IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

A comprehensive review of therapeutic approaches available for the treatment of cholera

Authors: Mr. Momin Abdul Faizan[1], Mr. Mohd Ameen Mahmood[2], Dr. Jameel Ahmed[3]
Department of Pharmacology, Kandhar college of pharmacy Balantwadi Post Ghodaj Tq Kandhar, Dist Nanded, Maharashtra, India.

ABSTRACT:-
Cholera, a diarrheal disease caused by Vibrio cholerae, remains a significant public health concern globally. Prompt and effective treatment is crucial to prevent dehydration, reduce mortality, and limit the spread of the disease. This review provides a comprehensive overview of the therapeutic approaches available for cholera treatment, including: - Objectives The oral rehydration solution is the most efficient method to treat cholera; however, it does not interfere in the action mechanism of the main virulence factor produced by Vibrio cholerae, the cholera toxin (CT), and this disease still stands out as a problem for human health worldwide. This review aimed to describe therapeutic alternatives available in the literature, especially those related to the search for molecules acting upon the physiopathology of cholera.

Key findings New molecules have offered a protection effect against diarrhoea induced by CT or even by infection from V. cholerae. The receptor regulator cystic fibrosis channel transmembrane (CFTR), monosialoganglioside (GM1), Enkephalinase, AMP-activated protein kinase (AMPK), inhibitors of expression of virulence factors and activators of ADP-ribosylarginine hydrolase are the main therapeutic targets studied. Many of these molecules or extracts still present unclear action mechanisms.
Interventions to reduce morbidity and mortality. By understanding the various therapeutic options available, healthcare providers can optimize cholera This review highlights the need for a multifaceted approach to cholera treatment, emphasizing the importance of timely and appropriate treatment and contribute to global efforts to control and prevent this disease.

Keywords: Cholera, therapy, viral disease, rehydration etc

INTRODUCTION:-

Cholera is an acute diarrheal infection is caused by Gram negative bacterium Vibrio cholerae of the O1 or O139 sero group [1]. It being a contagious disease is caused by contaminated water and food. It can result in watery diarrhea like condition which quickly leads to dehydration. If not treated in early stage, dehydration can lead to demise within few hours [2]. The bacteria is transmitted either through direct infection from environment or through fecal-oral route [3]. V. cholerae is Gram negative, facultative anaerobe, comma shaped bacteria having flagellum and pilli at one cell pole and is ubiquitously present in saltwater and brackish water, aquatic plants [4]. V. cholera was first observed by Pacini, more than 160 years ago. On the basis of antigen O of lipopolysaccharides, there were around 200 sero groups of V.cholerae. Of these, only two seogroups, O139 and O1 can cause scourge cholera. V. cholerae O1 has two biotypes, El Tor and classical, serotype Ogawa or Inaba, which differ in ubiquity with time. V. cholerae O139 was

initially reported in South Asia and which further caused outbreaks in Thailand or Bangladesh [5].
Cholera outbreaks are reported every year in more than 50 countries worldwide, both in emergency and non-emergency contexts. Every year there are an estimated 3–5 million cholera cases, and 100,000–120,000 deaths. Case fatality rates as high as 50% have been reported for untreated cholera, but can be reduced to 1% with rapid and comprehensive treatment. During cholera epidemics the number of cases can rapidly escalate to hundreds of patients in need for immediate therapy, and severe cases will survive only if effectively, timely and safely treated.

Recently, cholera has affected vulnerable communities such as the post-earthquake in Haiti, Iraq and Yemen, where natural disasters, refugee movements, wars and conflicts increase the risk of infection and outbreaks.[6] Although safe access to advanced sanitation systems has made cholera a treatable and limited disease in developed countries, new antibiotic-resistant strains, together with increasing severe weather events, can lead to a risk of reintroduction of cholera by travelers, tourists or workers in these countries.[7] The main mechanism by which V. cholerae causes its pathophysiological effects, responsible for the characteristic dehydration observed during cholera, is through the secretion of cholera toxin (CT) in the small intestine during its infection.[8]

Cholera:-

Cholera is a highly infectious diarrheal disease caused by the bacterium Vibrio cholerae. It's typically spread through:-

