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Original Research Article

The Development of Cholera Vaccine Production: A Literature Review

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ABSTRACT

Introduction. Cholera is a diarrheal disease that causes dehydration and rapid death due to infection with bacterium Vibrio cholerae that develops in the colon. Cholera generally develops in countries with poor sanitation, poverty, and unavailability of clean water such as Africa and South Asian. One of the efforts to prevent cholera transmission to tourists who will visit the country can be conducted through vaccines. **Method.** This research was made to find out the development of cholera vaccine using the literature search method through PubMed, Elsevier, Google Scholar databases, and credible websites. **Result and Analysis.** From the results of several literature searches, there is a monovalent O1 serogroup vaccine that contains killed whole-cell bacteria such as Ducoral and live-attenuated bacteria, called Vaxchora. **Discussion.** In addition, there are bivalent vaccines O1 and O139 serogroups that contain whole-cell killed bacteria such as Shanchol, Euvichol, mORC-Vax, and Cholvax.

Keywords: Cholera, Cholera Vaccines, Vaccine Production

INTRODUCTION

Vaccines are used to stimulate the immune system to form specific antibodies without having to experience illness due to exposure to certain pathogens. The body has recognized the incoming pathogen and is prepared with specific antibodies that have already been formed from a vaccine that includes parts or all of inactivated or weakened particular viruses or bacteria (Centers for Disease Control and Prevention, 2012) Immunization is the process of strengthening the body's immune system for it is resistant to specific diseases; this technique has been around for hundreds of years, for example monks who drank snake venom as a vaccine to provide immunity to snake bites and in 17th century, people in China went to extreme of smearing cowpox on skin that was deliberately scratched or torn to make it immune to smallpox. Edward Jenner is regarded as the father of vaccination because he started working on the smallpox vaccine in 1796 and improving its efficacy, safety, and ease of use. Many scientists are still working on developing bacterial and viral vaccines in 2021, as they did in 19th century, between 1890 and 1950. The cholera vaccine itself was developed by Pasteur Louis in 1897 (Immunisation Advisory Centre, 2022). There are three inactivated or non-live oral cholera vaccines that have been approved by WHO called Dukoral (manufactured by SBL Vaccines); ShanChol (manufactured by Shantha Biotec in India), and Euvichol-

Plus/Euvichol (manufactured by Eubiologics), but recently the FDA has approved a single dose live oral cholera vaccine namely Vaxchora® (lyophilized CVD 103-HgR) in United States of America (Centers for Disease Control and Prevention, 2022).

The number of cholera-related deaths in both sexes increased globally between 1990 and 2019, rising from 83,045 in 1990 to 117,167 in 2019. Cholera caused around 3.0 million fatalities worldwide. 2019 had the greatest cholera fatality rates for both sexes in Nigeria (ARS = 39.19) and the Central African Republic (ARS = 38.80), with Eritrea (ARS = 17.62) and Botswana (ARS = 13.77) following closely behind. Male cholera-related mortality globally dramatically declined (AAPC = 0.4%, 95% CI = 0.7 to 0.1), whereas female cholera-related mortality globally showed a constant trend (AAPC = 0.1%, 95% CI = 0.4 to 0.2) (Ilic, 2023). In 2011, IDEA (Initiative Against Diarrheal and Enteric Diseases in Africa and Asia) was established. IDEA is an independent, multidisciplinary network of professionals from cholera-prone nations in Asia and Africa. Its main objective is to facilitate and support the implementation of relevant prevention and control interventions on water, sanitation, and hygiene (WASH), on the use of oral cholera vaccine (OCV), by exchanging knowledge and by bringing attention to country-specific cholera situation.

In Asia and Africa, four IDEA workshops were successfully completed between 2015 and 2016. Experts from 10 cholera-prone Asian nations (Bangladesh, Cambodia, India, Indonesia, Malaysia, Nepal, Philippines, Pakistan, Thailand, and Vietnam) attended the fifth IDEA meeting in Hanoi, Vietnam (6–9 March 2017), along with representatives from the WHO, the US National Institutes of Health, the International Vaccine Institute, the Agence de médecine préventive, and NGOs (Save the Children, StC), as well as UNICEF (Ahmed *et al.*, 2018). In Indonesia, numerous reports have been successful in identifying V. cholera in the environment, aquaculture, food, and beverages, as well as in clinical instances of V. cholera infection (Ka Praja *et al.*, 2021).

