae* and reduce its colonisation in an interaction mediated by quorum sensing [103].

Cholera vaccines

Vaccination is an important element in the battle to prevent cholera transmission, in conjunction with access to safe water, sanitation and hygiene (WASH) [119,120]. There are currently three oral cholera vaccines (OCVs) prequalified by WHO, namely Dukoral®, Shanchol® and Euvichol®, all of which are killed/inactivated vaccines [120].

Dukoral®, which is manufactured by SBL Vaccines, contains heat- and formalin-killed *V. cholerae* and CT subunit B (CTB) and has shown efficacies ranging between 50% and 88% [121]. It is licensed for use in individuals over two years of age in many countries. It has also been shown to confer indirect (herd) protection as well as direct protection in a sub-Saharan African setting [122]

Shanchol®, which is manufactured by Shantha Biotec in India, contains killed whole cells of both *V. cholerae* O1 El Tor and Classical biotypes, and of O139 [120]. Its safety and immunogenicity has been confirmed in both adults and children as young as one year [123]. It is cheaper to produce than Dukoral® because it does not contain CTB and therefore does not need buffer. It has been used in vaccination programs in many countries. In Haiti, Shanchol® was used to vaccinate 97 774 people as part of a reactive vaccination campaign in 2012 in the context of the ongoing epidemic, as part of a package of measures that also included establishment of cholera treatment centers, promotion of WASH practices, and appropriate use of antibiotics [124–126]. This represented the first use of an OCV in an outbreak situation in urban and rural areas since prequalification by WHO. The effectiveness of the measures were confirmed in follow-up studies conducted 37 months after vaccination which demonstrated an efficacy of 97.5% [127]. It prompted the Ministry of Health in Haiti to incorporate use of OCVs into its national cholera control strategy [127]. However, despite this demonstration of the effectiveness of a reactive use of Shanchol®, administering such a scheme in Yemen remains complicated by issues such as the local movement of people and the difficulties in achieving WASH measures with a compromised healthcare system and sanitation and hygiene infrastructure, as well as issues such as global shortage of OCVs and the recommendations that the OCVs be administered in a two dose regimen [38]. Shanchol® was shown to be more effective when delivered as part of routine government services and combined with behavioral interventions aimed at encouraging safe drinking water and hand washing in a large cluster-randomized open-label trial in Bangladesh [128]. Another double-blind, cluster-randomized, placebo-controlled trial in Kolkata in India confirmed the long-term efficacy during a five-year follow-up study which showed cumulative protective efficacy of 65%, with no evidence of decreased efficacy [129]. Effectiveness of Shanchol® has also been demonstrated in significantly reducing cholera transmission in settings such as refugee camps [130].

Euvichol®, which is manufactured by EuBiologics in South Korea, contains killed whole cells of both *V. cholerae* O1 El Tor and Classical biotypes, and of O139 and is similar in formulation to Shanchol®. Its safety and immunogenicity was confirmed in a Phase I trial in South Korea [131], while its efficacy was confirmed in a Phase III trial on 1219 adults and children in the Philippines, in comparison to Shanchol® [132]. It had similar vibriocidal responses to Shanchol® in both adults and children [132].

Despite the effectiveness of these prequalified OCVs, there are constraints on their uptake and use. One major issue for developing countries in particular is cost. In considering cost-effectiveness of OCV use in Bangladesh, the Foundation Mérieux and the International Centre for Diarrheal Disease Research (ICDDR) found that use of lower cost OCVs such as Shanchol® would be potentially useful in combination with a range of WASH measures and clinical and rehydration treatments [45]. Cost-effectiveness was considered greatest for targeting of children aged

between 1 and 14 years, while other potentially cost-effective strategies would include targeting everyone aged over 1 year in high risk areas, or targeting urban slums or rural areas with poor water supply quality [45]. However affordability was identified as a major driver of which strategy would be chosen, while adequate global vaccine availability was also pinpointed as a crucial factor. Data from a vaccination campaign carried out in 2011 using Shanchol® in a high-risk area in Dhaka in Bangladesh was used to determine that total vaccination cost per person was US$3.98, given that 123 661 people received two doses and 18 178 people received one dose [133]. Vaccine purchase cost accounted for 58% of the cost overall, suggesting that reduction of cost-per-dose would impact significantly on future vaccination campaigns. Cost and global vaccine availability are both factors that have driven studies in which efficacy of using only one vaccine dose, as opposed to the recommended two, has been tested. Clinical trial data from Bangladesh [134] and from South Sudan [40] suggest that single-dose regimens can be efficacious. However, efficacy in the Bangladesh study was limited to adults and to children aged over five years, but not younger children [134]. Another approach would be to consider whether an alternative dosing schedule to the current two-week schedule could be used. In a randomized control trial with 356 participants conducted in Kolkata in India, vibriocidal response rates against *V. cholerae* O1 Ogawa in both adults and children were comparable for both two-week and four-week Shanchol® dosing schedules [135]. Less strict requirements for two-week schedules could improve vaccine uptake in situations where infrastructure is compromised and there is less reliable access to healthcare facilities. It is important that WHO and vaccine manufacturers clarify recommendations on dosing regimens in scenarios such as that currently existing in Yemen, where local logistical issues in exercising adequate surveillance in a country at war are compounding the more general problems. These include the global OCV shortage, the administrative difficulties inherent in submission of requests to the International Coordination Group and accessing sufficient vaccine doses for a reactive campaign in the face of competing demands in other endemic and epidemic regions [40]. Mathematical modeling has suggested that the optimal strategy for allocation of OCVs from the global stockpile is allocation of most doses to reactive campaigns, unless requests are made late in the epidemic, particularly when stocks are getting low [136]. This further emphasizes the desirability of having sufficient surveillance mechanisms in place that allow early reporting of epidemics.

