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REVIEW

Diagnosis, Management, and Future Control of Cholera

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SUMMARY Cholera, caused by *Vibrio cholerae*, persists in developing countries due to inadequate access to safe water, sanitation, and hygiene. There are approximately 4 million cases and 143,000 deaths each year due to cholera. The disease is transmitted fecally-orally via contaminated food or water. Severe dehydrating cholera can progress to hypovolemic shock due to the rapid loss of fluids and electrolytes, which requires a rapid infusion of intravenous (i.v.) fluids. The case fatality rate exceeds 50% without proper clinical management but can be less than 1% with prompt rehydration and antibiotics. Oral cholera vaccines (OCVs) serve as a major component of an integrated control package during outbreaks or within zones of endemicity. Water, sanitation, and hygiene (WaSH); health education; and prophylactic antibiotic treatment are additional components of the prevention and control of cholera. The World Health Organization

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(WHO) and the Global Task Force for Cholera Control (GTFCC) have set an ambitious goal of eliminating cholera by 2030 in high-risk areas.

KEYWORDS *Vibrio cholerae*, WaSH, oral cholera vaccine, treatment

INTRODUCTION

Vibrio cholerae is a major cause of severe dehydrating diarrheal disease and remains a major public health problem in low- and middle-income countries (LMICs) where water, sanitation, and hygiene (WaSH) are inadequate (1). Severe cholera can rapidly lead to hypovolemic shock after the onset of diarrhea and vomiting. However, with appropriate oral and intravenous (i.v.) fluid therapy, clinicians can lower the case fatality rate from over 50% to less than 1% (2). There are two fecal-oral mechanisms of *V. cholerae* transmission: one involves direct spread from person to person as a consequence of eating bacterium-contaminated food or water, and the other involves drinking environmentally polluted water from ponds, lakes, or rivers (3, 4). Fomites and flies may help disseminate *V. cholerae* by acting as mechanical vectors (3).

The World Health Organization (WHO) approximates 1.4 million to 4.0 million cases and 21,000 to 143,000 deaths globally due to the disease per annum (5). A zone where cholera is endemic is defined as an area with confirmed cases detected over the past 3 years (5). Cholera is endemic in Asia, Latin and Central America, and sub-Saharan Africa (1, 5, 6). *V. cholerae* originated in the Ganges River Delta and remains highly prevalent in Asia and Africa (7). Outbreaks of cholera are unpredictable and can occur in both zones where cholera is endemic and those where it is nonendemic depending on environmental conditions, with refugee camps being particularly susceptible (8). *V. cholerae* is always evolving, with new phenotypes and genotypes emerging with outbreaks and as a result of increased antibiotic resistance (9). The WHO's Global Task Force for Cholera Control (GTFCC) recommends access to both oral cholera vaccination and improved WaSH to avert cholera transmission as well as continued diarrheal disease surveillance (10).

EPIDEMIOLOGY

Geographical Distribution and Burden of Cholera

In resource-poor countries of endemicity, data from the last decade have shown that the burden of cholera has increased, and it has become an important public health problem (8). Figure 1 illustrates the global geographical distribution of cholera between 2016 and 2019 (11–14). Cholera has been historically endemic in the Asian subcontinent (e.g., Indonesia, India, Bangladesh, Vietnam, Thailand, Pakistan, Nepal, and Iraq) but is now endemic in Africa (e.g., South Africa, Mozambique, Botswana, Zambia, Sierra Leone, Nigeria, Angola, the Democratic Republic of the Congo [DRC], Yemen, Zimbabwe, the United Republic of Tanzania, and Guinea), Latin America (Brazil, Peru, Chile, Columbia, and Ecuador), and the Caribbean (Haiti, Cuba, and the Dominican Republic) (11–14). Due to weak or absent surveillance systems, many countries do not report cholera cases or deaths (3). Some countries are cautious in announcing cholera outbreaks to avoid economic losses in tourism and exports and to prevent general panic in society. But early reporting of cholera outbreaks has resulted in shorter durations of epidemics (15). Since 2006, 52 developing countries have reported increasing numbers of cholera cases (6).

In the Bengal Delta region, cholera epidemics typically follow a seasonal pattern, with one peak in spring (March to May) and another following the rainy season (September to November) (16–20). In Africa, epidemics occur in different regions during the rainy season, with recent outbreaks being reported in Zanzibar, the Eastern DRC, Angola, and West Africa. Haiti experienced a recent outbreak from 2017 to 2018 due to Hurricane Matthew, and the WHO reported 800,000 cholera cases and approximately 10,000 deaths from cholera since the outbreak (3). Mathematical modeling of cholera transmission suggests that outbreaks are reliant on variations in the environment and herd protection (21).

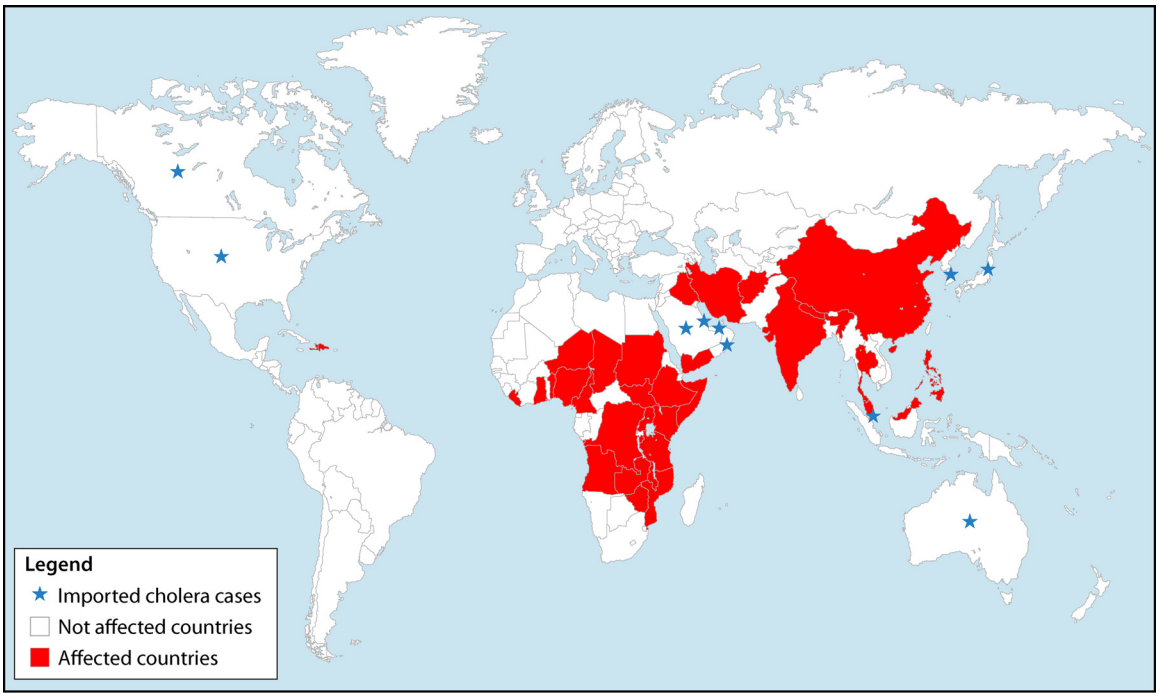

FIG 1 Global distribution of cholera between 2016 and 2019. ★, the imported cases were from elsewhere in the country where cholera is not common. The red zones represent the affected countries where cholera is endemic during the 3-year period.

Risk Factors

The risk factors for acquiring cholera are associated with poverty, including inadequate sanitation, contaminated drinking water, and poor food hygiene (e.g., street foods). Handwashing with soap before and after meals and after defecation is associated with a reduced risk (22).

