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Cholera: a great global concern

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ABSTRACT

Cholera, caused by the infection of toxigenic *Vibrio cholerae (V. cholerae)* to humans, is a life threatening diarrheal disease with epidemic and pandemic potential. The *V. cholerae*, both O1 and O139 serogroups, produce a potent enterotoxin (cholera toxin) responsible for the lethal symptoms of the disease. The O1 serogroup has two biotypes (phenotypes), classical and El Tor; each of which has two major serotypes (based on antigenic responses), Ogawa and Inaba and the extremely rare Hikojima. *V. cholerae* O1 strains interconvert and switch between the Ogawa and Inaba serotypes. Fluid and electrolyte replacement is the mainstay of treatment of cholera patients; the severe cases require antibiotic treatment to reduce the duration of illness and replacement of fluid intake. The antibiotic therapy currently has faced difficulties due to the rapid emergence and spread of multidrug resistant *V. cholerae* causing several outbreaks in the globe. Currently, cholera has been becoming endemic in an increasing number of geographical areas, reflecting a failure in implementation of control measures. However, the current safe oral vaccines lower the number of resistant infections and could thus represent an effective intervention measure to control antibiotic resistance in cholera. Overall, the priorities for cholera control remain public health interventions through improved drinking water, sanitation, surveillance and access to health care facilities, and further development of safe, effective and appropriate vaccines. Thus, this review describes the facts and phenomena related to the disease cholera, which is still a great threat mainly to the developing countries, and hence a grave global concern too.

1. Introduction

Cholera, an ancient and devastating acute diarrheal illness, is caused due to toxigenic *Vibrio cholerae (V. cholerae)* infection to humans (both adults and children), and currently is a serious global problem. The disease causes profuse watery diarrhea and can quickly lead to severe dehydration and death if treatment is not promptly given. An enterotoxin, called cholera toxin (CT), produced by toxigenic *V. cholerae*, is responsible for the manifestation of the disease cholera. *V. cholerae* was first identified by the Italian scientist Filippo Pacini, in 1854, though the discovery was not known until Koch (1894), who originally mentioned that cholera is caused due to *V. cholerae* infection.

The *V. cholerae* includes both pathogenic and nonpathogenic strains, differing in their virulence gene contents and polysaccharide surface antigens, and thus, among *V. cholerae*, the two toxigenic serogroups O1 and O139 are regarded as the etiologic serogroups causing the disease, which can be epidemic, endemic or pandemic in nature. This historically-feared disease still remains a major public health problem in many parts of Africa, Asia and Latin America, and though rare in developed countries, it is still an important infection worldwide, and thus, the disease has been categorized as the "emerging and reemerging infection" threatening many parts in the globe. The current burden of cholera is estimated to reach many million cases a year in both Asia and Africa, with fewer cases in Latin America[1].

It has been endemic in India, especially in the deltas of the Ganges and Brahmaputra including West Bengal, which has been recognized as the "homeland of *V. cholerae* causing cholera". The World Health Organization (WHO) describes cholera as a global threat to public health and one of the key indicators of social development, stating that with the increased reporting of cholera in 2006, almost every developing country is with an outbreak or the threat

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of an epidemic[2]. In this Review, we define various updated facts and phenomena related to the epidemiology of cholera, based upon the extensive searches in several biomedical science journals and web-based official organization reports.

2. Etiology

2.1. Identity confirmation

The *V. cholerae* is a gram-negative rod (slightly curved) and is characterized by the presence of a single polar flagellum, for which the bacterium is motile. The bacterium with positive oxidase reaction, β-haemolysis on a blood agar plate, growth of yellow colonies on thiosulfate citrate bile salts sucrose agar, susceptibility to 10 μg and 150 μg of vibriostatic agent O-129, and tolerability and intolerability to 1% and 10% salt solutions, respectively, has been defined as the *V. cholerae*. The biochemicl profiles for *V. cholerae* include positive tests *viz*., nitrate reduction, indole production, catalase production, citrate utilization, ornithine decarboxylase production and lysine decarboxylase production, and negative tests such as arginine dihydrolase production and urea hydrolysis. The evolving identity of choleragenic *V. cholerae* does not remain the same with continuous process of evolution, and the recent strains are also in the process of dynamic genetic changes[3].