The cholera vaccine is usually given to people who will be traveling to areas of active cholera transmission, i.e. areas with regularly reported cases of cholera infection, areas with confirmed poor sanitation and limited access to safe drinking water. The regions are Africa including Benin, Burundi, Cameroon, Democratic Republic of Congo, Ethiopia, Kenya, Malawi, Mozambique, Nigeria, Somalia, Sudan, Uganda, Asia including Bangladesh, India, Yemen, the Americas (Haiti) and Pacific (Philippines). Therefore, the use of cholera vaccines is expected to protect travelers or tourists from Vibrio cholerae bacteria. Besides the use of vaccines, there are several ways to prevent cholera infection, such as by eating food that has been cooked, washing vegetables and fruits before eating it and peeling its skin for fruit, drinking factory-sealed drinks, avoiding drinking ice water, and consuming pasteurized milk (Centers for Disease Control and Prevention, 2022).

The use of cholera vaccine in Indonesia itself is widely used by pilgrims who will go to Saudi Arabia to prevent cholera because Saudi Arabia borders to Yemen. According to Indonesian Ministry of Health 2017, the prevalence of Vibrio cholerae infection commonly known to the public, called diarrhea and vomiting is rarely found in Indonesia.

METHOD AND ANALYSIS

This research is used literature review based on the article search using the keywords “cholera”, “cholera etiology”, “cholera vaccine”, “vaccine design” and “vaccine production” through 2001 until 2021 in PubMed library, Elsevier, Google Scholar, and reliable websites. Five steps are used in a literature review are establishing research questions, identifying relevant studies, selecting articles, outlining data, assembling, summarizing, and presenting the findings (Rahimah and Fadhilah, 2022). In early screening, the researcher got 12 articles with the exclusion based on cholera and non-cholera. The article that will be processed after the screening is 10 articles, including 4 journal review, 4 non-review journal, and 2 credible websites. The reviewed article are 10 articles that 2 articles referred to in introduction, 1 article references the disease discussion, 1 article reference the discussion of disease causes, and 6 articles references for the discussion of vaccine design and production.

RESULT

Cholera

Cholera is a diarrheal disease caused by the bacterium Vibrio cholerae, which can lead to dehydration and rapid death. Cholera is closely associated with poverty, poor sanitation and lack of clean drinking water. Cholera is very common in African countries and countries in southern Asia. Cholera can be endemic and cause epidemics. The transmission of cholera bacteria spreads by fecal-oral route through contamination or direct ingestion of water or food that has been contaminated by cholera bacteria (Deen, Mengel and Clemens, 2020). The incubation period of cholera itself is less than 24 hours to 5 days. Some symptoms that indicate of cholera include:

1. Vomiting
2. Watery diarrhea
3. Leg cramps
4. Anxious
5. Severe dehydration resulting in dry mucous membranes
6. Low blood pressure.

Vibrio cholerae bacteria can live in brackish river environments and coastal waters which can affect marine animals such as shellfish. Vibrio cholerae bacteria that can be obtained through contaminated food and drinks can infected into human body which secretes enterotoxins that develop in small intestine, especially in the colon. The infecting Vibrio cholerae bacteria will invade the epithelial cells of intestinal mucosa. The toxin from Vibrio cholerae bacteria plays a role in causing cholera disease is Cholerae Toxin (CT) and Toxin Coregulated Pilus (TCP) (Bhattacharya *et al.*, 2013).

The diagnosis of cholera obtained from culture isolation of vibrio cholerae bacteria found in patient's feces. The appropriate medium for isolating and culturing these bacteria is *selective thiosulfate citrate bile salts agar* (TCBS). Besides the vaccines, there are therapeutic guidelines for someone who has been infected with cholera, called rehydration therapy, antibiotics, and zinc therapy.