There are some biological factors that have been recognized as risk factors for cholera, and these are female gender, blood group O, and retinol deficiency, and hypochlorhydria (i.e., in people who take histamine receptor blockers, antacids, and proton pump inhibitors) also increases the risk of contracting cholera (23–28). Moreover, *Helicobacter pylori* infection and gastrectomy are two major factors contributing to severe disease (1). Malnutrition increases susceptibility, especially among young children (1). The incidence of cholera in countries of endemicity is highest among children less than 5 years of age as they have low levels of acquired immunity compared to adults (29).

Secretory immunoglobulin A (SIgA), which is secreted in breast milk, protects against severe cholera, and thus, exclusive breastfeeding for the first 6 months of life is recommended for women living in communities of endemicity (30). Long palate, lung, and nasal epithelium clone protein 1 (LPLUNC1) is expressed in Paneth cells of the intestinal mucosa and is strongly associated with modulating host inflammatory responses to *V. cholerae* infection and disease severity (25). Concomitant infection of the gut with other bacteria like enterotoxigenic *Escherichia coli* (ETEC) or parasites increases the chance of *V. cholerae* infection (31, 32).

A household contact study of cholera patients in Bangladesh showed that first-degree relatives (parents, offspring, and siblings) have a greater chance of acquiring cholera than second-degree relatives (grandchildren, grandparents, uncles, and aunts) staying in the same household (15).

PATHOLOGY

Microbiology and Pathogenesis

V. cholerae is a Gram-negative, comma-shaped bacterium that is classified serologically into over 200 serogroups. Of them, *V. cholerae* O1 and O139 have caused recent cholera epidemics (33). Based on biochemical structure, *V. cholerae* O1 is categorized

into two biotypes, classical and El Tor, and, more recently, the altered El Tor biotype. Furthermore, each biotype is differentiated into three serotypes, Ogawa, Inaba, and the rare Hikojima type (34).

V. cholerae releases a heat-labile exotoxin based on the AB5 multimeric protein, named cholera toxin (CT), which adheres to the small intestinal mucosa of the gut. CT consists of two subunits (one A subunit [CTA] and five B subunits [CTB]), which cause fluid and electrolyte loss. The B subunit fixes onto eukaryotic cells, whereas the A subunit is shifted into the cell, which assists in increasing cyclic AMP (cAMP) and subsequently leads to secretory diarrhea, which causes severe dehydration. The toxin-coregulated pilus (TcpA), which is required for colonization, serves as a receptor for the cholera toxin phage (CTXphi), which is the *V. cholerae*-specific filamentous bacteriophage and carries the gene for CT (35).

V. cholerae exists in stagnant water or marine environmental reservoirs where the water source is infected with human and/or animal waste. *V. cholerae* in freshly shed stool appears to be hyperinfectious for 24 h after release into the environment (36). *V. cholerae* flourishes in 30°C water with 15% salinity and a pH of 8.5 (37). During spring and after the monsoon season, more adverse environmental conditions can trigger an outbreak of *V. cholerae*. Real-time recording of climatic parameters along with active surveillance systems can assist public health warning systems in minimizing risk factors (37). The intake of polluted water and contaminated food are the main sources of infection, although acidic gastric enzymes kill most of the bacteria, and the rest colonize the small intestine. The infective dose of *V. cholerae* is 10^3 to 10^8 CFU when ingested with water, but a minimum dose of $\sim 10^2$ to 10^4 CFU can cause diarrhea when ingested with food. (https://www.medscape.com/answers/962643-54707/what-is-the-infectious-dose-of-vibrio-v-cholerae-required-to-cause-cholera). A high infectious dose, 10^8 CFU, is required to cause diarrhea in healthy individuals, while a very small inoculum of 10^5 CFU can cause diarrhea in individuals with low levels of gastric acid (38). The incubation period of *V. cholerae* may range from 12 h to 5 days (39).

Molecular Epidemiology of *V. cholerae*

One focus of molecular epidemiological analysis of *V. cholerae* is the CTX phage, which contains the CT genes. The cholera toxin-encoding genes *ctxAB* carried by toxigenic *V. cholerae* have undergone numerous genetic mutations, which include two main components of CTX phage (CTX^{cla} and CTX-1) and repeat sequence 1 (RS1), along with point mutations in *ctxB* (40). Since 1817, a total of seven cholera pandemics have occurred worldwide, with the most recent pandemic continuing until the present (3). It appears that the O1 classical biotype was the causative agent of the first five pandemics (1817 to 1896). Following this, the O1 classical biotype CTX^{cla} was responsible for the sixth pandemic (1899 to 1923) (40, 41). During the pre-seventh-pandemic period (1923 to 1961), only a few sporadic outbreaks were reported with the El Tor biotype (41). The current cholera pandemic (7th cholera pandemic [7CP]) began in 1961 in Indonesia, with the El Tor biotype as the causative agent, and spread to South Asia after 2 years and then to Africa (1970), South America (1990), and the Caribbean (2010).

A new *V. cholerae* serogroup, O139, emerged in the Indian subcontinent in 1992 and transmitted throughout the Asian subcontinent by the mid-2000s. In 2004, another new *V. cholerae* O1 type was isolated in Asia and Africa as a hybrid El Tor biotype encoded with classical CT (40, 41).

Eight diverse phyletic lineages (L1 to L8) have been classified by genomic analysis, based on single nucleotide polymorphisms (SNPs) from the O1 and O139 serogroups. The classical (L1) and El Tor (L2) biotypes were separated into two distinctly evolved lineages. The L1 and L3 to -6 lineages represent the first six pandemics, while the L2 lineage, also known as 7 P *V. cholerae* El Tor (7PET), is responsible for 7CP having three waves and several phylogenetic sublineages of transmission events (T1 to -13 and Latin American transmission 1 [LAT-1] to LAT-3). T1 to T12 originated from Africa, while T13 originated from East Africa and Yemen (40, 41).

The wave 1 (T1 to -6 and LAT-1 and -2) isolates were composed of CTX-1, the repressor gene (*rstR*) of the El Tor biotype ($rstR^{El\ Tor}$) on chromosome 1, and CT

TABLE 1 Clinical assessment of dehydration[d]

Condition	Assessment of dehydration severity		
	No signs of dehydration	Some dehydration	Severe dehydration
Physical appearance	Well, alert	Restless/irritable[a]	Lethargic/unconscious[a]
Eyes	Normal	Sunken	Very sunken and dry
Tears[b]	Present	Absent	Absent
Mouth and tongue	Moist	Dry	Very dry
Thirst	Drinks normally, not thirsty	Thirsty, drinks eagerly[a]	Drinks poorly/unable to drink[a]
Skin pinch[c]	Goes back quickly	Goes back slowly[a]	Goes back very slowly[a]
Radial pulse	Normal	Rapid, low vol[a]	Weak or absent[a]
Diagnosis	No signs of dehydration	If the patient has 2 or more signs, including at least 1 sign[a] of mandatory criteria, there is some dehydration	If the patient has 2 or more signs, including at least 1 sign[a] of mandatory criteria, there is severe dehydration

[a]Mandatory criteria for the diagnosis of different grades of dehydration.
[b]Tear sign is applicable only to infants and younger children.
[c]The skin pinch is less useful in severely malnourished children or overweight patients.
[d]Adapted from WHO guidelines on the management of patients with cholera (58, 144).

genotype 3 (*ctxB3*) of *V. cholerae* O1 added with the toxin-linked cryptic (TLC) component and persisted from 1961 to 1999. Wave 1 spread from Indonesia to Southeast Asia, Mozambique, Angola, the Middle East, East Europe, Ethiopia, Angola, the U.S. Gulf Coast, and Latin America. Wave 2 (T7 and -8) isolates were composed of CT genotype 1 (*ctxB1*), a recurrence of *ctx-2* on chromosome 2, *ctx-1*, and RS1 on chromosome 1. The wave was prominent between 1978 and 1984. It originated in India and spread to East Asia and Africa. *V. cholerae* O139 contains *ctxB4* to *ctxB6* and was prevalent from 1999 to 2005 in Bangladesh. Wave 3 (T9 to -13 and LAT-3) isolates are composed of *rstR*$^{\text{El Tor}}$ and TLC:RS1:CTX3 to TLC:RS1:CTX6 in CT genotype 1 (1991 to 2010) and carry the integrating and conjugative element (ICE) (specifically, ICEVchInd5) of the SXT/R391 type (encoding resistance to chloramphenicol, streptomycin, tetracycline, sulfamethoxazole, and trimethoprim). Wave 3 isolates are divided into three or more types. Wave 3 originated in India in 2006 and caused outbreaks in Haiti, Yemen, and Mariupol, Ukraine, in 2019 (40).