2.2. Etiologic serogroup

The *V. cholerae* erogroups are recognized by the antigenicity of the O-antigen part of the lipopolysaccharides. Among more than 200 O-antigen serogroups that have been identified and characterized, only two serogroups, O1 and O139, are known to cause cholera. Thus, the choleragenic *V. cholerae* is classified, on the basis of its somatic antigens (O antigens), into two serogroups, the *V. cholerae* agglutinable with polyvalent O1 antiserum belong to the serogroup O1, and the *V. cholerae* non-agglutinable with polyvalent O1 antiserum belong to the serogroup non-O1. The non-O1 *V. cholerae* are agglutinable by their own antisera, and are designated as the *V. cholerae* serogroup O139; other *V. cholerae* serogroups, though occasionally cause human illness, have not evolved into an epidemic form.

2.3. The O1 biotype and serotype

The O1 serogroup has two biotypes, classical and El Tor, and two major sereotypes, Ogawa and Inaba; a third serotype, known as Hikojima, exists that is rare as well as unstable, and the Ogawa is most prevalent one.

The cholera biotypes (classical and El Tor) are the distinct phenotypes that differ with respect to the severity of their infections, ability to survive outside the host body, and seasonality patterns[4]. The identity of *V. cholerae* O1 classical and El Tor biotypes can be differentiated based on the number of traits: the El Tor, and classical biotypes are characterized by their sensitivity to Mukerjee El Tor phage 5, and polymyxin B (50 IU) and Mukerjee classical phage IV, respectively, and among the two biotypes, only the El Tor is agglutinated by chicken erythrocytes and positive to Voges - Proskauer reaction.

The serotype of *V. cholerae* O1 depends on which of the genes encoding somatic antigens A, B and C are expressed, and thus, the serotypes differ from one another only with respect to antigenic determinants that are found on their O-antigen capsule. The Ogawa and Inaba serotypes are positive to Ogawa and Inaba antisera, respectively, whereas the Hikojima is positive to both the antisera. The strains of the Ogawa serotype express the A and B antigens, whereas Inaba strains have antigenic determinants A and C, and the Hikojima strains contain all the three antigens A, B and C. These antigenic determinants play role in inducing host immunity, and because of the presence of common 'A' antigenic determinant, cross-immunity between serotypes exists; however, differences in antigenic determinants in the serotypes do not play role to express differences in phenotypic characteristics, *viz*., duration of infectious period, strength of the hosts' immune response or recovery rate.

3. Cholera pandemics

Cholera has affected human health for many decades, and there is statement, found in Sanskrit, of a disease with symptoms resembling cholera on the Indian subcontinent thousands of years ago. The world has already faced seven cholera pandemics in the past two centuries (Figure 1). The first six pandemics all seem to have originated in Bangladesh, and are thought to be due to the infection of the Classical O1 biotype[5]. The seventh cholera pandemic was initially caused by the O1 El Tor biotype; the 0139 biotype appeared in 1992, and this ongoing cholera pandemic started, in Indonesia, in 1961 resulting in a large number of cholera cases as well as deaths per year[6], and continues to the present day. A cumulative total of 838 315 cases had been notified to the WHO during 2004-2008, where as 676 651 cases were reported during 2000-2004; this represents a 24% increase in the number of cases reported with respect to the cases recorded in between 2000 and 2004[7].