Live OCVs have some advantages in some situations over the killed OCVs used in mass vaccination campaigns. Live-attenuated vaccines tend to require only one dose and produce robust and rapid mucosal and protective immunity responses. The live-attenuated vaccine CVD-103-HgR is a single dose vaccine and the only cholera vaccine licensed for use in the United States of America (USA) [17]. It was approved in 2016 by the Food and Drug Administration (FDA) for use in adults traveling to cholera endemic or epidemic areas. Its efficacy against severe secretory diarrhea has been estimated at 90% ten days post-vaccination and 80% three months post-vaccination [17]. However, live-attenuated vaccines are expensive to produce, may require refrigeration and may be risky for use in immunocompromised individuals [120]. Live attenuated vaccine currently under development include the VA 1.3 and VA 1.4 vaccines, both of which have undergone Phase I and II trials in India showing their safety and immunogenicity [137,138]. They are based on a non-toxigenic *V. cholerae* O1 El Tor strain and provide a potential tool for single-dose vaccination strategies which could be useful in reactive vaccination campaigns and emergencies. However, phase III trials are required to establish their efficacy and reservations remain on the use of live-attenuated vaccines in immunocompromised individuals such as HIV patients. Another live-attenuated cholera vaccine, derived from the 638 *V. cholerae* O1 El Tor Ogawa strain has also been shown to be safe and immunogenic in a placebo-controlled, double-blind randomized control trial on 120 adults in an endemic cholera area of Mozambique [139]. Conjugated vaccines are presently at the pre-clinical stage but may eventually provide an option for non-oral vaccination routes, or for supplementation of OCVs in vaccination of children [140,141]. Promising results have been obtained *in vivo* in mouse models for conjugate vaccines based on the O-specific polysaccharide (OSP) of the lipopolysaccharide (LPS) antigen of O1 Inaba and O1 Ogawa serotype strains [140,141].

Summary and perspectives

Cholera is a disease of poverty, as well as a frequent consequence of events such as war and/or natural disasters which undermine hygiene and health services. It remains rampant in many developing countries despite being readily preventable by access to adequate sanitation and hygiene measures and clean drinking water. Its devastating effects have been clear in the epidemic that occurred in Haiti in the aftermath of the 2010 earthquake and in the ongoing epidemic in Yemen, as well as in multiple seasonal outbreaks which occur regularly in sub-Saharan Africa and in Asia. It is readily treated by rehydration therapy if identified in time, although antibiotic treatment in the case of more serious infections has become more complicated in the light of the rise in antibiotic resistance. There are three killed OCVs which have been prequalified by WHO, but there is also a global shortage of vaccines despite the stockpile managed by the International

Coordination Group under the WHO Secretariat. Also, in situations of conflict or in the aftermath of natural disasters, problems with disease surveillance complicate early identification of cholera outbreaks and timely applications to the International Coordination Group in the event of an outbreak. While this system has been designed to ensure that scarce vaccine stocks are effectively targeted, the administration process and the vaccine shortage complicates the prompt distribution of vaccines to where they are urgently needed. Vaccination programs are most effective as part of a suite of measures also tackling infrastructure, sanitation and hygiene, which is difficult to establish in conflict situations such as that currently ongoing in Yemen. Clarity is needed on factors such as recommendations on dosing regimens in reactive vaccination campaigns and easier access to the vaccine stockpile in emergency situations. It is also important to be able to clarify which *V. cholerae* strains are dominant in the Yemen outbreak, and their antibiotic sensitivity and resistance profiles. Meanwhile, research is ongoing on several potential therapeutic options, including therapies aimed at viral virulence, host-directed options, or use of bacteriophages, to try to overcome the rising antibiotic resistance of *V. cholerae* strains. There are also promising developments in research on live-attenuated vaccines and conjugated vaccines which may be useful in one-dose administration in emergency situations, or for administration by alternative means than the oral route.