Clinical and Metabolic Manifestations

Cholera infection may be asymptomatic, mild, moderate, or severe (42). Diarrhea in cholera patients is usually painless and may contain bile or fecal matter in the early stages of infection. "Rice water stools" are unique to cholera patients. They are starchy in color, look more like water that contains uncooked rice or has been used to wash rice, and have a fishy odor (42, 43). An adult cholera patient can produce up to 1,000 mL per h of loose watery stool, leading to hypovolemia, shock, and death, termed "cholera gravis." The rate of excretion rate stool in children with severe cholera is generally between 10 and 20 mL/kg/h (44). This stool contains potassium, sodium, and bicarbonate. Signs of dehydration (e.g., sunken eyes, tears, dry mouth, thirst, rapid pulse, lethargy, cold skin, loss of skin elasticity, or crumpled hands and feet) are present due to profuse watery diarrhea (Table 1). Deep and rapid respiration due to hyperventilation (Kussmaul breathing) and acidosis (loss of bicarbonate in the stool) are some of the striking features of severe cholera. Symptomatic cholera patients can shed bacteria in their stool for 2 days to 2 weeks from the onset of infection (Fig. 2), whereas asymptomatic carriers shed for only a few days ($<$7 days) (15). In settings where cholera is endemic, spatiotemporal analysis has shown that index cholera cases can be very infectious during the first 5 days of infection and can spread the bacteria within a 200-m radius of their home. Household contacts have a 100-times-higher risk of acquiring cholera than those outside the radius (45).

Approximately 5 to 10% of patients develop severe dehydration from an increased frequency of profuse watery stool and excessive vomiting, which can rapidly deplete a large volume of water from the body, leading to kidney failure, shock, sepsis, and even death within a few hours if left untreated (1). Electrolyte imbalance is a common complication of

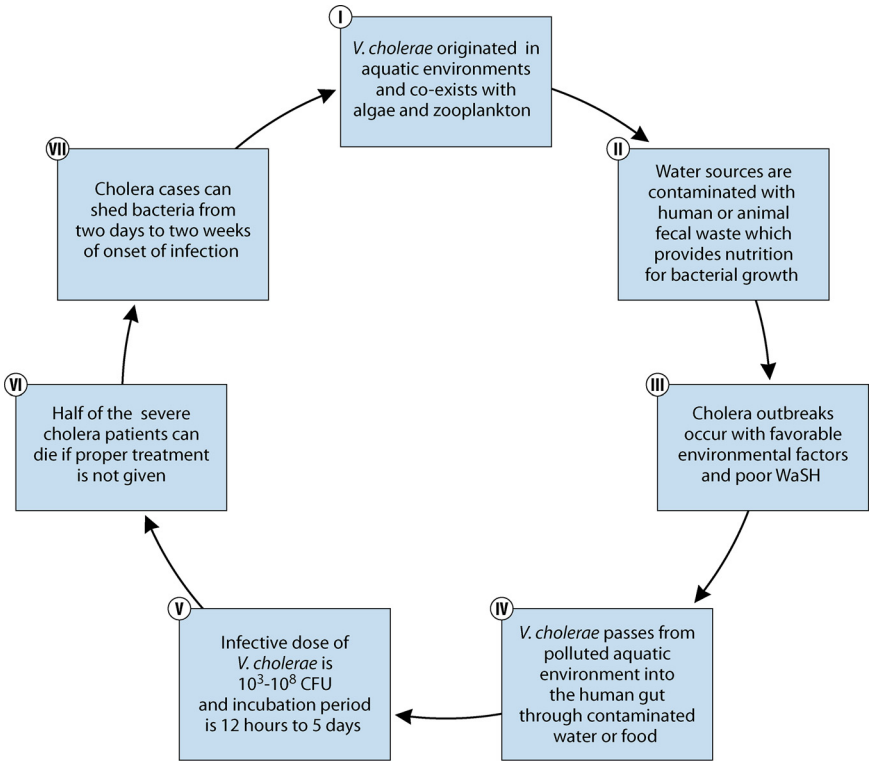

FIG 2 Life cycle of *V. cholerae*.

cholera, which includes hyponatremia or hypernatremia, hypocalcemia, and hypokalemia (42). Renal failure due to decreased urinary output and aspiration pneumonia are also common in children (46). The accumulation of fluid in the intestinal lumen (cholera sicca) is very uncommon. Children under 5 years of age may develop chronic enteropathy and malnutrition. Inadequate rehydration may cause metabolic abnormalities among patients suffering from severe dehydration (42). Reduced food intake during acute illness may lead to hypoglycemia, a lethal complication that is more common in children (1).

DIAGNOSIS

The diagnosis of cholera is frequently based on clinical signs and symptoms (Table 1) in resource-limited areas of endemicity where laboratory facilities are not available (42). In nonendemic settings, cholera is suspected if a patient has severe dehydration or someone has died from acute watery diarrhea (AWD). But in a cholera epidemic setting, if a patient (>5 years of age) has AWD more than three times with or without vomiting within 24 h, cholera is indicated (47).

Generally, in the developed laboratory setting, for the diagnosis of cholera, stool or rectal swab culture is the gold-standard reference method and costs approximately $10 (40). The specimens are placed into an enrichment broth made of alkaline peptone water, which enhances the sensitivity of the culture, and are later subcultured on selective thiosulfate citrate bile salt (TCBS) agar or taurocholate tellurite gelatin agar (TTGA), which is the ideal culture medium (48). Hence, performing stool culture requires trained personnel and a laboratory facility (49). Cary-Blair medium is commonly used as the medium for transport from field settings to the laboratory (40).

Different methods (e.g., biochemical, immunochemical, or molecular) with specific antiserum or monoclonal antibodies are used to characterize the biochemical properties of *V. cholerae* O1 such as classical, El Tor, or altered variants (43). Dark-field microscopy can be used to rapidly detect *V. cholerae* in stool samples before culture (50). However, more than half of the dark-field-microscopy-negative samples are found to

TABLE 2 Compositions of different rehydration solutions[a]

Solution	Content (mmol/L)				
	Na^+	K^+	Cl^-	HCO_3^-	Carbohydrate
i.v. lactated Ringer's solution	130	4	109	28	
i.v. normal saline, 0.9%	154	0	154	0	
i.v. cholera saline (Dhaka solution)	133	13	98	48	140
ORS (standard)	90	20	80	10 (citrate)	111
ORS (WHO 2002)[a]	75	20	65	10 (citrate)	75 (glucose)
Rice-based ORS (e.g., Cera ORS 75)	75	20	65	10 (citrate)	27 g rice syrup solids

[a]The data on the compositions of rehydration solutions are adapted from standard guidelines (56, 58, 145).

be positive for *V. cholerae* when cultured (51). *V. cholerae* diagnosis using PCR is highly sensitive, but this technique needs an enhanced laboratory capacity, which is often lacking in most LMICs (43, 46). PCR can be used to detect molecular markers of certain phenotypes with target genes such as *ctxA*, *tcpA*, and *ompW* (52). Even though PCR costs are approximately the same as those of stool culture and PCR requires a specific laboratory setup, the results can be obtained much earlier than with stool culture.