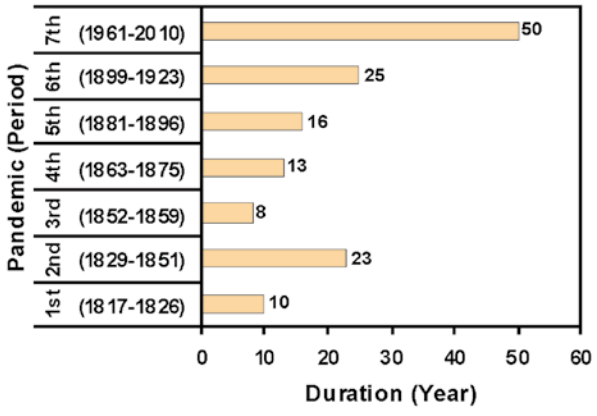

Figure 1. Duration and period of seven cholera pandemics.

4. Serogroup and serotype switching among toxigenic *V. cholerae*

4.1. Occurrence of serogroup switching

It has been stated that the first six pandemics of cholera were due to *V. cholerae* O1 classical biotype, and the ongoing seventh was due to *V. cholerae* O1 El Tor biotype, which made its appearance in 1905, in the village of El Tor, Egypt, and from mid-sixties this strain completely displaced the classical one in the globe, and thus the seventh pandemic has been initiated[8]. Among the two biotypes, the El Tor strains have better adaptability to survive in the environment and also in the human host. But a novel toxigenic strain, *V. cholerae* serogroup O139 (synonym Bengal), emerged in 1992, as a new causative agent and rapidly spread to all cholera-endemic areas in India as well as in the neighboring countries[9].

In the beginning, the new strain (serogroup O139) totally displaced, in Calcutta (currently Kolkata) and other parts of India, the existing *V. cholerae* O1, the only serogroup responsible for epidemics and pandemics of cholera at that time in Kolkata. In 1993, causing severe epidemic in the Indian continent, an explosive appearance of *V. cholerae* O139 occurred. The serogroup O139 disappeared soon, and a new clone of the El Tor biotype of O1 serogroup again became the major causative agent. The *V. cholerae* O139 strains, in 1996, reappeared and shared with the *V. cholerae* El Tor strains of the O1 serogroup[10], suggesting the clonal shift in *V. cholerae* a basic feature of cholera dynamics. Currently, the El Tor *V. cholerae* serogroup O1 has been the major causative agent of the disease and the frequency of serogroup O139 has considerably reduced over the last few years. The classical biotype is believed to be extinct[11]; however, studies have shown that the classical CT producing El Tor strains (harbouring classical cholera toxin gene) replaced the seventh pandemic El Tor strains[12,13], and that the variant strain is potentially more virulent than the usual *V. cholerae* O1 El Tor[12].

4.2. Occurrence of serotype switching

The *V. cholerae* O1 strains have been demonstrated to interconvert and to undergo serotype switching between Ogawa and Inaba; many authors from different parts of the globe reported that the emergence of *V. cholerae* O1 serotype Inaba strains were from the prevailing Ogawa stains[14,15]. In Kolkata, India, *V. cholerae* O1 Ogawa serotype predominated the Inaba serotype during 2004; the reverse was true for 2005[16]. The frequency of conversion of Ogawa to Inaba is about 105 and the conversion of Inaba to Ogawa is rare, and the latter may be strain dependent, and thus, the *V. cholerae* O1 serotypes, Inaba and Ogawa, are capable of unequal reciprocal interconversion[8], which occur during epidemics or in parts where cholera is endemic. It appears that as an alternate to the Ogawa serotype, Inaba has emerged to aid the persistence of cholera, and thus the spread of *V. cholerae* O1 El Tor is continued. The serotype switching between Ogawa and Inaba strains has been the consequence of the genetic reversal occurring *in vivo* and *in vitro*, and is possibly mediated by the immune response in the population[15].