Compliance with ethics guidelines

Ali A. Rabaan declares no conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

1. Ali M, Nelson AR, Lopez AL, Sack DA. Updated global burden of cholera in endemic countries. PLoS Negl Trop Dis 2015; 9(6): e0003832
2. WHO. Cholera 2015. Weekly Epidemiological Report 2016; 91: 433–440
3. WHO. 2016. Cholera Fact sheet.http://www.who.int/mediacentre/factsheets/fs107/en/ (Accessed June 14 2017)
4. Luquero FJ, Rondy M, Boncy J, Munger A, Mekaoui H, Rymshaw E, Page AL, Toure B, Degail MA, Nicolas S, Grandesso F, Ginsbourger M, Polonsky J, Alberti KP, Terzian M, Olson D, Porten K, Ciglenecki I. Mortality rates during cholera epidemic, Haiti, 2010–2011. Emerg Infect Dis 2016; 22(3): 410–416
5. Ohene S, Klenyuie W, Sarpeh M. Assessment of the response to cholera outbreaks in two districts in Ghana. Infect Dis Poverty 2016; 5(1): 99
6. Steinberg EB, Greene KD, Bopp CA, Cameron DN, Wells JG, Mintz ED. Cholera in the United States, 1995–2000: trends at the end of the twentieth century. J Infect Dis 2001; 184(6): 799–802
7. Loharikar A, Newton AE, Stroika S, Freeman M, Greene KD, Parsons MB, Bopp C, Talkington D, Mintz ED, Mahon BE. Cholera in the United States, 2001–2011: a reflection of patterns of global epidemiology and travel. Epidemiol Infect 2015; 143(4): 695–703
8. Li XQ, Wang M, Deng ZA, Shen JC, Zhang XQ, Liu YF, Cai YS, Wu XW, Di B. Survivability and molecular variation in *Vibrio cholerae* from epidemic sites in China. Epidemiol Infect 2015; 143 (2): 288–297
9. WHO. 2017. Yemen: cholera outbreak. Daily Epidemiology Update. June 20 2017
10. BBC World News. 2017. Yemen crisis: Who is fighting whom? http://www.bbc.com/news/world-middle-east-29319423 (accessed June 14 2017)
11. WHO. 2016. Survey reveals extent of damage to Yemen's health system. http://www.who.int/hac/crises/yem/releases/en/ (Accessed June 14 2017)
12. Sakazaki R, Shimada T. Serovars of *Vibrio cholerae* identified during 1970–1975. Jpn J Med Sci Biol 1977; 30(5): 279–282
13. Sakazaki R, Shimada T. Additional serovars and inter-O antigenic relationships of *Vibrio cholerae.* Jpn J Med Sci Biol 1977; 30(5): 275–277
14. Shimada T, Arakawa E, Itoh K, Okitsu T, Matsushima A, Asai Y, Yamai S, Nakazato T, Nair GB, Albert MJ, Takeda Y. Extended serotyping scheme for *Vibrio cholerae.* Curr Microbiol 1994; 28 (3): 175–178
15. Banerjee R, Das B, Balakrish Nair G, Basak S. Dynamics in genome evolution of *Vibrio cholerae.* Infect Genet Evol 2014; 23: 32–41
16. Kaper JB, Morris JG, Levine MM. Cholera. Clin Microbiol Rev 1995; 8(1): 48–86
17. Wong KK, Burdette E, Mahon BE, Mintz ED, Ryan ET, Reingold AL. Recommendations of the Advisory Committee on Immunization Practices for Use of Cholera Vaccine. Atlanta: U.S. Center for Disease Control, 2017
18. Kuna A, Gajewski M. Cholera — the new strike of an old foe. Int Marit Health 2017; 68(3): 163–167
19. Nishiura H, Tsuzuki S, Yuan B, Yamaguchi T, Asai Y. Transmission dynamics of cholera in Yemen, 2017: a real time forecasting. Theor Biol Med Model 2017; 14(1): 14
20. Naseer M, Jamali T. Epidemiology, determinants and dynamics of cholera in Pakistan: gaps and prospects for future research. J Coll Physicians Surg Pak 2014 11; 24(11): 855–860
21. Siriphap A, Leekitcharoenphon P, Kaas RS, Theethakaew C, Aarestrup FM, Sutheinkul O, Hendriksen RS. Characterization and genetic variation of *Vibrio cholerae* isolated from clinical and environmental sources in Thailand. PLoS One 2017; 12(1): e0169324
22. Chowdhury F, Mather AE, Begum YA, Asaduzzaman M, Baby N, Sharmin S, Biswas R, Uddin MI, LaRocque RC, Harris JB, Calderwood SB. Vibrio cholerae serogroup O139: isolation from cholera patients and asymptomatic household family members in Bangladesh between 2013 and 2014. PLoS Negl Trop Dis 2015; 9 (11): e0004183
23. Li BS, Xiao Y, Wang DC, Tan HL, Ke BX, He DM, Ke CW,