In rural or underdeveloped health care settings where there is a scarcity of culture medium/PCR and/or trained personnel (5), rapid diagnostic tests (RDTs) of stool samples cost only $2, and these can be performed without special training. RDTs can also provide an early warning for public health experts when a cholera outbreak is imminent. Different categories of RDTs are available on the market, with a wide range of sensitivities and specificities. Monoclonal antibodies in Crystal VC (Span Diagnostics Ltd., Surat, India) can easily detect the lipopolysaccharide (LPS) antigens of both *V. cholerae* O1 and O139 serogroups. Crystal VC has shown 97% sensitivity and 76% specificity (53). Cholkit is available in Bangladesh, with acceptable sensitivity (76%) and good specificity (90%), and is less expensive (less than $2) than the existing RDTs (54). However, this kit detects only the *V. cholerae* O1 serogroup and hence needs to be used in settings where cholera is endemic and where this serogroup is predominant. Currently, *V. cholerae* O1 is the predominant serogroup, although *V. cholerae* O139 strains are occasionally isolated in Bangladesh (3, 55).

CLINICAL MANAGEMENT

Fluid Replacement

Early diagnosis and rapid management of dehydration are crucial for increasing positive outcomes. Most cholera cases are usually mild to moderate and easily managed with an oral rehydration solution (ORS) (https://www.who.int/news-room/fact-sheets/detail/cholera). Currently, the WHO and UNICEF recommend low-osmolarity ORS, which contains sodium, chloride, potassium, citrate, and anhydrous glucose prepared in 1,000 mL of sterile water (Table 2). This improved ORS recipe is safe, lowers hypertonicity, and decreases stool output (56). ORS can also be prepared at home by mixing 1/2 teaspoon of salt and 6 teaspoons of sugar in 1,000 mL of sterile water. Rice-based saline (i.e., rice powder) is also used for those above 6 months of age but is more difficult to prepare (57). After each purging of watery stool, ORS is given according to different age groups to counterbalance the amount of stool loss (Fig. 3). For young children up to 2 years of age, breastfeeding is vital, along with fluid replacement (8). Many countries have introduced low-osmolarity ORS plus zinc for the treatment of cholera.

Treatment with i.v. fluids can be considered if vomiting continues more than three times in 1 h or if ORS is not improving the patient's condition. Instant corrections of fluid and electrolyte deficits are the bases of rehydration therapy in order to counterbalance ongoing losses. Severe cholera cases require i.v. fluid (25). The WHO recommends Ringer's lactate solution (Na^+ at 130 mmol/L, K^+ at 4 mmol/L, Cl^- at 109 mmol/L, HCO_3^- at 28 mmol/L, and Ca^+ at 1.5 mmol/L [osmolarity of 273 mmol/L and pH of 6.5]) over normal saline (Na^+ at

No signs of dehydration

Observation for 2-4 hours and use of ORS and continued feeding. ORS to be given as per the following schedule

Age	Amount of ORS in ml after each stool
<2 years	50–100
2–9 years	100–200
≥10 years	as much as the patient wanted

Some signs of dehydration

Treatment with ORS, 75 mL/kg in 4 hours. The following age-specific ORS plan may be given.

Age	Body weight	ORS in ml
<4 months	<5 kg	200–400
4–11 months	5–7.9 kg	400–600
12–23 months	8–10.9 kg	600–800
2–4 years	11–15.9 kg	800–1200
5–14 years	16–29.9 kg	1200–2200
≥15 years	30 kg or more	2200–4000

Frequent vomiting (>3 times /1 hour) with persisting dehydration, need treatment with IV fluid.

- IV fluids will be needed if signs of severe dehydration appear.
- Continue normal feeding.
- Continue breastfeeding to young children.

Severe dehydration

Treatment with IV fluid immediately (100ml/kg)

Age	IV fluid in ml	
Children < 1 year	30ml/kg in the first 1 hour	70ml/kg in the next 5 hours
Children < 1 year to adult	30ml/kg in the first 30 minutes	70ml/kg in the next 2 and half hours
Encourage to take ORS as soon as the patient is able to drink		

Maintenance therapy: ORS after each stool
- Children of < 2 years: 50 – 100 mL
- Older children: 100 – 200 mL
- Adults: ORS as much as the patient can take

Antibiotic therapy:
- Children: Oral Azithromycin (20 mg/kg single dose)
- Adults: Oral Azithromycin (1g single dose)

Zinc treatment
- Children of 6 months to 5 years: Oral Zinc (20 mg for 10 days)

FIG 3 Management of cholera based on the severity of dehydration. These guidelines have been adapted from WHO guidelines for the treatment of diarrhea (58).

154 mmol/L and K^+ at 154 mmol/L [osmolarity of 308 mmol/L and pH of 4.5]) as it contains more potassium and bicarbonate. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), and other governing bodies of countries where cholera is endemic prefer to use the "Dhaka solution" or "cholera saline" (Na^+ at 133 mmol/L, K^+ at 13 mmol/L, Cl^- at 154 mmol/L, HCO_3^- at 48 mmol/L, carbohydrate at 140 mmol/L) (Table 2), which contains glucose as well as more potassium and bicarbonate than Ringer's lactate solution and can reduce adverse outcomes such as electrolyte imbalances (58, 59).

Severely dehydrated patients need an instant i.v. fluid infusion at a bolus dose of 100 mL/kg of body weight over 3 h and a one-third infusion in the first 30 min (59). Patients aged 1 year and above need 30 mL/kg in the first 30 min and 70 mL/kg in the next 2 1/2 h. The total duration of rehydration is 6 h for children less than 1 year of age (Fig. 3). Severe cholera patients should be kept on a cholera cot (e.g., a cot with a hole and an underlying bucket) to monitor the ongoing fluid loss so that the actual amount of fluid can be replaced. When a patient is able to drink, ORS is started again for hydration (5). For all patients, the fluid infusion should be given in repetitive measures if danger signs (hypovolemia, low radial pulse, and deep breathing, etc.) appear even after starting an i.v. infusion as a bolus therapy (Fig. 4). Malnourished children require a high-energy diet after correction of fluid deficiency to prevent hypoglycemia, hyponatremia, and hypokalemia (58).

Antibiotics and Antimicrobial Resistance

The WHO has recommended antibiotics for severe cholera patients irrespective of age and for patients who require hospitalization (5, 60). Several studies have shown that antibiotics shorten the length of infectious diarrhea (from 5 days to 1 to 2 days) as well as decrease the volume of stool output by up to 50% (1, 43, 61). Tetracycline, fluoroquinolones, co-trimoxazole, doxycycline, ciprofloxacin, trimethoprim-sulfamethoxazole, erythromycin, and azithromycin are the most commonly used antimicrobials for treating cholera patients, but resistance has become a global concern (43). The choice of antibiotics depends on local antibiotic susceptibility patterns. In most countries, doxycycline is recommended as a first-line treatment as a 300-mg single oral dose for adults (including pregnant women) and as a 2- to 4-mg/kg single oral dose for children. If resistance to doxycycline is documented, azithromycin and ciprofloxacin are

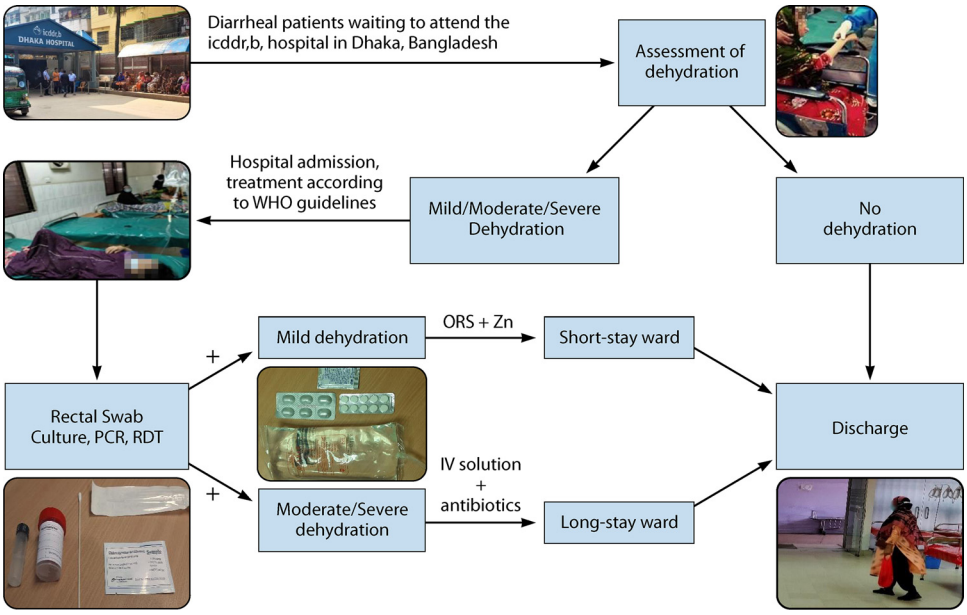

FIG 4 Flow diagram of the course of a cholera patient from admission to recovery.