1. Contaminated food or water
2. Fecal-oral transmission
3. Poor sanitation and hygiene

Epidemiology:
Endemic in over 40 countries, particularly in Africa and Asia. Outbreaks often occur in areas with poor sanitation, conflict, or natural disasters. Affects approximately 1.3 million to 4.0 million people worldwide each year

History:
1. First described in ancient India around 400 BCE
2. Seven major pandemics have occurred since 1817
3. Most recent pandemic (1961-1975) spread to over 100 countries

Impact:
1. High mortality rate if left untreated (up to 60%)
2. Significant economic burden due to healthcare costs and lost productivity
3. Social and emotional impact on affected communities

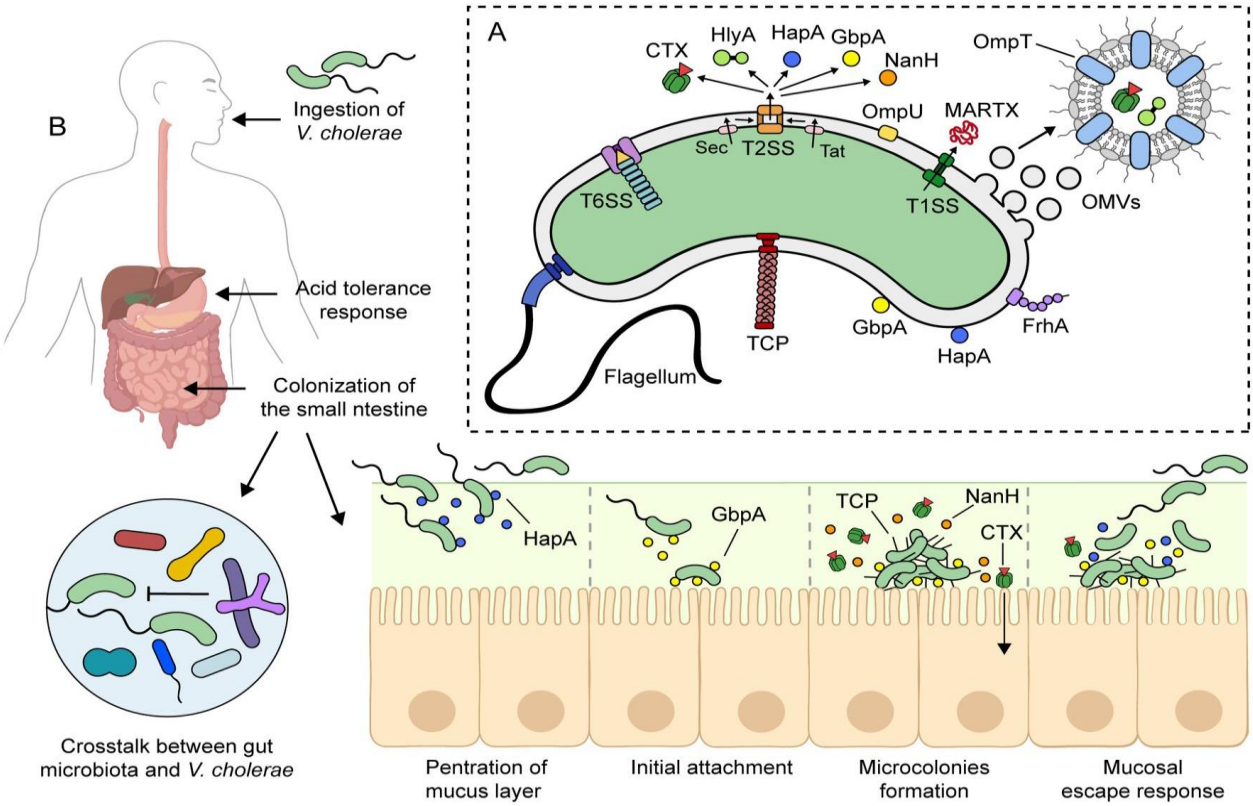
Prevention:
1. Access to clean water and sanitation
2. Proper food handling and hygiene practices
3. Vaccination (oral cholera vaccines)

PATHOGENESIS:-

V. cholerae enters the mouth through fecal contaminated water or food [9-10]. Most of bacteria entering the gastrointestinal canal are killed due to the low pH maintained by gastric acid [10]. However, if the bacterial load is too high, bacteria reach in small intestine and adhere to epithelial cell by special TCP (toxin-co regulated pilus) [11].