Zhang YH. Genetic relatedness of selected clinical *Vibrio cholerae* O139 isolates from the southern coastal area of China over a 20-year period. Epidemiol Infect 2016; 144(12): 2679–2687

24. Aldová E, Lázničková K, Štěpánková E, Lietava J. Isolation of nonagglutinable vibrios from an enteritis outbreak in Czechoslovakia. J Infect Dis 1968; 118(1): 25–31
25. Kamal AM. Outbreak of gastro-enteritis by non-agglutinable (NAG) vibrios in the republic of the Sudan. J Egypt Public Health Assoc 1971; 46: 125–159
26. Rahaman MH, Islam T, Colwell RR, Alam M. Molecular tools in understanding the evolution of *Vibrio cholerae.* Front Microbiol 2015; 6: 1040
27. Blake PA. *Vibrio cholerae* and Cholera: Molecular to Global Perspectives. In: Wachsmuth IK, Blake PA, Olsvik Ø, eds. Washington, DC: Am So. Microbiol, 1994: 293–295
28. Dziejman M, Balon E, Boyd D, Fraser CM, Heidelberg JF, Mekalanos JJ. Comparative genomic analysis of *Vibrio cholerae*: genes that correlate with cholera endemic and pandemic disease. Proc Natl Acad Sci USA 2002; 99(3): 1556–1561
29. Eppinger M, Pearson T, Koenig SS, Pearson O, Hicks N, Agrawal S, Sanjar F, Galens K, Daugherty S, Crabtree J, Hendriksen RS, Price LB, Upadhyay BP, Shakya G, Fraser CM, Ravel J, Keim PS. Genomic epidemiology of the Haitian cholera outbreak: a single introduction followed by rapid, extensive, and continued spread characterized the onset of the epidemic. MBio 2014; 5(6): e01721–e14
30. Langa JP, Sema C, De Deus N, Colombo MM, Taviani E. Epidemic waves of cholera in the last two decades in Mozambique. J Infect Dev Ctries 2015; 9(6): 635–641
31. Nsagha DS, Atashili J, Fon PN, Tanue EA, Ayima CW, Kibu OD. Assessing the risk factors of cholera epidemic in the Buea Health District of Cameroon. BMC Public Health 2015; 15(1): 1128
32. Nair GB, Faruque SM, Bhuiyan NA, Kamruzzaman M, Siddique AK, Sack DA. New variants of *Vibrio cholerae* O1 biotype El Tor with attributes of the classical biotype from hospitalized patients with acute diarrhea in Bangladesh. J Clin Microbiol 2002; 40(9): 3296–3299
33. Das B, Halder K, Pal P, Bhadra RK. Small chromosomal integration site of classical CTX prophage in Mozambique *Vibrio cholerae* O1 biotype El Tor strain. Arch Microbiol 2007; 188(6): 677–683
34. Faruque SM, Tam VC, Chowdhury N, Diraphat P, Dziejman M, Heidelberg JF, Clemens JD, Mekalanos JJ, Nair GB. Genomic analysis of the Mozambique strain of *Vibrio cholerae* O1 reveals the origin of El Tor strains carrying classical CTX prophage. Proc Natl Acad Sci USA 2007; 104(12): 5151–5156
35. Barua D. History of cholera. In: Barua D, Greenough WB (eds). Cholera. New York: Springer US, 1992: 1–36
36. Bwire G, Munier A, Ouedraogo I, Heyerdahl L, Komakech H, Kagirita A, Wood R, Mhlanga R, Njanpop-Lafourcade B, Malimbo M, Makumbi I, Wandawa J, Gessner BD, Orach CG, Mengel MA. Epidemiology of cholera outbreaks and socio-economic characteristics of the communities in the fishing villages of Uganda: 2011–2015. PLoS Negl Trop Dis 2017; 11(3): e0005407
37. Kachwamba Y, Mohammed AA, Lukupulo H, Urio L, Majigo M, Mosha F, Matonya M, Kishimba R, Mghamba J, Lusekelo J, Nyanga S, Almeida M, Li S, Domman D, Massele SY, Stine OC. Genetic characterization of *Vibrio cholerae* O1 isolates from outbreaks between 2011 and 2015 in Tanzania. BMC Infect Dis 2017; 17(1): 157
38. Dalusi L, Lyimo TJ, Lugomela C, Hosea KMM, Sjöling S. Toxigenic *Vibrio cholerae* identified in estuaries of Tanzania using PCR techniques. FEMS Microbiol Lett 2015; 362(5): fnv009
39. Moore S, Miwanda B, Sadji AY, Thefenne H, Jeddi F, Rebaudet S, De Boeck H, Bidjada B, Depina JJ, Bompangue D, Abedi AA, Koivogui L, Keita S, Garnotel E, Plisnier PD, Ruimy R, Thomson N, Muyembe JJ, Piarroux R. Relationship between distinct African cholera epidemics revealed via MLVA haplotyping of 337 *Vibrio cholerae* isolates. PLoS Negl Trop Dis 2015; 9(6): e0003817
40. Parker LA, Rumunu J, Jamet C, Kenyi Y, Lino RL, Wamala JF, Mpairwe AM, Ciglenecki I, Luquero FJ, Azman AS, Cabrol JC. Adapting to the global shortage of cholera vaccines: targeted single dose cholera vaccine in response to an outbreak in South Sudan. Lancet Infect Dis 2017; 17(4): e123–e127
41. Bwire G, Mwesawina M, Baluku Y, Kanyanda SS, Orach CG. Cross-border cholera outbreaks in Sub-Saharan Africa, the mystery behind the silent illness: what needs to be done? PLoS One 2016; 11(6): e0156674
42. Masoumi-Asl H, Gouya MM, Rahbar M, Sabourian R. The epidemiology and antimicrobial resistance of cholera cases in Iran during 2013. Iran J Microbiol 2016; 8(4): 232–237
43. Ngwa MC, Liang S, Kracalik IT, Morris L, Blackburn JK, Mbam LM, Pouth SF, Teboh A, Yang Y, Arabi M, Sugimoto JD. Cholera in Cameroon, 2000–2012: spatial and temporal analysis at the operational (health district) and sub climate levels. PLoS Negl Trop Dis 2016; 10(11): e0005105
44. Jutla A, Aldaach H, Billian H, Akanda A, Huq A, Colwell R. Satellite based assessment of hydroclimatic conditions related to cholera in Zimbabwe. PLoS One 2015; 10(9): e0137828
45. Nelson CB, Mogasale V, Bari TI, Clemens JD. Considerations around the introduction of a cholera vaccine in Bangladesh. Vaccine 2014; 32(52): 7033–7036
46. Righetto L, Zaman RU, Mahmud ZH, Bertuzzo E, Mari L, Casagrandi R, Gatto M, Islam S, Rinaldo A. Detection of *Vibrio cholerae* O1 and O139 in environmental waters of rural Bangladesh: a flow-cytometry-based field trial. Epidemiol Infect 2015; 143(11): 2330–2342
47. Kumar P, Mishra DK, Deshmukh DG, Jain M, Zade AM, Ingole KV, Goel AK, Yadava PK. *Vibrio cholerae* O1 Ogawa El Tor strains with the *ctxB*7 allele driving cholera outbreaks in south-western India in 2012. Infect Genet Evol 2014; 25: 93–96
48. Klinzing DC, Choi SY, Hasan NA, Matias RR, Tayag E, Geronimo J, Skowronski E, Rashed SM, Kawashima K, Rosenzweig CN, Gibbons HS. Molecular tools in understanding the evolution of *Vibrio cholerae.* Front Microbiol 2015; 6: 1040
49. Das SK, Begum D, Ahmed S, Ferdous F, Farzana FD, Chisti MJ, Latham JR, Talukder KA, Rahman MM, Begum YA, Faruque ASG, Malek MA, Qadri F, Ahmed T, Alam N. Geographical diversity in seasonality of major diarrhoeal pathogens in Bangladesh observed between 2010 and 2012. Epidemiol Infect 2014; 142(12): 2530–2541
50. Rashed SM, Hasan NA, Alam M, Sadique A, Sultana M, Hoq MM, Sack RB, Colwell RR, Huq A. *Vibrio cholerae* O1 with reduced susceptibility to ciprofloxacin and azithromycin isolated from a