alternative options. For children under 12 years of age, azithromycin is prescribed as a 20-mg/kg (maximum, 1-g) single oral dose, and ciprofloxacin is prescribed as a 20-mg/kg (maximum, 1-g) single oral dose. A single oral dose of azithromycin (1 g) or ciprofloxacin (1 g) is prescribed to adults suffering from severe cholera (https://www.cdc.gov/cholera/treatment/antibiotic-treatment.html).

Antimicrobial resistance (AMR) against trimethoprim-sulfamethoxazole, nalidixic acid, furazolidone, tetracycline, and ciprofloxacin developed between 1990 and 2010 (62). Hence, frequent performance of antimicrobial testing on *V. cholerae* clinical isolates is recommended for the treatment of cholera patients in settings where cholera is endemic as multidrug resistance (MDR) has developed due to frequent chromosomal mutation. Over the past decade, whole-genome sequencing of *V. cholerae* isolates has shown mobile genetic elements (MGEs) from other bacterial species, plasmids, conjugative elements, superintegrons, and supplemental sequences, all of which could lead to AMR (63). Studies from Africa showed increased resistance of *V. cholerae* O1 serotype strains to co-trimoxazole, chloramphenicol, ampicillin, and tetracycline (64, 65). Countries such as India and Nepal have also reported *V. cholerae* AMR to nalidixic acid, ciprofloxacin, tetracycline, and furazolidone, and there is an increasing number of MDR *V. cholerae* El Tor strains (66, 67).

Between 2009 and 2014, 17% of *V. cholerae* strains were resistant to third-generation cephalosporins, and 93% MDR strains were detected, exhibiting resistance to streptomycin, nalidixic acid, tetracycline, and trimethoprim-sulfamethoxazole (68, 69). An alarming fact is that the isolates were also becoming resistant to ciprofloxacin and azithromycin as these antibiotics were the drugs of choice for the management of cholera cases (70).

Self-medication with antibiotics has been reported in 83% (120/144) of infected and symptomatic household contacts in Bangladesh (71). Antibiotics are easily obtainable without a doctor's prescription, and the habit of frequent and incomplete courses is quite common in developing countries (72, 73). Most recently, a randomized controlled trial of electronic decision-based diarrheal management through mobile phones using the mHealth Diarrhea Management (mHDM) platform in 10 district hospitals in Bangladesh showed a 10% reduction in the ordering of antibiotics in hospitalized patients compared to paper-based decisions in clinical facilities. Additionally, that study also found a remarkable decrease in prescribing of nonindicated antibiotics

using mHDM among patients <5 years (28.5%) and >18 years (11.8%) of age (74). Such findings may help with antimicrobial stewardship in Bangladesh.

V. cholerae susceptibility to antibiotics is usually tested by two methods, disk diffusion and MIC methods for antibacterial properties (75, 76). To better understand the pattern of declining sensitivity of *V. cholerae* to antibiotics and its alarming resistance surge in the world, several molecular techniques are being conducted globally. National surveillance systems to identify changing sensitivity patterns are being used in order to identify the most appropriate drugs for cholera management (77). Moreover, the advantage of stool culture with sensitivity tests relative to PCR or RDTs is the added ability to perform antimicrobial susceptibility testing (AST) and monitor resistance patterns.

Antibiotic Prophylaxis

Findings from a systematic review have revealed that household contacts of cholera cases obtained protection against the disease when antibiotics were given to them as prophylaxis (78). In another study, one dose of doxycycline (300 mg) was administered orally to all prisoners and prison staff to prevent the spread of cholera in a prison in Cameroon in 2004, and no new cholera cases were reported over the next 4 months (79). However, mass chemoprophylaxis with an antibiotic is not recommended by the WHO for cholera control as it can lead to antibiotic resistance, but selective chemoprophylaxis may be provided for the prophylactic treatment of close contacts of a cholera patient (80). More studies are needed to inform the future use of targeted chemoprophylaxis in high-risk subpopulations.

Micronutrients

Zinc supplementation in children <5 years of age can also reduce the length and stool volume of diarrhea. Studies in various countries have shown that the addition of zinc to ORS reduces the severity of diarrhea and subsequently limits the use of antimicrobials (81). Zinc inhibits basolateral potassium channels by blocking cAMP-induced chloride-dependent fluid secretion. Zinc also regenerates the intestinal epithelium and increases the secretion of enzymes and the absorption of water and electrolytes, thus enhancing the immune response (82, 83). Vitamin A supplementation is suggested for children 6 months to 5 years of age to avert further occurrences of diarrhea (84). A high-calorie diet may reduce hypokalemia, hypoglycemia, and malnutrition, even when diarrhea is present (85).

FUTURE TREATMENTS

Supplementation with probiotics is relatively new for cholera management (86). Cholera toxin alters the gut microbiota (87). Probiotics can restore the gut microbiome and thus can potentially be used to clinically manage cholera. The use of probiotics in cholera management may limit AMR by reducing antibiotic use. Studies have shown the important contribution of the gut microbiome to fighting cholera and other diarrheal diseases (82, 83). There are several bacterial species in the gut that have been found to suppress cholera infection. *Ruminococcus obeum* has a positive correlation with cholera recovery (88). Another study showed that *Lactobacillus rhamnosus* strain GG (ATCC 53103) and *Bifidobacterium longum* 46 (DSM 14583) in coculture with *V. cholerae* were capable of removing CT from its environment (89). The therapeutic use of lytic bacteriophages, which is known as "phage therapy," is another novel approach to the treatment of cholera (90). Phages are efficient at killing MDR bacteria. Studies conducted in infant mice and rabbits found that a combination of three isolated *V. cholerae*-specific virulent phages (ICP1, ICP2, and ICP3) was able to decrease the bacterial load of *V. cholerae* and prevent cholera-like diarrhea (91). Experimental studies targeting the inhibitor of cystic fibrosis channel transmembrane (CFTR), inhibitors of virulence factors, and a monosialoganglioside (GM1) antagonist found that they reduced intestinal secretion induced by CT of *V. cholerae* (92).

INTEGRATED CONTROL

Water, Sanitation, and Hygiene

Water, sanitation, and hygiene (WaSH) are of the utmost importance for the prevention and control of cholera and other enteric infections in the developing world (88, 89). Treatment of water at the source will not prevent the disease as contamination may occur at any time from collection to consumption. Water storage can also lead to contamination (93). When an outbreak is declared, interdisciplinary action is crucial for containing the pathogen, along with prioritizing which components of WaSH need to be implemented (93). Behavioral change practices (e.g., handwashing, use of soapy water, chlorination of household drinking water) have been shown to improve the protective efficacy of oral cholera vaccination when administered together (94). The Philippines, Thailand, and Vietnam have initiated cholera control strategies comprising surveillance, health promotion, and the supply of safe water. But in the cross-border areas of Malaysia, great challenges remain where the scarcity of sterile water, poor hygiene, and open defecation are noticeable concerns. Vietnam has reported zero cholera cases since 2012, and the Philippines has implemented a zero-open-defecation program along with other WaSH interventions to control the disease (95). Countries with high cholera disease burdens, such as Bangladesh, Pakistan, Nepal, and India, have minimal piped water in many localities, limited access to potable water in rural areas, shared latrines among the urban poor, open defecation in rural areas, and poor compliance with handwashing (96). Reports on evidence of household water treatment reducing cholera incidence have been published, but it is not effective in high-risk households due to financial constraints, poor education, and practices resulting in the low uptake of interventions (96, 97). Few high-risk households (12% in Nigeria, 19% in Nepal, and 24% in Haiti) have reported using water treatment (e.g., filtration, boiling, UV purification, or chlorine disinfectant use) (97). Without the support of national and local governments and nongovernment organizations, such implementations are impossible to sustain. Additionally, effective sewage systems with safe waste disposal and mechanisms to prevent untreated waste from reentering the environment are essential for controlling cholera (97). A strategy implemented in Mozambique, following the impact of Cyclone Idai in March 2019, was successful in controlling an outbreak of cholera. This included the establishment of a real-time surveillance system, WaSH, and vaccination (98). The WHO also recommends reinforcement and access to improved potable water, standard sanitation laws for food industries, execution of handwashing practices with soap, and safe handling of food as part of the cholera prevention strategy (5).