V. cholerae secrete protein exotoxin, which is made up of two subunit - A subunit and 5 B subunit [12]. These are enterotoxin which is antigenically identified as heat-labile enterotoxin of E.coli and can stimulate the creation of antibodies, and act on interior of the cell. Ganglioside GM1 fills in as the mucosal receptor for subunit B, which advances passage of subunit A into the eukaryotic cell. Subunit B never enter inside the eukaryotic cell because of its pentameric nature and is responsible for the binding with cholera toxin receptor.

GM1 is responsible for cell signaling. Subunit A enters inside the cell and get cleaves into A1 and A2. A1 increase adenylatecylase activity which further increases levels of intracellular cAMP inside the intestinal cell. This results in prolonged hypersecreation of electrolytes and water outside [1].



Lacking of treatment, the date rate of cholera case is 30% [13]. The first measure to control the illness is fluid replacement. For this, intravenous rehydration should be performed. Oral hydration should be encountered if emesis is not occurring frequently and after these antibiotics can be given to diminish the measure of stool volume and to diminish the term of ailment [14,15]. The methods of treatment for the cholera are described below:

Oral rehydration therapy (ORT)

The endeavors to use ORT started in 1964. Based on literature studies, it was confirmed that the availability of glucose was necessary to encourage retention of water in the gut [16, 17], oral glucose saline was utilized by US Navy Capt. Robert Phillips to effectively treat cholera in two patients in Philippines (18)..V. cholerae contamination causes epithelial cell lining in the intestine to lose large measures of electrolyte. World Health Organisation, has recommended the usage of sodium and potassium chloride and glucose ORT for treating cholera.However, these recommended constituents and their concentration has been modified several times. Glucose based formulations shortens the cholera symptoms by stimulating adsorption of electrolytes in the small intestine. Similarly, the rice based formulations decreases stool volume by 36% (20). . ORT replaces lost fluids and electrolytes during infection. The mortality rate of cholera has been diminished by over 97% by utilizing ORT and 99% patients survived V. cholerae infections after given ORT [19, 15].

It stimulates synthesis of short chain fatty acid (SCFA) via fermentation of carbohydrates that are not degraded and absorbed in small intestine by the action of colonic normal flora (17). In Haiti, rice based oral rehydration formulations were successfully tested for the treatment of cholera (16). ORT composed of starch, shows resistance to metabolic degradation in gut, thus it persists for longer time than glucose (17, 20). Also, starch and glucose could have a synergistic effect in suppressing cholera symptoms due to enhanced ion absorption (17). There are still cases

where ORT was unable to curb the spread of cholera. Combined treatment with vaccines, antibiotic and antimicrobials is needed for reducing the cholera symptoms when ORT is ineffective (20,21, 22).

Antibiotic treatment

In addition to rehydration, antibiotic treatment is recommended. Antimicrobial therapy is useful for (a) prompt eradication of the vibrio, (b) diminish the duration of diarrhea, and (c) decrease the fluid loss. Antibiotics should be administered to moderate or severe cases. The drug sensitivity test of the strains isolated to date in Haiti confirmed the resistance to trimethroprim–sulfamethoxazole, furazolidone, nalidixic acid and streptomycin.

1] Adults:-

Option 1:- Doxycycline, 300 mg po single dose

Option 2:- Ciprofloxacin, 1g po single dose

OR azithromycin, 1g po single dose.

2] Pregnant Women:-

Option 1:- Erythromycin 500 mg/ 6 hours for 3 days

OR azithromycin5, 1g po single dose

3] Children over 3 year, who can swallow tablets:-

Option 1:- Erythromycin 12.5 mg/kg/ 6 hours for 3 days.

OR azithromycin, 20 mg/kg, in a single dose, without exceeding 1 g

Option 2:- Ciprofloxacin, suspension or tablets, 20 mg/kg, in a single dose

OR doxycycline, suspension or tablets, 2-4 mg/kg po in single6 dose.