rural coastal area of Bangladesh. Front Microbiol 2017; 8: 252
51. Kar SK, Pal BB, Khuntia HK, Achary KG, Khuntia CP. Emergence and spread of tetracycline resistant *Vibrio cholerae* O1 El Tor variant during 2010 cholera epidemic in the tribal areas of Odisha, India. Int J Infect Dis 2015; 33: 45–49
52. Bhattacharya D, Dey S, Roy S, Parande MV, Telsang M, Seema MH, Parande AV, Mantur BG. Multidrug-resistant *Vibrio cholerae* O1 was responsible for a cholera outbreak in 2013 in Bagalkot, North Karnataka. Jpn J Infect Dis 2015; 68(4): 347–350
53. Torane V, Kuyare S, Nataraj G, Mehta P, Dutta S, Sarkar B. Phenotypic and antibiogram pattern of *V. cholerae* isolates from a tertiary care hospital in Mumbai during 2004–2013: a retrospective cross-sectional study. BMJ Open 2016; 6(11): e012638
54. Reimer AR, Van Domselaar G, Stroika S, Walker M, Kent H, Tarr C, Talkington D, Rowe L, Olsen-Rasmussen M, Frace M, Sammons S. Comparative genomics of *Vibrio cholerae* from Haiti, Asia, and Africa. Emerg Infect Dis 2011; 17(11): 2113
55. Hendriksen RS, Price LB, Schupp JM, Gillece JD, Kaas RS, Engelthaler DM, Bortolaia V, Pearson T, Waters AE, Upadhyay BP, Shrestha SD. Population genetics of *Vibrio cholerae* from Nepal in 2010: evidence on the origin of the Haitian outbreak. MBio 2011; 2(4): e00157–e11
56. Orata FD, Keim PS, Boucher Y. The 2010 cholera outbreak in Haiti: how science solved a controversy. PLoS Pathog 2014; 10(4): e1003967
57. Piarroux R, Barrais R, Faucher B, Haus R, Piarroux M, Gaudart J, Magloire R, Raoult D. Understanding the cholera epidemic, Haiti. Emerg Infect Dis 2011; 17(7): 1161–1168
58. Ivers LC, Walton DA. The "first" case of cholera in Haiti: lessons for global health. Am J Trop Med Hyg 2012; 86(1): 36–38
59. Kahler AM, Haley BJ, Chen A, Mull BJ, Tarr CL, Turnsek M, Katz LS, Humphrys MS, Derado G, Freeman N, Boncy J, Colwell RR, Huq A, Hill VR. Environmental surveillance for toxigenic *Vibrio cholerae* in surface waters of Haiti. Am J Trop Med Hyg 2015; 92 (1):118–125
60. Alam MT, Weppelmann TA, Longini I, De Rochars VMB, Morris JG, Ali A. Increased isolation frequency of toxigenic *Vibrio cholerae* O1 from environmental monitoring sites in Haiti. PLoS One 2015; 10(4): e0124098
61. Lantagne D, Nair GB, Lanata CF, Cravioto A. The cholera outbreak in Haiti: where and how did it begin? In: Nair GB, Takeda Y. Cholera Outbreaks. Berlin Heidelberg: Springer, 2013: 145–164
62. Lewnard JA, Antillón M, Gonsalves G, Miller AM, Ko AI, Pitzer VE. Strategies to prevent cholera introduction during international personnel deployments: a computational modeling analysis based on the 2010 Haiti outbreak. PLoS Med 2016; 13(1): e1001947
63. Satchell KJF, Jones CJ, Wong J, Queen J, Agarwal S, Yildiz FH. Phenotypic analysis reveals that the 2010 Haiti cholera epidemic is linked to a hypervirulent strain. Infect Immun 2016; 84(9): 2473–2481
64. Grandesso F, Allan M, Jean-Simon PS, Boncy J, Blake A, Pierre R, Alberti KP, Munger A, Elder G, Olson D, Porten K, Luquero FJ. Risk factors for cholera transmission in Haiti during inter-peak periods: insights to improve current control strategies from two case-control studies. Epidemiol Infect 2014; 142(8): 1625–1635
65. Page A, Ciglenecki I, Jasmin ER, Desvignes L, Grandesso F, Polonsky J, Nicholas S, Alberti KP, Porten K, Luquero FJ. Geographic distribution and mortality risk factors during the cholera outbreak in a rural region of Haiti, 2010–2011. PLoS Negl Trop Dis 2015; 9(3): e0003605
66. Aureli M, Mauri L, Ciampa MG, Prinetti A, Toffano G, Secchieri C, Sonnino S. GM1 ganglioside: past studies and future potential. Mol Neurobiol 2016; 53(3): 1824–1842
67. Broeck DV, Horvath C, De Wolf MJ. *Vibrio cholerae*: cholera toxin. Int J Biochem Cell Biol 2007; 39(10): 1771–1775
68. Heggelund JE, Burschowsky D, Bjørnestad VA, Hodnik V, Anderluh G, Krengel U. High-resolution crystal structures elucidate the molecular basis of cholera blood group dependence. PLoS Pathog 2016; 12(4): e1005567
69. Phongsisay V, Iizasa EI, Hara H, Yoshida H. Evidence for TLR4 and FcRγ–CARD9 activation by cholera toxin B subunit and its direct bindings to TREM2 and LMIR5 receptors. Mol Immunol 2015; 66(2): 463–471
70. Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 1996; 272(5270): 1910–1914
71. Heilpern AJ, Waldor MK. CTXΦ infection of *Vibrio cholerae* requires the tolQRA gene products. J Bacteriol 2000; 182(6): 1739–1747
72. Moyer KE, Kimsey HH, Waldor MK. Evidence for a rolling-circle mechanism of phage DNA synthesis from both replicative and integrated forms of CTXΦ. Mol Microbiol 2001; 41(2): 311–323
73. Das B, Bischerour J, Val ME, Barre FX. Molecular keys of the tropism of integration of the cholera toxin phage. Proc Natl Acad Sci USA 2010; 107(9): 4377–4382
74. Quinones M, Kimsey HH, Waldor MK. LexA cleavage is required for CTX prophage induction. Mol Cell 2005; 17(2): 291–300
75. Waldor MK, Rubin EJ, Pearson GD, Kimsey H, Mekalanos JJ. Regulation, replication, and integration functions of the *Vibrio cholerae* CTXΦ are encoded by region RS2. Mol Microbiol 1997; 24(5): 917–926
76. Martínez E, Paly E, Barre F. CTXψ replication depends on the histone-like HU protein and the UvrD helicase. PLoS Genet 2015; 11(5): e1005256
77. DiRita VJ, Parsot C, Jander G, Mekalanos JJ. Regulatory cascade controls virulence in *Vibrio cholerae.* Proc Natl Acad Sci USA 1991; 88(12): 5403–5407
78. Lowden MJ, Skorupski K, Pellegrini M, Chiorazzo MG, Taylor RK, Kull FJ. Structure of *Vibrio cholerae* ToxT reveals a mechanism for fatty acid regulation of virulence genes. Proc Natl Acad Sci USA 2010; 107(7): 2860–2865
79. Matson JS, Withey JH, DiRita VJ. Regulatory networks controlling *Vibrio cholerae* virulence gene expression. Infect Immun 2007; 75(12): 5542–5549
80. Global Task Force on Cholera Control (GTFCC). Cholera outbreak: assessing the outbreak response and improving preparedness. Geneva: World Health Organization, 2010. Available from: http://www.who.int/cholera/publications/OutbreakAssessment/en/ (Accessed June 27 2017)
81. Leibovici-Weissman Y, Neuberger A, Bitterman R, Sinclair D, Salam MA, Paul M. Antimicrobial drugs for treating cholera. Cochrane Database Syst Rev 2014; (6): 1
82. Baron S, Lesne J, Jouy E, Larvor E, Kempf I, Boncy J, Rebaudet S, Piarroux R. Antimicrobial susceptibility of autochthonous aquatic