Vaccine Design and Production

Oral cholera vaccines that currently licensed/under development

Vaccine	**Dukoral®**	**Shanchol™**	**Euvichol®**	**mORC-Vax™**	**Cholvax**	**Vaxchora™**
Manufacturers	Valneva, France	Shantha Biotechnics, (Hyderabad India) Sanofi Company	Eubiologis, Seoul, South Korea	Vabiotech, Hanoi, Vietnam	Vaksin Inceota Bangladesh	Pax Vax Inc, United States of America
Developer	Solna, University of Gothenburg Sweden	IVI, Shantha	IVI, Eubiologis	IVI, Vabiotech	IVI, icddr, b	Pax Vax Inc. (US) University of Kentucky and University of Maryland
Type	Monovalent, Killed whole-cell vaccine O1 serogroup and recombinant cholera toxin B subunit	Bivalent, killed whole-cell (O1 and O139 serogroup)	Bivalent, killed whole-cell (O1 and O139 serogroup)	Bivalent, killed whole-cell (O1 and O139 serogroup)	Bivalent, killed whole-cell (O1 and O139 serogroup)	Monovalent, live, attenuated
Age range	>2 years	1 year and above	1 year and above	2 year and above	1 year and above	18-64 years
Regimen	2 doses given for 7-14 days apart (3 doses for children aged 2-5 years)	2 doses 14 days apart	2 doses 14 days apart	2 doses 14 days apart	2 doses 14 days apart	Single doses

Booster	Every 2 years for > 6 years (every 6 months for children 2-5 years)	No recommendation from manufacturer	No recommendation from the manufacturer	No recommendation from the manufacturer	-	No recommendation from the manufacturer
Route	Oral	Oral	Oral	Oral	Oral	Oral
Buffer	Natrium bicarbonate	Not required	Not required	Not required	Not required	Na bicarbonate
Protection Duration	2 years (6 months in children 2-5 years)	Minimum of 5 years	Unavailable	3 years	-	Unavailable
Storage	2-8^{o}C	2-8^{o}C	2-8^{o}C	2-8^{o}C	2-8^{o}C	(-25) - (-15) ^{o}C
Shelf life	36 months	30 months	24 months	24 months	24 months	24 months
License	60 countries	28 countries	Zambia, Nepal, and Pakistan	Vietnam	Under development	Newly approved by US FDA, June 2016
WHO Prequalification	25 October 2001	29 September 2011	23 December 2015/2017	No	-	No

DISCUSSION

Several safe and effective vaccines are available on the market to prevent cholera (Wierzba, 2019; Deen, Mengel and Clemens, 2020), including;

1. WC-rBS, Killed Whole-Cell Monovalent (O1) Vaccines with a Recombinant B Subunit of Cholera Toxin (Dukoral®)

Dukoral® is an oral killed whole-cell vaccine developed in the 1980s, manufactured by SBL Vaccin AB and internationally licensed in Sweden in 1991 and licensed in Europe in 2004 (Lopez *et al.*, 2014). In February 2015, Dukoral was acquired by Valneva, Lyon, France. Dukoral was WHO

prequalified in October 2001 and is currently registered in more than 60 countries worldwide. Dukoral® was WHO prequalified in October 2001 and is currently available in >60 countries. Dukoral ® contains recombinant cholera toxin B-subunit (CTB) and three killed strains of V. cholerae O1 bacteria (V. cholerae O1 Inaba classic [heat inactivated], V. cholerae O1 Inaba El Tor [formalin inactivated], V. cholerae O1 Ogawa classic [heat inactivated and formalin inactivated]) (Wierzba, 2019). Dukoral ® is indicated for active immunization against disease caused by V. cholerae serogroup O1 in adults and children from 2 years old who will be visiting endemic/epidemic areas, but there are no data to support the use of Dukoral ® under 2 years of age. The vaccine works by inducing antibodies against bacterial and CTB components; bacterial-induced antibodies inhibit intestinal colonization by V. cholerae O1 by preventing bacterial attachment to intestinal wall, and antitoxin antibodies attenuate diarrhea symptoms by preventing cholera toxin from binding to the intestinal mucosa (Gabutti *et al.*, 2020). Dukoral® was found to be safe and immunogenic in individuals 2 years and older. However, as an inactivated vaccine, Dukoral® does not have the potential to reverse genetics into virulence. This vaccine has not been shown to protect against cholera caused by V. cholerae serogroup O139 or other Vibrio species. In addition, these vaccines have not been routinely adopted for public health use due to their high cost, limited duration of protection, and logistical issues with vaccine administration. The vaccine requires the addition of sodium bicarbonate buffer (effervescent granule in sachets) to protect the acid-unstable CTB component from degradation by stomach acid (Lopez *et al.*, 2014). The bicarbonate buffer must be dissolved in water and mixed with the vaccine before use.