4] Children under 3 year, or infants who cannot swallow tablets:-

Option 1:- Erythromycin, suspension, 12.5 mg/kg/ 6 hours for 3 days

OR azithromycin, suspension 20 mg/kg, in a single dose.

Option 2:- Ciprofloxacin, suspension, 20 mg/kg, in a single dose

OR doxycycline, syrup, 2-4 mg/kg po in a single dose2.

Probiotics:-

The potential of host micro biome to inhibit the infection is a growing concept in microbiology. V. cholerae cause severe damage to the gastrointestinal microbiome during infections [23]. V.cholerae Type VI releases toxin against gut micro biome which is directly involved in colonization of bacteria (24, 25). Therefore, the colonization by probiotic bacteria is helpful to deal with cholera. Colonization of Ruminococcusabeum in human gut inhibits multiplication and colonization of V.cholerae in human gut (26). There is a positive correlation between R. obeum and prevention of cholera. According to study, the Lactococcus. lactis increase the production of lactic acid in response to V.cholerae. V. cholerae being is sensitive to acidic environment is killed in presence of lactic acid [27, 28].

Vaccination

In 1880s, the first injectable killed whole cell vaccine was devleoped producing (29, 30). In 20th century, these vaccines were extensively used by travellers (31). Certain disadvantages like limited efficacy, high cost, and inefficiency of the vaccine to control expansion of disease were among few reasons that the vaccine was not much recommended (29, 30 and 31).

Various new vaccines against cholera were developed and tested in different countries. Oral cholera vaccines (OCV) which weaken or reduce the effect of cholera were advocated. According to WHO, these vaccines provide effective response in combination with other therapies (32, 33). OCV helps to produce antibodies (IGA) against the antigen produced by V.cholerae. These antibodies work well against O1 specific polysaccharides present on the surface of

V.cholerae (34). Antibodies provide immunity for 6 months which further activates B cells and plasma cells to provide protection against the pathogen (34).

Most popular whole cell vaccine is 'Dukoral' which is the combination of inactivated V.cholerae O1 (E1) tor and classical biotype of recombinant B subtypes of CT (CTB) (35, 36, 37, and 38). It is widely used in endemic region due to cost effectiveness and efficiency rate of around 55-88% effect (39, 40). For adults, Dukoral is recommended after 2 years, for the children between 2 to 6 years dosage should be given 6 months [41].

Other vaccines, Shanchol and Euvichol are composed of deactivated O1 ogawa and O139 strains (36, 42). Shanchol has protection rate of approximately 65 %(32). These both vaccines are not recommended for pregnant women (36, 33). The oral live attenuated vaccine, vaxchora (CVD 103-HgR) is a single dose oral vaccine produced from classical O1 strain which is GMS of cholera (43). This vaccine has the ability to produce a desired or intended result against V.cholerae classical biotype with 65% effective response against V.cholerae E1 tor biotype (44). The vaxchora vaccine has high efficiency of 90% (45, 37, 46). Several other forms of vaccines developed from outer membrane vesicle (OMV), can also give rise to immunogenic responses (35) and provide protection against V.cholerae. Pandemic research indicated that as different region or area may be affected by different strains of cholera, new vaccines should be developed. The three vaccines are used for treatment of cholera are discussed below:

1. Killed whole cell monovalent (O1) vaccine with cholera toxin B subunit (WC-BS and WC-rBS):-

This vaccine is manufactured and marketed with the brand name Dukoral. Dukoral was developed and certified in Sweden in the year 1991. This vaccine is made by combining subunit B of cholera toxin and heat killed whole cells. The whole cell consists of monovalent V. cholerae O1 representing serotype Ogawa and Inaba, biotype classical and EI Tor. Cholera toxin (B-subunit) was initially synthetically developed (WC-BS). However currently developed by recombinant innovation (WC-rBS), both BS and rBS produced indistinguishable immune response (47). The vaccine doesnot contain cholera toxin subunit A and is free from its toxic impacts. Since the heat labile toxin (LT) of E. coli cross respond with cholera toxin, this vaccine has appeared to provide short term cross protection against diarrhea caused by enterotoxigenic E. coli (48). Two doses for grown-ups and three doses for youngsters beneath five years old are required for the vaccine. The vaccine requires coorganization of a bicarbonate buffer in safe water to prevent debasement of the toxin B subunit. Sur et al., (2009) reported 85% efficacy for 6 months vaccination or 50% efficacy of 3 year for older children or adults in randomized controlled trial involving ninety thousand children (age 2 years) (37).