Vibrio cholerae in Haiti. Front Microbiol 2016; 7: 1671
83. Carraro N, Rivard N, Ceccarelli D, Colwell RR, Burrus V. IncA/C conjugative plasmids mobilize a new family of multidrug resistance islands in clinical *Vibrio cholerae* Non-O1/Non-O139 isolates from Haiti. MBio 2016; 7(4): e00509–e00516
84. Ceccarelli D, Alam M, Huq A, Colwell RR. Reduced susceptibility to extended-spectrum β-lactams in *Vibrio cholerae* isolated in Bangladesh. Front Public Health 2016; 4: 231
85. Wang R, Li J, Kan B. Sequences of a co-existing SXT element, a chromosomal integron (CI) and an IncA/C plasmid and their roles in multidrug resistance in a *Vibrio cholerae* O1 El Tor strain. Int J Antimicrob Agents 2016; 48(3): 305–309
86. Mehla K, Ramana J. DBDiaSNP: an open-source knowledgebase of genetic polymorphisms and resistance genes related to diarrheal pathogens. OMICS 2015; 19(6): 354–360
87. Bhattacharya MK, Kanungo S, Ramamurthy T, Rajendran K, Sinha A, Bhattacharya A, Sarkar BS. Comparison between single dose azithromycin and six doses, 3 day norfloxacin for treatment of cholera in adult. Int J Biomed Sci 2014; 10(4): 248–251
88. Daniels NA, Shafaie A. A review of pathogenic *Vibrio* infections for clinicians. Infect Med 2000; 17(10): 665–685
89. Ghosh A, Ramamurthy T. Antimicrobials and cholera: are we stranded? Indian J Med Res 2011; 133(2): 225
90. Pugliese N, Maimone F, Scrascia M, Materu SF, Pazzani C. SXT-related integrating conjugative element and IncC plasmids in *Vibrio cholerae* O1 strains in Eastern Africa. J Antimicrob Chemother 2009; 63(3): 438–442
91. Spagnoletti M, Ceccarelli D, Rieux A, Fondi M, Taviani E, Fani R, Colombo MM, Colwell RR, Balloux F. Acquisition and evolution of SXT-R391 integrative conjugative elements in the seventh-pandemic *Vibrio cholerae* lineage. MBio 2014; 5(4): e01356–e14
92. Wang R, Yu D, Zhu L, Li J, Yue J, Kan B. IncA/C plasmids harboured in serious multidrug-resistant *Vibrio cholerae* serogroup O139 strains in China. Int J Antimicrob Agents 2015; 45(3): 249–254
93. Wang R, Yu D, Yue J, Kan B. Variations in SXT elements in epidemic *Vibrio cholerae* O1 El Tor strains in China. Sci Rep 2016; 6(1): 22733
94. Goel AK, Jiang SC. Genetic determinants of virulence, antibiogram and altered biotype among the *Vibrio cholerae* O1 isolates from different cholera outbreaks in India. Infect Genet Evol 2010; 10(6): 814–818
95. Mutreja A, Kim DW, Thomson NR, Connor TR, Lee JH, Kariuki S, Croucher NJ, Choi SY, Harris SR, Lebens M, Niyogi SK, Kim EJ, Ramamurthy T, Chun J, Wood JLN, Clemens JD, Czerkinsky C, Nair GB, Holmgren J, Parkhill J, Dougan G. Evidence for several waves of global transmission in the seventh cholera pandemic. Nature 2011; 477(7365): 462–465
96. Wozniak RA, Fouts DE, Spagnoletti M, Colombo MM, Ceccarelli D, Garriss G, Déry C, Burrus V, Waldor MK. Comparative ICE genomics: insights into the evolution of the SXT/R391 family of ICEs. PLoS Genet 2009; 5(12): e1000786
97. Waldor MK, Tschäpe HE, Mekalanos JJ. A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in *Vibrio cholerae* O139. J Bacteriol 1996; 178(14): 4157–4165
98. Ceccarelli D, Spagnoletti M, Bacciu D, Danin-Poleg Y, Mendiratta DK, Kashi Y, Cappuccinelli P, Burrus V, Colombo MM. ICEVchInd5 is prevalent in epidemic *Vibrio cholerae* O1 El Tor strains isolated in India. Int J Med Microbiol 2011; 301(4): 318–324
99. Sá LL, Fonseca ÉL, Pellegrini M, Freitas F, Loureiro EC, Vicente AC. Occurrence and composition of class 1 and class 2 integrons in clinical and environmental O1 and non-O1/non-O139 *Vibrio cholerae* strains from the Brazilian Amazon. Mem Inst Oswaldo Cruz 2010; 105(2): 229–232
100. Costa DS, Araújo TSL, Sousa NA, Souza LKM, Pacífico DM, Sousa FBM, Nicolau LAD, Chaves LS, Barros FCN, Freitas ALP, Medeiros JVR. Sulphated polysaccharide isolated from the seaweed *Gracilaria caudata* exerts an antidiarrhoeal effect in rodents. Basic Clin Pharmacol Toxicol 2016; 118(6): 440–448
101. Zahid MS, Awasthi SP, Asakura M, Chatterjee S, Hinenoya A, Faruque SM, Yamasaki S. Suppression of virulence of toxigenic *Vibrio cholerae* by anethole through the cyclic AMP (cAMP)-cAMP receptor protein signaling system. PLoS One 2015; 10(9): e0137529
102. Perveen S, Chaudhary HS. *In silico* screening of antibacterial compounds from herbal sources against *Vibrio cholerae.* Pharmacogn Mag 2015; 11(44): S550–S555
103. Thompson JA, Oliveira RA, Xavier KB. Can chatter between microbes prevent cholera? Trends Microbiol 2014; 22(12): 660–662
104. Cil O, Phuan P, Gillespie AM, Lee S, Tradtrantip L, Yin J, Tse M, Zachos NC, Lin R, Donowitz M, Verkman AS. Benzopyrimido-pyrrolo-oxazine-dione CFTR inhibitor (R)-BPO-27 for antisecretory therapy of diarrheas caused by bacterial enterotoxins. FASEB J 2017; 31(2): 751–760
105. Sarker P, Banik A, Stromberg R, Gudmundsson GH, Raqib R, Agerberth B. Treatment with entinostat heals experimental cholera by affecting physical and chemical barrier functions of intestinal epithelia. Antimicrob Agents Chemother 2017;61(7): e02570-16
106. Mandal RS, Ta A, Sinha R, Theeya N, Ghosh A, Tasneem M, Bhunia A, Koley H, Das S. Ribavirin suppresses bacterial virulence by targeting LysR-type transcriptional regulators. Sci Rep 2016; 6(1): 39454
107. Yen M, Cairns LS, Camilli A. A cocktail of three virulent bacteriophages prevents *Vibrio cholerae* infection in animal models. Nat Commun 2017; 8: 14187
108. Solís-Sánchez A, Hernández-Chiñas U, Navarro-Ocaña A, De LM, Xicohtencatl-Cortes J, Eslava-Campos C. Genetic characterization of ØVC8 lytic phage for *Vibrio cholerae* O1. Virol J 2016; 13(1): 47
109. Al-Fendi A, Shueb RH, Foo PC, Ravichandran M, Yean CY. Complete genome sequence of lytic bacteriophage VPUSM 8 against O1 El Tor Inaba *Vibrio cholerae.* Genome Announc 2017; 5(21): e00073–e17
110. Woodbrey AK, Onyango EO, Pellegrini M, Kovacikova G, Taylor RK, Gribble GW, Kull FJ. A new class of inhibitors of the AraC family virulence regulator *Vibrio cholerae* ToxT. Sci Rep 2017; 7: 45011
111. Withey JH, Nag D, Plecha SC, Sinha R, Koley H. Conjugated linoleic acid reduces cholera toxin production *in vitro* and *in vivo* by inhibiting *Vibrio cholerae* ToxT Activity. Antimicrob Agents