Inexpensive WaSH interventions such as solar power water purifiers, handwashing facilities, soapy water dispensers, Aquatabs (hypochlorous acid) for water purification, and safe storage containers (Fig. 5) have been modestly effective in lowering the numbers of cholera cases in community trials (99). During outbreaks, emergency WaSH interventions, including inexpensive WaSH strategies, health education, and media coverage, are crucial for reducing mortality as well as preventing further outbreaks (99). Community engagement plays an important role in long-term behavior changes and the prevention of cholera. Awareness campaigns using advertisements on the radio, television, billboards, and text messages can be used to educate people on when to seek medical attention and the use of ORS.

Vaccination

In areas where cholera is endemic, the WHO recommends cholera vaccination as part of the national cholera control program along with WaSH (10). Several studies have shown that the early introduction of vaccines during an outbreak offers 79% protection against cholera (100, 101), and even one dose of the cholera vaccine significantly reduces the risk (5, 102–104).

Broadly speaking, there are four different types of cholera vaccines (Table 3): (i) killed whole-cell (WC) monovalent *V. cholerae* O1 (classical Inaba, and Ogawa, and El

FIG 5 Low-cost WaSH interventions for the control of cholera during an outbreak.

Tor Inaba) vaccines with a recombinant B subunit of cholera toxin, (ii) killed WC modified bivalent *V. cholerae* O1 and *V. cholerae* O139 vaccines without the B subunit, (iii) live attenuated oral cholera vaccines (OCVs), and (iv) parenteral cholera vaccines (5, 105, 106). Presently, only two OCVs are available worldwide: (i) the killed WC monovalent vaccine with a recombinant B subunit of cholera toxin (Dukoral) and (ii) killed and modified WC (O1 and O139) vaccines without the B subunit (Shanchol, Euvichol, Euvichol Plus, and mORC-Vax [106]).

Killed whole-cell cholera vaccines. (i) Monovalent WC *V. cholerae* O1 oral cholera vaccine with a recombinant B subunit of cholera toxin. The killed WC monovalent OCV was first manufactured in Sweden and gained licensure in 1991. Currently, it is available in more than 60 countries (5). It is found under the trade name Dukoral, manufactured by Valneva, France, and achieved WHO prequalification in October 2001 (106). The vaccine was formulated in combination with a recombinant B subunit of cholera toxin and formalin- or heat-killed WC *V. cholerae* O1 (classical Inaba and Ogawa and El Tor Inaba). The B unit of *V. cholerae* toxin is analogous to the heat-labile toxin (LT) of enterotoxigenic *E. coli* (ETEC) in its composition and functional capability and elicits cross-protection against ETEC (5). The vaccine is administered as a three-dose regimen 1 week apart for those aged 2 to 6 years and as a two-dose regimen at least 1 week apart for individuals >6 years of age. The added B subunit in the vaccine can be neutralized by gastric acids, and hence, to protect its functionality, the vaccine is required to be administered along with a buffer solution. The Dukoral vaccine does not defend against the *V. cholerae* O139 serogroup or other types of vibrios and has also been reported to elicit higher immune responses in cholera-naive populations than in populations where cholera is endemic (5, 105, 106).

OraVacs is a monovalent, killed WC vaccine containing the O1 serogroup (classical or El Tor biotype) and a recombinant cholera toxin B (rCTB) subunit and is indicated for traveler's diarrhea. A clinical trial of this vaccine revealed that it is safe and immunogenic for children older than 2 years of age and adults. However, no efficacy trial data are available (106). This vaccine is manufactured by Shanghai United Cell Biotechnology, China, and is licensed in China and the Philippines (Table 3).

(ii) Bivalent modified WC *V. cholerae* O1 and *V. cholerae* O139 vaccines. In the mid-1980s, scientists in Vietnam developed a modified killed WC vaccine comprising the *V. cholerae* O1 serogroup excluding CTB, ORC-Vax, with technology transfer from Sweden (105), and including both *V. cholerae* O1 and O139; hence, this vaccine was called the bivalent killed WC vaccine and was first licensed in Vietnam in 1997 (5, 105, 106). It was manufactured by Vabiotech (Hanoi, Vietnam), and it was included in the national Expanded Program for Immunization (EPI) and used during cholera outbreaks in that country. However, this vaccine was not WHO prequalified as Vietnam's National Regulatory Agency (NRA) was not recognized by the WHO (105). Hence, to comply with international guidelines, the vaccine's manufacturing technology was transferred to the International Vaccine Institute (IVI) (Seoul, South Korea) in 2004 from Vabiotech.

TABLE 3 Comparison of oral cholera vaccines[a]

Vaccine	Description for vaccine							
	Dukoral	OraVacs	Shanchol	Euvichol/Euvichol Plus	mORC-Vax	Cholvax	Vaxchora	Hillchol
Generic name	WC-rBS[b]	WC-rBS[b]	Modified bivalent WC	Modified bivalent WC	Modified bivalent WC	Modified bivalent WC	CVD 103-HgR	
Type	Oral killed; buffer is needed	Oral killed; capsule	Oral killed; no buffer	Oral killed; no buffer	Oral killed; no buffer	Oral killed; no buffer	Oral live attenuated; buffer is needed	Oral killed; no buffer
Composition	Monovalent; killed whole-cell vaccine O1 serogroup (classical Inaba, and Ogawa, and El Tor Inaba) and recombinant cholera toxin B subunit	Monovalent; killed whole-cell vaccine O1 serogroup (classical Inaba and El Tor Inaba) and recombinant cholera toxin B subunit	Bivalent; killed modified whole-cell bivalent (O1 and O139) vaccine without the B subunit	Bivalent; killed modified whole-cell bivalent (O1 and O139) vaccine without the B subunit	Bivalent; killed modified whole-cell bivalent (O1 and O139) vaccine without the B subunit	Bivalent; killed modified whole-cell bivalent (O1 and O139) vaccines without the B subunit	Monovalent; live attenuated serogroup O1 classical Inaba strain 569B	Single strain; whole-cell recombinant Hikojima strain MS1568
Age range (yrs)	≥2	≥2	≥1	≥1	≥1	≥1	18–64	1–45
Dose regimen(s)	2 doses given 1–6 wks apart, 3 doses for children aged 2–5 yrs		2 doses, 14 days apart	2 doses, 14 days apart	2 doses, 14 days apart	3 doses, days 0, 7, and 28	Single dose	2 doses, 14 days apart
Manufacturer	Developed by SBL, Sweden; now Valneva, France	Shanghai United Cell Biotechnology, China	Shantha Biotechnics, India	Eubiologics, South Korea	Vabiotech, Hanoi, Vietnam	Incepta Vaccine Ltd., Bangladesh	PaxVax Inc., USA	Incepta Vaccine Ltd., Bangladesh
First licensing country(ies), yr(s)	Sweden, 1991	China and Philippines	India, 2009	South Korea, 2004	Vietnam, 1997, 2009	Bangladesh, 2019	USA, 2016	Under development
WHO prequalification date (mo and yr)	October 2001	No	September 2011	December 2014/2017	No	No	No	No

[a]Information on the different oral cholera vaccines is adapted from references 5 and 106.
[b]Whole-cell recombinant cholera B subunit.