4.3. Molecular basis of the conversion

The serotype conversion in *V. cholerae* O1 might be due to a change in the genetic make-up of the *wbeT* gene (also known as *rfbT* gene that codes for O1 antigen biosynthesis), which occurred either by a point mutation or a deletion, or both, and this may occur as a result of selection due to pressure of lytic phages and immune response during cholera infection[17]. The DNA sequence analysis revealed that *wbeT* was homologous in Inaba isolates from different parts of India, and in all the isolates, a novel mutation (substitution of C for T at position 538) was detected, which changed serine to proline[18].

Molecular epidemiological studies suggest that O139 strains are closely related to O1 El Tor strains, and the O139 strains have evolved from El Tor strains[16]; the conversion of the ancestral O1 El Tor strain involved insertion of a large foreign genomic region encoding the O139 antigen-specific genes and simultaneous deletion of most of the O1 antigen-specific genes[19].

5. Cholera toxin

The cholera toxin (CT), the primary toxin produced by *V. cholerae* O1 and O139, is responsible for most of the manifestations of the disease cholera. The CT is an oligomeric protein of 84 000 daltons, and consists of a single A subunit surrounded by five B subunits. Based on the B subunit of CT, two non identical but immunologically related epitypes have been designated: CT1, which is the prototype elaborated by classical biotype strains, and CT2, which is produced by the El Tor biotype and O139 strains.

The CT causes the infected person to hypersecrete electrolytes and water, sometimes with fatal results. The B subunit of the CT is responsible for the binding of the holotoxin to a specific receptor, the monosialosyl ganglioside GM1, on mammalian intestinal mucosa cell membrane and facilitates entrance of the A subunit into the cell. The A subunit after proteolytic cleavage gives rise to the enzymatically active A1-peptide that catalyses ADP-ribosylation of the A subunit of the heterotrimeric GTP-binding protein Gs. This renders adenylyl cyclase constitutively active, thereby increasing the intracellular level of cAMP. This activity in turn causes secretion of chloride and bicarbonate into the small intestine; as a result, water is drawn from the intravascular and extracellular spaces of the body, and rapidly lost into the gut lumen.

6. Spectrum of illness

The *V. cholerae* is a non-invasive organism that colonizes the lining epithelium of the gut after penetrating the mucus layer, and it affects the small intestine through the CT causing cholera. The hallmark of cholera is painless purging of very large volume of stool resembling rice-water, with varying degrees of dehydration that ranged from none to

severe and life-threatening diarrhea; the spectrum of the disease is thus wide, with mild and asymptomatic illness occurring more frequently than severe disease[20].

The disease is characterized by incubation period 18 h to 5 d, followed by profuse watery diarrhea, which may be associated with vomiting, muscle cramps, and complications related to dehydration and metabolic acidosis. In its extreme manifestation, cholera is one of the most rapidly fatal infectious diseases known; within 3-4 h of the onset of symptoms, a healthy person may become hypotensive and may die within 6-8 h. More commonly, fatal cases progress to shock within 6-12 h with death.

WHO suggests that around 90 % of episodes of cholera are of mild to moderate severity and are difficult to distinguish clinically from other causes of acute diarrhea[21]. However, cholera can be rapidly fatal in severe cases, and if left untreated, can result in up to 50% mortality, but, prompt administration of fluid replacement and supportive therapy can reduce mortality to 1%[21].

7. Transmission of the disease

Two routes of transmission of *V. cholerae* have been recognized, the first one occurs from aquatic reservoirs in the environment (primary transmission), and the second one occurs from previously infected individuals (secondary transmission); once the primary transmission has initiated an outbreak, secondary transmission causes epidemics in the endemic areas[22].

In endemic areas, water is usually the main vehicle of transmission, although this may occur via food, and thus infection due to *V. cholerae* begins with the ingestion of contaminated water or food. Transmission of cholera in non-endemic areas is more commonly associated with consumption of foods, such as raw or undercooked seafood, imported from cholera-endemic regions.