Chemother 2015; 59(12): 7471–7476
112. Leonardi W, Zilbermintz L, Cheng LW, Zozaya J, Tran SH, Elliott JH, Polukhina K, Manasherob R, Li A, Chi X, Gharaibeh D, Kenny T, Zamani R, Soloveva V, Haddow AD, Nasar F, Bavari S, Bassik MC, Cohen SN, Levitin A, Martchenko M. Bithionol blocks pathogenicity of bacterial toxins, ricin, and Zika virus. Sci Rep 2016; 6(1): 34475
113. Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents 2014; 44(5): 377–386
114. Bhattaram V, Upadhyay A, Yin H, Mooyottu S, Venkitanarayanan K. Effect of dietary minerals on virulence attributes of *Vibrio cholerae.* Front Microbiol 2017; 8: 911
115. Faruque SM, Naser IB, Islam MJ, Faruque AS, Ghosh AN, Nair GB, Sack DA, Mekalanos JJ. Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. Proc Natl Acad Sci USA 2005; 102(5): 1702–1707
116. Bhowmick TS, Koley H, Das M, Saha DR, Sarkar BL. Pathogenic potential of vibriophages against an experimental infection with *Vibrio cholerae* O1 in the RITARD model. Int J Antimicrob Agents 2009; 33(6): 569–573
117. Jaiswal A, Koley H, Ghosh A, Palit A, Sarkar B. Efficacy of cocktail phage therapy in treating *Vibrio cholerae* infection in rabbit model. Microbes Infect 2013; 15(2): 152–156
118. Jaiswal A, Koley H, Mitra S, Saha DR, Sarkar B. Comparative analysis of different oral approaches to treat *Vibrio cholerae* infection in adult mice. Int J Med Microbiol 2014; 304(3–4): 422–430
119. Chaignat CL. What about cholera vaccines? Expert Rev Vaccines 2008; 7(4): 403–405
120. Saha A, Rosewell A, Hayen A, MacIntyre CR, Qadri F. Improving immunization approaches to cholera. Expert Rev Vaccines 2017; 16(3): 235–248
121. Lucas ME, Deen JL, von Seidlein L, Wang XY, Ampuero J, Puri M, Ali M, Ansaruzzaman M, Amos J, Macuamule A, Cavailler P, Guerin PJ, Mahoudeau C, Kahozi-Sangwa P, Chaignat CL, Barreto A, Songane FF, Clemens JD. Effectiveness of mass oral cholera vaccination in Beira, Mozambique. N Engl J Med 2005; 352(8): 757–767
122. Khatib AM, Ali M, von Seidlein L, Kim DR, Hashim R, Reyburn R, Ley B, Thriemer K, Enwere G, Hutubessy R, Aguado MT, Kieny MP, Lopez AL, Wierzba TF, Ali SM, Saleh AA, Mukhopadhyay AK, Clemens J, Jiddawi MS, Deen J. Effectiveness of an oral cholera vaccine in Zanzibar: findings from a mass vaccination campaign and observational cohort study. Lancet Infect Dis 2012; 12(11): 837–844
123. Saha A, Chowdhury MI, Khanam F, Bhuiyan MS, Chowdhury F, Khan AI, Khan IA, Clemens J, Ali M, Cravioto A, Qadri F. Safety and immunogenicity study of a killed bivalent (O1 and O139) whole-cell oral cholera vaccine Shanchol, in Bangladeshi adults and children as young as 1 year of age. Vaccine 2011; 29(46): 8285–8292
124. Ivers LC, Teng JE, Lascher J, Raymond M, Weigel J, Victor N, Jerome JG, Hilaire IJ, Almazor CP, Ternier R, Cadet J. Use of oral cholera vaccine in Haiti: a rural demonstration project. Am J Trop Med Hyg 2013; 89(4): 617–624
125. Ivers LC, Hilaire IJ, Teng JE, Almazor CP, Jerome JG, Ternier R, Boncy J, Buteau J, Murray MB, Harris JB, Franke MF. Effectiveness of reactive oral cholera vaccination in rural Haiti: a case-control study and bias-indicator analysis. Lancet Glob Health 2015; 3(3): e162–e168
126. Rouzier V, Severe K, Juste MA, Peck M, Perodin C, Severe P, Deschamps MM, Verdier RI, Prince S, Francois J, Cadet JR. Cholera vaccination in urban Haiti. Am J Trop Med Hyg 2013; 89 (4): 671–681
127. Sévère K, Rouzier V, Anglade SB, Bertil C, Joseph P, Deroncelay A, Mabou MM, Wright PF, Guillaume FD, Pape JW. Effectiveness of oral cholera vaccine in Haiti: 37-month follow-up. Am J Trop Med Hyg 2016; 94(5): 1136–1142
128. Qadri F, Ali M, Chowdhury F, Khan AI, Saha A, Khan IA, Begum YA, Bhuiyan TR, Chowdhury MI, Uddin MJ, Khan JA, Chowdhury AI, Rahman A, Siddique SA, Asaduzzaman M, Akter A, Khan A, Ae You Y, Siddik AU, Saha NC, Kabir A, Riaz BK, Biswas SK, Begum F, Unicomb L, Luby SP, Cravioto A, Clemens JD. Feasibility and effectiveness of oral cholera vaccine in an urban endemic setting in Bangladesh: a cluster randomised open-label trial. Lancet 2015; 386(10001): 1362–1371
129. Bhattacharya SK, Sur D, Ali M, Kanungo S, You YA, Manna B, Sah B, Niyogi SK, Park JK, Sarkar B, Puri MK, Kim DR, Deen JL, Holmgren J, Carbis R, Dhingra MS, Donner A, Nair GB, Lopez AL, Wierzba TF, Clemens JD. 5 year efficacy of a bivalent killed whole-cell oral cholera vaccine in Kolkata, India: a cluster-randomised, double-blind, placebo-controlled trial. Lancet Infect Dis 2013; 13(12): 1050–1056
130. Phares CR, Date K, Travers P, Déglise C, Wongjindanon N, Ortega L, Bhuket PR. Mass vaccination with a two-dose oral cholera vaccine in a long-standing refugee camp, Thailand. Vaccine 2016; 34(1): 128–133
131. Baik YO, Choi SK, Kim JW, Yang JS, Kim IY, Kim CW, Hong JH. Safety and immunogenicity assessment of an oral cholera vaccine through phase I clinical trial in Korea. J Korean Med Sci 2014; 29(4): 494–501
132. Baik YO, Choi SK, Olveda RM, Espos RA, Ligsay AD, Montellano MB, Yeam JS, Yang JS, Park JY, Kim DR, Desai SN, Singh AP, Kim IY, Kim CW, Park S. A randomized, non-inferiority trial comparing two bivalent killed, whole cell, oral cholera vaccines (Euvichol vs Shanchol) in the Philippines. Vaccine 2015; 33(46): 6360–6365
133. Sarker AR, Islam Z, Khan IA, Saha A, Chowdhury F, Khan AI, Cravioto A, Clemens JD, Qadri F, Khan JA. Estimating the cost of cholera-vaccine delivery from the societal point of view: a case of introduction of cholera vaccine in Bangladesh. Vaccine 2015; 33 (38): 4916–4921
134. Qadri F, Wierzba TF, Ali M, Chowdhury F, Khan AI, Saha A, Khan IA, Asaduzzaman M, Akter A, Khan A, Begum YA, Bhuiyan TR, Khanam F, Chowdhury MI, Islam T, Chowdhury AI, Rahman A, Siddique SA, You YA, Kim DR, Siddik AU, Saha NC, Kabir A, Cravioto A, Desai SN, Singh AP, Clemens JD. Efficacy of a single-dose, inactivated oral cholera vaccine in Bangladesh. N Engl J Med 2016; 374(18): 1723–1732
135. Kanungo S, Desai SN, Nandy RK, Bhattacharya MK, Kim DR, Sinha A, Mahapatra T, Yang JS, Lopez AL, Manna B, Bannerjee B, Ali M, Dhingra MS, Chandra AM, Clemens JD, Sur D, Wierzba TF. Flexibility of oral cholera vaccine dosing—a randomized