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This reformulated vaccine was named mORC-Vax and is now licensed in Vietnam. Several clinical trials have evaluated the two-dose schedule of this reformulated vaccine and found it to be safe and to produce antibody responses to O1, but immunogenicity was less pronounced against the O139 serotype (107, 108).

The IVI transferred the modified technology to Shantha Biotechnics (Hyderabad, India) to distribute mORC-Vax internationally. The vaccine was reformulated once again to WHO standards and met the requirements of safety and immunogenicity following several clinical trials. The modified version of the vaccine was called Shanchol (Shantha Biotechnics-Sanofi Pasteur), and it was licensed in 2009 and subsequently WHO prequalified in 2011 (105, 106). This final version of the vaccine was found to have fewer adverse reactions and higher levels of antibody responses in India, Bangladesh, Vietnam, and Ethiopia (107–110). In Kolkata, India, and Bangladesh, large phase II and III trials were conducted, and it was proven to be safe and efficacious. During the 2-, 3-, and 5-year follow-up periods, the vaccine provided 67%, 66%, and 65% protection against *V. cholerae* O1, respectively (111–113). A single dose of the Shanchol OCV is also effective in individuals above 5 years of age in settings where cholera is highly endemic (114, 115). The vaccine is safe and stable at elevated temperatures and is presently stockpiled (116). To meet the increasing demand for the OCV internationally, the IVI transferred the vaccine manufacturing technology to Eubiologics (Seoul, South Korea) to manufacture Euvichol, similar to Shanchol. The Euvichol (glass vial) vaccine was licensed in 2004 by the Government of South Korea, and the vaccine was prequalified by the WHO in 2015 (117). Clinical trials were conducted in South Korea and the Philippines, and the study outcomes revealed that robust antibody responses after two doses of Euvichol were comparable to those elicited by Shanchol in adults (82% versus 76%) and children (87% versus 89%) (117). Euvichol Plus (plastic vial) was developed at a lower cost, is easier to store, and received WHO prequalification in 2017 (105, 106). Euvichol is now stockpiled.

Live attenuated oral cholera vaccine. In the 1970s, researchers from the Center for Vaccine Development (CVD) at the University of Maryland School of Medicine manufactured a live attenuated OCV with a *V. cholerae* O1 strain (106, 118). The live attenuated oral vaccine (like other live vaccines) provides greater efficacy, a more rapid immune response, and greater long-term protection among naive populations than among populations in areas where cholera is endemic (106, 119). To date, four live attenuated oral cholera vaccines have been produced, one of which is currently available, CVD 103-HgR (119).

CVD 103-HgR is the first live attenuated single-dose OCV (monovalent classical Inaba O1). Commercially, this vaccine was produced by the Swiss Serum and Vaccine Institute (SSVI), Berne, with the market names Orochol and Mutacol. Unfortunately, these vaccines were withdrawn from the market in 2003 for economic reasons (120, 121). In 2016, PaxVax Inc., a pharmaceutical company in the United States, acquired the licensure of CVD 103-HgR with the market name Vaxchora for U.S. adult travelers (122). Single-dose administration of Vaxchora showed a $>$90% vibriocidal seroconversion rate and gave protection among U.S. adult study participants following vaccination (119, 120, 122, 123). It had 79% protective efficacy when administered during a mass cholera vaccination campaign following an epidemic in Micronesia (119). Moreover, recently, satisfactory antibody responses and very few adverse events were seen in U.S. adults, children, and HIV patients (119, 122).

Licensures of other live attenuated OCVs are currently under development, such as Peru-15, composed of the O1 El Tor Inaba strain (Cholera Garde; Harvard Medical School, USA); OCV VA 1.4 (Government of India); a live attenuated El Tor Ogawa strain (638); IEM 108 (China); and HaitiV (United States) (106, 124, 125).

Parenteral vaccines. Several parenteral cholera vaccines have been developed, among which are a killed WC vaccine, a purified lipopolysaccharide vaccine, a killed WC vaccine in combination with different adjuvants, and a polysaccharide-cholera toxin conjugate vaccine (126, 127). Only the killed WC vaccine was broadly available for several years but was not recommended by the WHO as this vaccine provided only

a short duration of protective efficacy (43% protection for 3 months), induced a high level of adverse events (106, 126, 127), and was not recommended for pregnant women (126, 128). Most recently, a preclinical study with a newly developed parenteral cholera conjugate vaccine composed of Inaba O-specific polysaccharide (OSP) and the recombinant tetanus toxoid showed significant boosting of vibriocidal immune responses in mice following a single dose (CVD 103-HgR) (129). The parenteral cholera vaccine does not protect against cholera caused by *V. cholerae* O139 and did not show great effectiveness during cholera outbreaks. It also has a high-adverse-event profile, especially after intramuscular (i.m.) or subcutaneous (s.c.) administration, including local pain, erythema, induration, fever, malaise, and headache in most individuals, in comparison to oral cholera vaccines. OCVs are also more feasible to administer in an emergency setting than any parenteral vaccine and are also safe for pregnant women (126).

New vaccines under development. Incepta Vaccine Ltd., a Bangladeshi company, has developed two OCVs, Cholvax and Hillchol. The technology for Cholvax vaccine production was transferred from the IVI (106). Cholvax has the same formulation as that of the Shanchol vaccine regarding strains and other formulations required for maintaining international good manufacturing practice (GMP) standards and WHO production guidelines (130). The manufacturing process for the Cholvax vaccine is also less expensive than those for the other available WHO-prequalified OCVs, and Cholvax has been found to be safe, immunogenic, and noninferior to the Shanchol vaccine (106, 131). Cholvax has been approved by the Directorate General of Drug Administration (DGDA) and was licensed in Bangladesh in 2019. The annual capacity for the production of Cholvax is 20 million to 40 million doses, which will be helpful to reduce the cholera burden in Bangladesh (106).

Hillchol is a formaldehyde-inactivated WC single-strain vaccine that originated from recombinant Hikojima *V. cholerae* strain MS1568 and was generated from an El Tor *V. cholerae* O1 parent/ancestor Inaba strain (Phil6973). This strain contains 50% LPS of each of the Ogawa and Inaba serotypes (132). The new monovalent Hillchol vaccine is manufactured with a solitary inactivation process and is anticipated to have a lower price than other current OCVs. The Hillchol vaccine was found to be safe, immunogenic, and noninferior to Shanchol among study participants (healthy adults and older and younger children) in Bangladesh (133).

The amended heat-stable Hillchol-B vaccine is composed of WC, formalin-inactivated Ogawa and Inaba strains in combination with rCTB in an enteric-coated capsule and can be easily administered during cholera outbreaks and among travelers to areas where cholera is endemic. Very recently, this vaccine was named the DuoChol capsule (124). Another OCV is in preclinical development in Sweden and is composed of formalin-killed cocultured isogenic El Tor Ogawa and Inaba serogroups (112). Eubiologics and the IVI are developing a formalin-killed classical Ogawa (Cairo50) and El Tor Inaba (Phil6973) vaccine, which has completed preclinical trials and is now set to move forward with a clinical trial in South Korea (124).

Vaccine enhancement in vulnerable populations. Many low-income countries where cholera is endemic have reported a high cholera prevalence among young children. Current vaccines have shown a minimal level of protection and a short duration of protection in those aged 2 to 5 years (10, 126). Many approaches have been suggested to improve vaccine efficacy for this age cohort. Different regimes of vaccine administration, such as 3 doses at 4-week intervals or 2 doses at 8-week intervals, have been suggested to enhance immunogenicity in children. One study showed that withholding breastfeeding 3 h before vaccination increases vaccine efficacy along with supplementation with 20 mg of zinc per day for 42 days (134). Blood group, gut microbiota, malnutrition, environmental enteropathy, and the presence of multiple copathogens may also have a strong association with lower immunity in children (119).