Mass vaccination was noted to be productive in averting cholera in population with 20-30% sero prevalence of HIV [49, 50]. In a case study, where 14000 individuals were injected with a minimum dose, 78% of population was protected from the disease whereas serious illness was prevented in 89% cases.

2. Modified and killed whole cell vaccines (WC) - mORCVAX :-

In 1980s, the government of Vietnam started production of local cheap oral O1 serogroup whole cell vaccines. Composition of mORCVAX is same to WC-rBS apart from B subunit toxin. During widespread occurrence of cholera, this vaccine showed 66% protection (51). In 1997, mORCVAX was licensed through Vabiotech in Hanoi, Vietnam with the properties of bivalent formulation (O1 and O139). In this vaccine there is no co-administration of oral buffer because of lack of toxin. In Vietnam, 20 millions of doses were practiced. But due to quality aspects, vaccine did not prequalify the standard of WHO. Scientist of different nation together worked again on its production and improved quality, ingredients and which was then finally approved and the resulting vaccine is shanchol. In 2011, shanchol was prescreened by WHO by testing in Vietnam, Ethiopia and India (52-53). Trails conducted on more than 67000 youngsters and adult in Kolkata, India, have confirmed that shanchol has granted 67 % protection (54, 55). But the level of protection was not even for all age groups. In children between age between 1 to 5 years have shown efficacy of 42% (56). In Bangladesh, vaccine has shown efficiency upto 65%.

3. Attenuated and live oral cholera vaccines:-

Attenuated vaccines are conceivably more reactive and effective after just single-dose of vaccination. It has been postulated that attenuated immunizations can mirror normal infections, and therefore they ought to give dependable and explicit responses (57). They may have a few downsides, for example, presenting excessively high reactogenicity (58). The only authorized attenuated V. cholerae vaccine is CVD103-HgR, which is marketed as Orochol TM (Berna, Crucell, Switzerland). Orochol comes in sachets of two that contains attenuated vaccine and neutralizing buffer which protects it from gastric environment (59). Orochol TM comprises the hereditarily modified V. cholerae O1 Inaba strain 569B, likewise called CVD103-HgR. This derivative is created by deleting 550 bps in the ctxA gene. This expelled about 94% of the area encoding area of peptide A1 of the cholera toxin. A valuable quality gene mer is added. The antibodies produced in response to the vaccine are demonstrated to be immunogenic and safe (60).
This literature review and data search provide evidence that

Conclusion:-

New molecules or new biotech applications are being developed or can be applied to the development of effective and easy-to-use drugs for the treatment of cholera. It was also possible to observe that the CFTR channel, GM1 receptor, Enkephalinase, AMPK protein kinase, inhibitors of expression of virulence factors and activators of ADP-Ribosylarginine hydrolase are the primary therapeutic targets studied (Figure 1). Several plants used in folk medicine of different countries have been targets of studies for the treatment of cholera, in the sense of isolation of molecules of different classes of secondary metabolites.

Based on this literature search, it was possible to observe that several animal research models were developed for the study of cholera. Most of these models were developed using rodents or rabbits in which the two pathogenic subtypes of V. cholerae or their virulence factors, mainly CT, were inoculated. Some works are also developed using human intestinal epithelial cells (T84) in association with CT.

REFERENCE:-

[1]. Morris Jr, J. G., & Acheson, D. (2003). Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clinical Infectious Diseases, 37(2), 272-28

[2]. Ali, M., Gupta, S. S., Arora, N., Khasnobis, P., Venkatesh, S., Sur, D.,&Ganguly, N. K. (2017). Identification of burden hotspots and risk factors for cholera in India: An observational study. PloS one, 12(8), e0183100.