People infected with cholera suffer acute diarrhea and excrete "rice-water stool" loaded with toxigenic *V. cholerae*, which can infect water that is to be used by other people. Thus, the major source of *V. cholerae* is faeces of persons acutely infected with the organism that reaches water most often through sewage. Individuals with reduced gastric acidity and blood group O are more susceptible to the infection, and in situations where poor environmental sanitation is coupled with poor domestic and personal hygiene, transmission results from ingestion of faecally contaminated water (as well as food), and hence it is usually a disease of developing countries or areas where improved water and adequate sanitation are lacking.

Person-to-person transmission of cholera is regarded as uncommon, and in human volunteer studies, the infective dose was determined to be 10^2-10^3 cells, as has been reported by Hartely *et al*[23]. However, the size of the inoculum needed to cause severe infection is on the basis of health status of the individual; a high infectious dose (10^5-10^8 bacteria) is necessary to produce disease in healthy individuals, and a very small inoculum can also manifest the disease in populations with low levels of gastric acid[24]. Transmission via the faeces of an infected individual may cause the disease with small inoculum, if this occurs within a few hours of exposure[25].

8. Cholera epidemic and outbreak

Epidemics or explosive outbreaks generally occur in underdeveloped areas with inadequate sanitation, poor hygiene, and limited access to safe water supplies, whereas in some countries, a seasonal relation for cholera epidemics has been observed[4,17]. WHO described a dramatic increase in the number of cholera cases and outbreaks[2], in the new communities and in communities where the disease had been absent for many years, with changing profiles[2], and so is often considered as a re-emerging disease.

The cholera endemicity in India is the potential risk of epidemics, and thus outbreaks of cholera including major epidemics have occurred from time to time at various places in the country[26-28]. A total of 68 outbreaks occurred in 18 states and union territories in India, and the overall number of cases was many folds higher than the number reported to WHO over the same time period[29], and according to data from population-based diarrhea surveillance, the incidence of cholera was 2.2 cases per 1 000 in Kolkata[30], the homeland of the disease that in the very first decade of the current century witnessed several outbreaks. Sur *et al*[31] reported an outbreak of cholera during 2004 in the eastern part of the city, and during 2004 and 2005, two outbreaks from different parts of the West Bengal state have been reported [32]. Mandal *et al*[33] reported 2007 cholera outbreak from Kolkata, due to the infection of multidrug resistant *V. cholerae* O1 strains.

In the year 2006, 52 countries officially reported a large number of cases, with fatality rate 2.7%, to the WHO[2]. Tavana *et al*[34] investigated the relationship of duration with outbreaks of cholera for seven years (2000-2006) in Iran. MDR cholera epidemics have been reported from the India's neighboring countries like Bangladesh[35], Pakistan[36] and Nepal[37]. In July 2002, cholera outbreak caused by *V. cholerae* O139 was detected in Karachi, Pakistan[36] and in March 2005, *V. cholerae* O139 emerged as the sole cause of a significant outbreak of cholera in southern costal area of Bangladesh[38]. WHO reported a 30% increase in cholera cases in 2005, compared with 2004[39], and China has reported that cases have increased in 2005 compared with 2004[40].

African countries experienced more epidemics and cases of cholera than countries in Asia and America, and most cholera cases reported to WHO originate in Africa: 95% in 2005[39], and 94% in 2004[41]. During 2004, major outbreaks of cholera occurred in Cameroon, Chad, Guinea, Mali, Niger, Senegal, and Zambia[41]. In between August 2008 and February 2009, 70 640 patients were reported with cholera in Zimbabwe, of which 3 467 died[42], and more accurately the 2008-2009 cholera outbreak, which involved nearly 100 000 people, the case fatality rate was 4.3%[43]. The Haitian populations have little access to safe sources of water, and hence remain vulnerable to epidemics of cholera [44]. In a current epidemic in Haiti, the case fatality rate for cholera remains at 6%; 18 382 hospital admissions, and 1 110 deaths due to cholera have been reported[45].