The WHO recommends OCVs for pregnant and lactating women as these vaccines have potential benefits that outweigh the negligible risks (5). During pregnancy, severe dehydration can lead to premature delivery, miscarriage, and fetal death. OCVs were found to be safe, with no adverse fetal outcomes observed in several studies (135–138). The

WHO now recommends the use of OCVs for pregnant mothers in areas where cholera is endemic to prevent severe dehydrating cholera that may harm the fetus.

Herd immunity and vaccines. It has been shown that inactivated OCVs can give significant herd protection in various study settings, which were analyzed using geographic information system (GIS) tools (138). The herd-protective effects of OCVs were measured using various study designs (individually or cluster randomized trials and cohort or case-control studies), and significant herd protection (both direct and indirect) against cholera was seen among unvaccinated persons and in the community (138). Mathematical modeling of cholera transmission (139) using Matlab showed that 93% of cholera infections in Bangladesh can be prevented with 50% OCV coverage (140). The model predicts an 89% drop in the incidence of cholera in the population that is not vaccinated. Study findings in Zanzibar showed that after mass cholera vaccination, OCVs also gave herd immunity (both direct and indirect) in this African setting (101). A large feasibility trial in Bangladesh revealed that children less than 3 years of age had a 47% reduction in the incidence of cholera if their mothers were given OCVs (139). Another analysis by Ali et al. showed that the chance of having cholera in the unvaccinated adult population was reduced by 14% with a 10% rise in OCV coverage in all age strata with a killed oral vaccine (138).

Challenges with oral cholera vaccines. In 2013, after the formation of the global cholera vaccine stockpile, the logistic complications of vaccine delivery and the consistent scarcity of the vaccine supply hampered successful OCV implementation. The main challenge is to deliver two doses of the vaccine at a 14-day interval in rural or urban field settings (141). Recently, a heat stability study conducted on Shanchol showed that the vaccine is thermostable (116). Fortunately, Shanchol can be used at an ambient temperature (up to 42°C) for up to 1 month, but the storage temperature should be between 2°C and 8°C according to WHO guidelines (5).

Future vaccine strategies. A single dose of an OCV induces a vibriocidal response among exposed populations, as observed in previous clinical trials (109, 111, 114, 142). A single dose of an OCV was shown to be efficacious (57%) among those above 5 years of age; however, no protective efficacy was observed for those below 5 years of age (114). A possible reason is a lack of acquired immunity. Thus, the memory B cell response against cholera-specific antigen develops over time due to recurrent natural exposure to *V. cholerae* (106, 142).

Studies on the use of booster doses of OCVs have been carried out in Bangladesh, which showed that children who received a single dose of an OCV 3 years earlier showed significantly increased vibriocidal antibody responses after receiving one booster dose of an OCV compared to those who did not receive an OCV earlier (142). These results suggest that boosting with one dose of an OCV augmented the immune responses in children, although more studies are required to adjust the primary booster doses of OCVs as well as to determine the duration between the prime and booster doses (142). Nevertheless, one dose of an OCV was also found to be protective during an outbreak among people (5 years of age and older) in Zambia who had less exposure to cholera (100).

Large campaigns of two doses of an OCV were carried out in 2017 among the Rohingya population (forcibly displaced Myanmar Nationals) in Cox's Bazar in Bangladesh. A trial was conducted to assess the immunological parameters before and after vaccination. The study revealed a significant increase in vibriocidal antibody titers 14 days following the first dose of the OCV (143). Similarly, another study conducted during a humanitarian crisis in South Sudan showed that only one dose of an OCV was immunogenic and induced short-term antibodies (106, 115).

CHOLERA ELIMINATION

The GTFCC of the WHO has launched an initiative, entitled Ending Cholera: a Global Roadmap to 2030, aiming for at least a 90% mortality reduction in 47 countries of endemicity. The global roadmap aligns health and WaSH resources and targets areas

most in need, saving lives, enhancing equity, and reducing the significant economic burden of cholera as well as other enteric diseases. The global roadmap focuses on three strategic priorities to control cholera. The first strategy includes the rapid detection of cholera cases and early responses to outbreaks through an early-warning surveillance system (EWARS) with an enhanced laboratory culture capacity along with dedicated health care facilities to treat cholera. The second strategy focuses on averting cholera recurrence in identified hot spots by improving WaSH and the delivery of OCVs. The third strategy is to develop a well-organized and efficient network to provide financial support to countries where cholera is endemic and bring national and international collaborators together to promote intersectorial coordination, supply mobilization, technical assistance, and strong cooperation to control cholera (https://www.gtfcc.org/wp-content/uploads/2019/10/gtfcc-ending-cholera-a-global-roadmap-to-2030.pdf).

Bangladesh is one of 20 countries of endemicity targeting cholera elimination. The incidence rate for cholera is 1.64 per 1,000 population annually. Bangladesh formulated the National Cholera Control Plan (NCCP) (https://www.gtfcc.org/wp-content/uploads/2020/08/6th-annual-meeting-gtfcc-bangladesh.pdf) in 2019, which is the guiding document to ensure OCV delivery to the target population according to a GTFCC strategic approach. A large OCV campaign was demonstrated among 1.2 million people as part of the NCCP in February 2020 in the capital city of Dhaka, comprising six high-risk cholera-prone areas (https://www.dtnext.in/world/2020/02/20/bangladesh-begins-1st-nationwide-anticholera-drive).

Along with Bangladesh, Uganda, Zambia, and Zanzibar are also in the process of trying to meet the Ending Cholera: a Global Roadmap to 2030 objectives. The Zambia government is working toward improving the WaSH sector in compliance with the roadmap supported by the GTFCC (https://www.gtfcc.org/wp-content/uploads/2020/08/5th-gtfcc-2018-zambia-francis-bwalya.pdf). Zanzibar is implementing the Zanzibar Comprehensive Cholera Elimination Plan (ZACCEP) (https://www.gtfcc.org/wp-content/uploads/2019/05/national-cholera-plan-zanzibar.pdf), which is a 10-year program to eliminate the indigenous transmission of cholera and promote a healthy and clean environment. Uganda has incorporated a 5-year plan to reduce cholera by 50% by 2022 through vaccination and the implementation of WaSH among 300,000 persons in the first 3 years, as reported at the 5th annual meeting of the GTFCC (https://www.gtfcc.org/wp-content/uploads/2020/08/5th-gtfcc-2018-uganda-immaculate-ampaire.pdf). Developed countries have eliminated cholera largely through improved sewage systems and clean water supplies. However, in the 21st century, high-risk populations and those in LMICs still do not have access to safe drinking water and formal sewage systems (97).

CONCLUSIONS

V. cholerae causes periodic cholera epidemics in several regions around the globe. The disease requires immediate treatment as it can cause death within hours in patients with moderate to severe cholera. With the development of i.v. fluids, ORS, and Zn therapy, progression to severe dehydration and mortality has been remarkably reduced. Antibiotics, micronutrients, and probiotics can further assist in recovery. In the developing world, treatment challenges are primarily due to delays in receiving prompt medical attention by health care professionals. The use of cholera RDTs as a point-of-care diagnostic during an outbreak along with PCR requires significant laboratory investment and skilled personnel. The ambitious global roadmap to end cholera by 2030 requires countries of endemicity to use evidence-based solutions to make this goal a reality (https://www.gtfcc.org/wp-content/uploads/2019/10/gtfcc-ending-cholera-a-global-roadmap-to-2030.pdf). To control cholera in endemic or outbreak situations at the domestic and communal levels, an OCV is considered an essential component of an integrated control package along with WaSH. Extensive and robust cholera surveillance, rapid diagnostics, treatment, and health education will be required for sustained control. Finally, to make all of this possible there must be political will at all levels of government. Without such support, the WHO 2030 targets will not be met.

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