[3]. Sharland, M., Butler, K., Cant, A., Dagan, R., Davies, G., de Groot, R.,&Giaquinto, C. (Eds.). (2016). Manual of childhood infections: the blue book. Oxford University Press.

[4]. Harris, J. (2012). F, Ryan, ET, and Calderwood, SB. Cholera. Lance.

[5]. Sharland, M., Butler, K., Cant, A., Dagan, R., Davies, G., de Groot, R.,&Giaquinto, C. (Eds.). (2016). Manual of childhood infections: the blue book. Oxford University Presst, 379, 2466-2476.

[6]. Dutta D et al. Vibrio cholerae nonO1,non-O139 serogroups and cholera-like diarrhea, Kolkata, India.merg Infect Dis 2013; 19: 464–467

[7]. Dick MH et al. Review of two decades of cholera diagnostics – how far have we really come? PLoS Negl Trop Dis 2012; 6: e1845.

[8].Randak CO. An elusive adenylate cyclase complicit in cholera is exposed. J Biol Chem 2018; 293:12960–12961.

[9]. Morris Jr, J. G., & Acheson, D. (2003). Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. Clinical Infectious Diseases, 37(2), 272-280.

[10]. Alam, M., Hasan, N. A., Sadique, A., Bhuiyan, N. A., Ahmed, K. U., Nusrin, S.,&Huq, A. (2006). Seasonal cholera caused by Vibrio choleraeserogroups O1 and O139 in the coastal aquatic environment of Bangladesh. Appl. Environ. Microbiol, 72(6), 40964104.

[11]. Rosa, R. Y., &DiRita, V. J. (1999). Analysis of an autoregulatory loop controlling ToxT, cholera toxin, and toxin-coregulatedpilus production inVibriocholerae. Journal of bacteriology, 181(8), 2584-2592.

[12]. Kabir, S. (2005). Cholera vaccines: the current status and problems. Reviews in Medical Microbiology, 16(3), 101-116.

[13]. Karlsson, E. K., Harris, J. B., Tabrizi, S., Rahman, A., Shlyakhter, I., Patterson, N., ...& Sheikh, A. (2013). Natural selection in a bangladeshi population from the choleraendemic ganges river delta. Science translational medicine, 5(192), 192ra86-192ra86.

[14]. Campbell, J. D., &Kaper, J. B. (2003). Vaccines against Vibrio cholerae.