Several biological factors may influence the success of *V.*

cholerae clones in cholera epidemics, including resistance to predation by phages, resistance to prevailing immune mechanisms in the human population and resistance to antibiotics used in the treatment[17]. The human immunity and the selection pressure due to lytic phages may provide explanation for periodic shift in the occurrence of cholera by *V. cholerae* strains of different serogroups and serotypes[17], while antibiotics directly influence the development of antibiotic resistance among the strains causing cholera endemic areas. Overall, the epidemics of cholera depend on the amplification of toxigenic *V. cholerae* in humans and their transmission by the faecal-oral route, and the host genetic factors (including blood group O) predispose individuals to severe cholera, and differ in prevalence between populations in endemic and non-endemic regions[43].

9. Treatment protocol for cholera

9.1. Rehydration therapy

Rehydration is the essence of cholera treatment, and thus individual patients should be treated with appropriate intravenous or oral rehydration fluids; for severely dehydrated patients, isotonic fluids (preferably lactated Ringer's solution) has preferably been suggested through intravenous route, until the detectable pulse is restored. Patients with severe cholera need 200 mL per kg of isotonic fluids in the first 24 h of therapy, but this amount might range from 100 mL per kg to more than 350 mL per kg[46]. Patients without severe dehydration can usually be treated with oral rehydration solution. The intravenous route can also be adopted for moderately dehydrated patients unable to tolerate the oral route, and for patients considered as high stool purgers (>10 mL/kg/h), at the time of maintenance phase. At the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh (ICDDR, B), which played a crucial role in providing oral rehydration therapy, has extensive experience of management of severe cholera patients, minimizing case fatality rate below 0.2%[1,47].

9.2. Antibiotic treatment regimen

Rehydration is the mainstay of cholera treatment, but antibiotics have been shown to be important in severe cases and in epidemic situations. The antibiotic therapy, which is considered as an useful adjunct to fluid replacement in treating cholera, can shorten the duration of diarrhea, and reduce stool volume and requirements for rehydration fluids thereby reducing the duration of hospitalization and breaking the transmissibility cycle in epidemic situations[48]. Following antibiotic therapy, Saha *et al*[49] and Hossain *et al*[48] reported cholera cases with shortening of the diarrhoeal illness course and the volume of diarrhoeal stool output by up to 50%.

Tetracycline (T) and doxycycline (Dx) have long been the antibiotics of choice for treating severe cholera[24], and these were found effective when used in a single dose, except for young children and pregnant women. Furazolidone (Fz), erythromycin (Er), trimethoprim-sulphamethoxazole (Tm-Smz), ampicillin (Am) and chloramphenicol (C) are effective against severe cholera caused by of *V. cholerae* susceptible to these agents; ciprofloxacin (Cp) is an important substitute drug for treatment of multidrug resistant (MDR) cholera. In children, Tm-Smz, Er and Fz are preferred, while pregnant women can be treated with Er or Fz[50]. The current safe alternatives for MDR *V. cholerae* with Cp resistance remain the third generation cephalosporins including ceftriaxone (Cf) and cefotaxime (Ct), which are very expensive and the supply is limited in poor countries; the other alternative includes azithromycin (Az)[51]. Cp and Az were reported to be more effective than Er or T, when used in single doses against *V. cholerae* infection due to the strains susceptible to these drugs[52]. Moreover, it has been suggested that the choice of antibiotic must be guided by local antibiogram patterns prevailing at a given time, and in order to select drugs in the first-line treatment options like drug efficacy, its current affordability, and the therapeutic ratio (which will be high), should also be considered. The current strain in circulation in Haiti is susceptible to Dx and Az and resistant to Nx, with reduced susceptibility to Cp; and thus prepared the appropriate antibiotic choice for the management of cholera in Haiti[43].