controlled trial measuring immune responses following alternative vaccination schedules in a cholera hyper-endemic zone. PLoS Negl Trop Dis 2015; 9(3): e0003574

136. Moore SM, Lessler J. Optimal allocation of the limited oral cholera vaccine supply between endemic and epidemic settings. J R Soc Interface 2015; 12(111): 20150703
137. Mahalanabis D, Ramamurthy T, Nair GB, Ghosh A, Shaikh S, Sen B, Thungapathra M, Ghosh RK, Pazhani GP, Nandy RK, Jana S, Bhattacharya SK. Randomized placebo controlled human volunteer trial of a live oral cholera vaccine VA1. 3 for safety and immune response. Vaccine 2009; 27(35): 4850–4856
138. Kanungo S, Sen B, Ramamurthy T, Sur D, Manna B, Pazhani GP, Chowdhury G, Jhunjhunwala P, Nandy RK, Koley H, Bhattacharya MK, Gupta S, Goel G, Dey B, M T, Nair GB, Ghosh A, Mahalanabis D. Safety and immunogenicity of a live oral recombinant cholera vaccine VA1. 4: a randomized, placebo controlled trial in healthy adults in a cholera endemic area in Kolkata, India. PLoS One 2014; 9(7): e99381
139. García HM, Thompson R, Valera R, Fando R, Fumane J, Jani I, Mirabal M, Armesto MI, Songane M, Luis S, Nzualo AM. A single dose of live-attenuated 638 *Vibrio cholerae* oral vaccine is safe and immunogenic in adult volunteers in Mozambique. Vaccimonitor 2011; 20(3): 1–8
140. Alam MM, Bufano MK, Xu P, Kalsy A, Yu Y, Freeman YW, Sultana T, Rashu MR, Desai I, Eckhoff G, Leung DT, Charles RC, LaRocque RC, Harris JB, Clements JD, Calderwood SB, Qadri F, Vann WF, Kováč P, Ryan ET. Evaluation in mice of a conjugate vaccine for cholera made from *Vibrio cholerae* O1 (Ogawa) O-specific polysaccharide. PLoS Negl Trop Dis 2014; 8(2): e2683
141. Sayeed MA, Bufano MK, Xu P, Eckhoff G, Charles RC, Alam MM, Sultana T, Rashu MR, Berger A, Gonzalez-Escobedo G, Mandlik A, Bhuiyan TR, Leung DT, LaRocque RC, Harris JB, Calderwood SB, Qadri F, Vann WF, Kováč P, Ryan ET. A cholera conjugate vaccine containing O-specific polysaccharide (OSP) of *V. cholerae* O1 Inaba and recombinant fragment of tetanus toxin heavy chain (OSP:rTTHc) induces serum, memory and lamina proprial responses against OSP and is protective in mice. PLoS Negl Trop Dis 2015; 9(7): e0003881