In New Bacterial Vaccines (pp. 339-349). Springer, Boston, MA.
[15]. Lopez, A. L., Clemens, J. D., Deen, J., &Jodar, L. (2008). Cholera vaccines for the developing world. Human vaccines, 4(2), 165-169. [16]. Kühn, J., Finger, F., Bertuzzo, E., Borgeaud, S., Gatto, M., Rinaldo, A., &Blokesch, M. (2014). Glucose-but not rice-based oral rehydration therapy enhances the production of virulence determinants in the human pathogen Vibrio cholerae. PLoS neglected tropical diseases, 8(12), e3347.
[17]. Binder, H. J., Brown, I., Ramakrishna, B. S., & Young, G. P. (2014). Oral rehydration therapy in the second decade of the twenty-first century. Current gastroenterology reports, 16(3), 376.
[18]. Bhattacharya SK: History of development of oral rehydration therapy. Indian J Public Health. 1994; 38(2): 39–43.
[19]. Weil, A. A., & Ryan, E. T. (2018). Cholera: recent updates. Current opinion in infectious diseases, 31(5), 455-461.
[20]. Milner, S. M., Green III, W. B., Asuku, M. E., Feldman, M., Makam, R., Noppenberger, D., ...& Van Loon, I. N. (2011). From cholera to burns: a role for oral rehydration therapy. Journal of health, population, and nutrition, 29(6), 648
[21]. Butler, T. (2017). Treatment of severe cholera: a review of strategies to reduce stool output and volumes of rehydration fluid. Transactions of The Royal Society of Tropical Medicine and Hygiene, 111(5), 204-210.
[22]. Binder, H. J., Brown, I., Ramakrishna, B. S., & Young, G. P. (2014). Oral rehydration therapy in the second decade of the twenty-first century. Current gastroenterology reports, 16(3), 376.
[23]. Hay, A., & Zhu, J. (2014). Microbiota talks cholera out of the gut. Cell host & microbe, 16(5), 549-550.
[24]. Zhao, W., Caro, F., Robins, W., &Mekalanos, J. J. (2018). Antagonism toward the intestinal microbiota and its effect on Vibrio cholerae virulence. Science, 359(6372), 210-213. [25]. Logan, S. L., Thomas, J., Yan, J., Baker, R. P., Shields, D. S., Xavier, J. B., ...&Parthasarathy, R. (2018). The Vibrio cholerae type VI secretion system can modulate host intestinal mechanics to displace gut bacterial symbionts. Proceedings of the National Academy of Sciences, 115(16), E3779-E3787. [26]. Hsiao, A., Ahmed, A. S., Subramanian, S., Griffin, N. W., Drewry, L. L., Petri, W. A., ... & Gordon, J. I. (2014). Members of the human gut microbiota involved in recovery from Vibrio cholerae infection. Nature, 515(7527), 423.
[27]. O'Hara, B. J., Barth, Z. K., McKitterick, A. C., & Seed, K. D. (2017). A highly specific phage defense system is a conserved feature of the Vibrio choleraemobilome. PLoS genetics, 13(6), e1006838.
[28]. Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World journal of gastrointestinal pharmacology and therapeutics, 8(3), 162
[29]. World Health Organization. (2000). Supplementary information on vaccine safety. Part 2: background rates of adverse events following immunization (No. WHO/V&B/00.36). Geneva: World Health Organization.
[30]. Preston, N. W. (2004). Prevention of cholera. The Lancet, 363(9412), 898. [31]. Acosta, C. J., Galindo, C. M., Deen, J. L., Ochiai, R. L., Lee, H. J., Von Seidlein, L., ... & Clemens, J. D. (2004). Vaccines against cholera, typhoid fever and shigellosis for developing countries. Expert Opinion on Biological Therapy, 4(12), 1939-1951.
[32]. Hsiao A, Hall AH, Mogasale V, et al.: The health economics of cholera: A systematic review. Vaccine. 2018; 36(30): 4404–4424. [33]. Harris JB: Cholera: Immunity and Prospects in Vaccine Development. J Infect Dis. 2018; 218(suppl_3): S141–S146.
[34]. Mengel, M. A., Delrieu, I., Heyerdahl, L., &Gessner, B. D. (2014). Cholera outbreaks in Africa. In Cholera outbreaks (pp. 117-144). Springer, Berlin, Heidelberg. [35]. Leitner DR, Lichtenegger S, Temel P, et al.: A combined vaccine approach against Vibrio cholerae and ETEC based on outer membrane vesicles. Front Microbiol. 2015; 6: 823. [36]. Hay AJ, Zhu J: Microbiota talks cholera out of the gut. Cell Host Microbe. 2014; a. 16(5): 549–50.
[37]. Wierzba TF: Oral cholera vaccines and their impact on the global burden of disease. Hum VaccinImmunother. 2018; 1–8.
[38]. Chen WH, Cohen MB, Kirkpatrick BD, et al.: Single-dose Live Oral Cholera Vaccine CVD 103-HgR Protects Against Human Experimental Infection With Vibrio cholerae O1 El Tor. Clin Infect Dis. 2016; 62(11):
[39]. Cabrera A, Lepage JE, Sullivan KM, et al.: Vaxchora: A Single-Dose Oral Cholera Vaccine. Ann Pharmacother. 2017; 51(7): 584–589. 1329–1335. [40]. Kirigia JM, Sambo LG, Yokouide A, et al.: Economic burden of cholera in the WHO African region. BMC Int Health Hum Rights. 2009; 9: 8.
[41]. Leibovici-Weissman Y, Neuberger A, Bitterman R, et al.: Antimicrobial drugs for treating cholera. Cochrane Database Syst Rev. 2014; (6): CD008625. [42]. Jertborn, M., Svennerholm, A. M., & Holmgren, J. (1992). Safety and immunogenicity of an oral recombinant cholera B subunit—whole cell vaccine in Swedish volunteers. Vaccine, 10(2), 130-132.
[43]. Lee EY, Lee S, Rho S, et al.: Immunogenicity of a bivalent killed thimerosal- free oral cholera vaccine, Euvichol, in an animal model. ClinExp Vaccine Res. 2018; 7(2): 104– 110. [44]. Chen, W. H., Cohen, M. B., Kirkpatrick, B. D., Brady,