9.3. Combined chemotherapy

Combination effect of antimicrobial agents appears to be of particular interest in the treatment of infection caused by microorganisms resistant to clinically achievable concentration of single drug, and also in order to reduce the chances of emergence of drug resistance[53,54]. Cotrimoxazole, which is a combination of Tm and Smz, has been in use against the infection of different enteric bacteria including *V. cholerae*. An *in vitro* synergistic activity of CP-Tm combination against *V. cholerae* O1 biotype El Tor serotype Ogawa showing resistance to Cp an Tm suggests that Cp in combination with Tm could be the potential treatment regimen against drug-resistant cholera[55]. However, more studies are required to establish various antibiotic combinations with synergistic interation, and their effective *in vivo* application against MDR cholera.

10. Incidence of multi-drug resistance among *V. cholerae*

Indiscriminate and rampant use of antibiotics in the treatment of cholera and other enteric diseases led emergence of antibiotic resistance among *V. cholerae* and other enteric bacteria. Multidrug resistant (MDR) *V. cholerae* with epidemic outbreaks (both classical and El Tor biotypes) have been reported worldwide. The profiles of major multiple antibiotic resistant *V. cholerae* as documented in Kolkata and other parts of India are: Am-Fz (ampicilllin-furazolidone), Am-Fz-Nm (Am-Fz-neomycin), Am-Fz-Nm-Str (Am-Fz-Nm-streptomycin) [56], and Am-Tm-T-Er-Nx (Am-trimethoprim-tetracycline-erythromycin-nalidixic acid) Tm-T-Er-Cp (Tm-T-Er-ciprofloxacin) and Am-Tm-Nx[33]. Antibiotic resistance of MDR *V. cholerae* O1 biotype El Tor serotype Ogawa isolates involved in an outbreak of cholera in 2007, based upon the minimum

inhibitory concentration determination following National Committee for Clinical Laboratory Standards guidelines, are represented in Table 1. It has been reported worldwide that there is great variation in antibiotic resistance among *V. cholerae* O1 strains[26-28]. Overall, the *V. cholerae* strains limit the therapeutic potential of antibiotics: some antibiotics are already unsuitable for certain group of population (such as T is not recommended for children and quinolones are not for pregnant women and children); the multi-drug resistance on the other hand presents additional challenges to disease management. Thus, multiple antibiotic resistances among *V. cholerae* have emerged as a major problem worldwide[57].

Plasmids are known to encode and transfer resistance in *V. cholerae*. Prescott and his colleagues (1968) reported the discovery of transferable R-plasmid in MDR *V. cholerae* isolated in Calcutta (Kolkata), India. Transfer of antimicrobial resistance has been documented among various serogroups of *V. cholerae*, and also between *V. cholerae* and the members of the family Enterobacteriaceae. Plasmid encoded resistance to Am-C-Str-Smz-Tr has been reported among *V. cholerae* O1 isolates from eastern Africa[58]. Plasmid mediated resistance to Tm-Smz-C-Am-Str in outbreak causing *V. cholerae* in Tanzania has been reported by Reid and Amyes[59]. The *V. cholerae* strains with plasmid, of different sizes, encoding multidrug resistance of various patterns have been reported from different parts of the globe[33,60].

Table 1

Minimum inhibitory concentration (MIC) values of antibiotics for outbreak causing *V. cholerae* O1 biotype El Tor serotype Ogawa isolates in Kolkata, India.

Strains	MICs of antibiotics (μ g/mL)								
	Am	C	Tm	T	Er	Nx	Cp	Ak	Cfx
Resistant isolates	75-200	-	75-200	75-100	64-128	32-64	10	-	-
Sensitive isolates	2-4	2-8	-	4	2	4-8	0.66	2-4	2-8

Am = ampicillin, Ak = amikacin, C = chloramphenicol, Cfx = cefotaxime, Cp = ciprofloxacin, Er = erythromycin, Nx = nalidixic acid, Tm = trimethoprim, T = tetracycline.

11. Cholera vaccine

A vaccine that is effective in lowering the total number of cases will also lower the number of resistant infections and could thus represent an effective intervention measure to control antibiotic resistance in cholera. Manna *et al*[61] reported vaccination against cholera, in conjunction with other prevention and control strategies, as an attractive additional tool to combat the disease in endemic areas. Page[62] described vaccines help limit epidemics in endemic areas where poor sanitation seems difficult to overcome. The injectable vaccines for cholera have been in use for long time, but with little lasting benefit. The currently available killed injectable vaccine is not recommended, since studies showed it to be less effective. However, the current availability of improved oral cholera vaccines has led to renewed interest in the use of vaccines during cholera epidemics. Ali *et al*[63] documented on significant herd immunity conferred by oral cholera vaccines in a large field trial in Bangladesh, and showed vaccinating more than 51% of the population in a geographic area led to a substantial reduction in cholera rates among individuals who did not take the vaccine.

Two oral cholera vaccines: one consisting of killed whole-cell *V. cholerae* O1 in combination with a recombinant B-subunit of cholera toxin (WC/rBS), and the other live attenuated vaccine containing the genetically manipulated classical *V. cholerae* strain CVD 103-HgR have been reported. Compared to the old parenteral vaccine, the current oral vaccines provide better and more long-lasting protection against cholera[64]. Mass vaccination, with WC/rBS vaccine, has been shown to be effective in refugee camps as well as in endemic regions, and a single-dose of CVD 103-HgR vaccine was found effective to limit the spread of a cholera outbreak[65, 66]. However, the manufacturer stopped production of CVD 103-HgR vaccine in 2004, and, although licensed it is no longer available[39], due to reason described by Richie *et al*[67]. Thus, WC/rBS is currently the cholera vaccine, prequalified by WHO, providing also some cross protection against enterotoxigenic *Escherichia coli* diarrhoea, is the one that has been used extensively worldwide.

12. Conclusion

Cholera is a life threatening diarrheal disease; while replacement of fluids and electrolytes remains the cornerstone of the management of cholera, antimicrobial therapy can significantly shorten the duration of diarrhoea, and reduce stool volume and requirements for rehydration fluids. However, like most bacteria of clinical and public health significance, *V. cholerae* O1 is continuously becoming more resistant to a variety of antimicrobial agents, necessitating the use of newer drugs which are more expensive and have more adverse effects to patients. In this situation, rotational use of anti-cholera antibiotics may lead to emergence of fully susceptible strains over time, which may allow for extension of use of the most effective therapies such as T as well as Cp.

The diversity of *V. cholerae* serogroups potential to cause cholera might be the survival advantage to the *V. cholerae* strains in the wake of host with less susceptibility to the pathogen. These strains, along with MDR clones, may form a group of emerging pathogens. Since the epidemics caused by the infection of MDR strains remain a threat, effective antibiotics are needed to reduce costs and treatment time, shorten the duration of illness; the antibiotic resistance patterns that change from time to time and place to place also require continual surveillance in order to provide

appropriate treatment.

Moreover, the continuous changes in the characteristics of the toxigenic *V. cholerae*, either in the serogroups predominating in outbreaks, antimicrobial resistance patterns, or its virulence, need monitoring of treatment and control of the disease. Compared with the parenteral vaccine, the new internationally available oral vaccines represent significant improvement in terms of protective efficacy, duration of protection, safety and easy method of administration.

Over all, the most useful measure in preventing the spread of cholera is the provision of safe drinking water, good food hygiene, and sanitary disposal of human feces; also, vaccination and improved sanitation have synergistic roles in controlling the disease[24]. Thus, the global reduction of the burden of cholera as a human health problem requires a multifactorial (both international and national) action to improve public health and to reduce the susceptibility to the infection of the poor communities, and thus, to reduce the threat of this global problem.

Conflict of interest statement

We declare that we have no conflict of interest.

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