R. C., Galloway, D., Gurwith, M., ...& Lyon, C. E. (2016). Single-dose live oral cholera vaccine CVD 103-HgR protectsagainst human experimental infection with Vibrio cholerae O1 El Tor. Clinical Infectious Diseases, 62(11), 1329-1335. [45]. Zuckerman, J. N., Rombo, L., &Fisch, A. (2007). The true burden and risk of cholera: implications for prevention and control. The Lancet infectious diseases, 7(8), 521-530. [46]. Rabaan AA: Cholera: an overview with reference to the Yemen epidemic.Front Med. 2019; 13(2): 213–228coli: results of a large-scale field trial. Journal of Infectious Diseases, 158(2), 372-377.
[47]. Butler T: Treatment of severe cholera: a review of strategies to reduce stool output and volumes of rehydration fluid. Trans R Soc Trop Med Hyg. 2017;111(5): 204–210. [48]. Ali, Mohammad, et al. "The global burden of cholera." Bulletin of the World Health Organization 90 (2012): 209-218.
[49]. Clemens, J. D., Sack, D. A., Harris, J. R., Chakraborty, J., Neogy, P., Stanton, B., ...&Ansaruzzaman, M. (1988). Cross-protection by B subunit-whole cell cholera vaccine against diarrhea associated with heat-labile toxin-producing enterotoxigenic Escherichia coli: results of a large-scale field trial. Journal of Infectious Diseases, 158(2), 372-377.
[50]. Trach, D. D., Clemens, J. D., Ke, N. T., Thuy, H. T., Son, N. D., Canh, D. G., ... &Rao, M. R. (1997). Field trial of a locally produced, killed, oral cholera vaccine in Vietnam. The Lancet, 349(9047), 231-235
[51]. Cavailler, P., Lucas, M., Perroud, V., McChesney, M., Ampuero, S., Guérin, P. J., ...&Kahozi, P. (2006). Feasibility of a mass vaccination campaign using a two-dose oral cholera vaccine in an urban cholera-endemic setting in Mozambique. Vaccine, 24(22), 4890-4895. [52]. Lucas, M. E., Deen, J. L., Von Seidlein, L., Wang, X. Y., Ampuero, J., Puri, M., ... &Cavailler, P. (2005). Effectiveness of mass oral cholera vaccination in Beira, Mozambique. New England Journal of Medicine, 352(8), 757-767. [53]. Desai, S. N., Akalu, Z., Teshome, S., Yamuah, L., Kim, D. R., Yang, J. S., ...&Aseffa, A. (2014). A randomized, double-blind, controlled trial to evaluate the safety and immunogenicity of killed oral cholera vaccine (Shanchol®) in healthy individuals in Ethiopia. International Journal of Infectious Diseases, 21, 431-432. [54]. Desai, S. N., Akalu, Z., Teferi, M., Manna, B., Teshome, S., Park, J. Y., ...&Digilio, L. (2016). Comparison of immune responses to a killed bivalent whole cell oral cholera vaccine between endemic and less endemic settings. Tropical Medicine & International Health, 21(2), 194-201.
[55]. Sur, D., Kanungo, S., Sah, B., Manna, B., Ali, M., Paisley, A. M., ...& Kim, D. R. (2011). Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. PLoS neglected tropical diseases, 5(10), e1289. [56]. Bhattacharya, S. K., Sur, D., Ali, M., Kanungo, S., You, Y. A., Manna, B., ...&Puri, M. K. (2013). 5 year efficacy of a bivalent killed whole-cell oral cholera vaccine in Kolkata, India: a cluster-randomised, double-blind, placebo-controlled trial. The Lancet infectious diseases, 13(12), 1050-1056.
[57]. Viret, J. F., Dietrich, G., & Favre, D. (2004). Biosafety aspects of the recombinant live oral Vibrio cholerae vaccine strain CVD 103-HgR. Vaccine, 22(19), 2457-2469.