2. BivWC, Killed Modified Whole-Cell Bivalent (O1 and O139) Vaccines without B Subunit

   Shanchol™, mORC-Vax™, Cholvax

   Sanchol is a modified vaccine from ORC-VAX, a two-dose regimen, killed whole-cell vaccine produced in Vietnam in the early 1990s. ORC-VAX has been used in more than 20 million doses for public health programs in Vietnam (Trach *et al.*, 2002; Lopez *et al.*, 2008). This modification of ORC-VAX was conducted due to the incompatibility of its use on an international scale based on several production and standardization issues, and because Vietnam's national regulatory authority was not approved by WHO, this development was conducted at Shantha Biotechnics in India, where the national regulatory authority was approved by WHO, initiated by International Vaccine Institute (IVI) in Seoul, Korea in 2004 (Clemens *et al.*, 2011). The modification result that was conducted in India is licensed as Sanchol. Sanchol is a modified whole-cell killed bivalent vaccine, oral without B subunit which derived from three killed V. cholerae O1 bacterial strains and one killed O139 strain but no toxin subunit of cholera B. Sanchol acts to prevent colonization of V. cholerae O1 and O139 in the gut and with easier

administration. Sanchol is indicated for all age groups over one year or more as an active immunization. Sanchol was licensed in India in 2009 and prequalified by WHO in September 2011 (Shaikh *et al.*, 2020). In addition to Sanchol, there is mORC-VAX™, which is a vaccine modified by VaBiotech, in Hanoi, Vietnam in 2010. This vaccine is also administered as a two-dose regimen for 1 year old or older and only available in Vietnam (Lopez *et al.*, 2014). The composition of Sanchol and mORC-VAX™ is basically identical. This vaccine contains formalin-inactivated O1 Inaba E1 Tor strain Phil 6973, heat-inactivated O1 Ogawa classic strain Cairo 50, formalin-inactivated O1 Ogawa classic strain Cairo 50, heat-inactivated O1 Inaba classic strain Cairo 48, and formalin-inactivated O139 strain 4260B, and contains thimerosal as a preservative (Shaikh *et al.*, 2020). Another similar vaccine, Cholvax, is currently undergoing development for Bangladesh market only. The primary immunization schedule consists of two doses given every two weeks, with the onset of initial protection at 7-10 days after completion. The effectiveness of protection against cholera was also evaluated during mass vaccination campaigns in Vietnam and India (Sur *et al.*, 2009; Lopez *et al.*, 2014; Wierzba *et al.*, 2015). From the 66,900 people vaccinated in India, 20 cases of cholera were reported in vaccine group, while 68 cases were reported in placebo group, and up to 67% efficacy was reported. During 3 year and 5 year of examination periods, the vaccine showed 66% and 65% cumulative protective efficacy (Sur *et al.*, 2009, 2011; Bhattacharya *et al.*, 2013). Vaccines provided the protection to individuals aged 1-4.9 years, 5-15.9 years, 15 years and older that did not differ significantly in efficacy ($p = 0.28$) (Sur *et al.*, 2009). In addition, the protection during third year of examination was 65% (one-sided) ($p < 0.001$) (Sur *et al.*, 2011). In a further experiment in India, of 51,488 eligible residents, 31,552 individuals received one dose and 23,751 residents received two doses of the vaccine. The protective effectiveness for people who received two doses was 69% (95% CI 14.5-88.8), and statistical analysis showed that a single dose still provided a protection (33%, $p = 0.0091$) (Wierzba *et al.*, 2015).

Euvichol®

Euvichol® is identical to Shanchol™ in terms of manufacturing process, quality, composition and administration route. Fed batch culture method is used for production that increasing the production by three to four times. Originally, the vaccines were produced in 100L fermenters with an annual production of 6 million doses of thimerosal-containing products. Considering the global demand for OCVs, the Eubiologics was upgraded from 100L to 600L that targeting production capacity of up to 25 million doses per year of thimerosal-free vaccines (Shaikh *et al.*, 2020). Euvichol® received the export license of Korean Ministry of Food and Drug Safety in 2014 and has met the WHO qualification in December 2015. Euvichol is produced in single-dose glass vials containing 1.5 mL of suspension and it has been developed

with a new plastic tube packaging, called Euvichol-Plus. Euvichol provides easier administration, storage, and affordable production costs. Euvichol Plus received WHO prequalification in 2017 (Odevall *et al.*, 2018).

3. Monovalent, live attenuated vaccine (Vaxchora™)

   Vaxchora™ (CVD 103-HgR) is a live attenuated V. cholerae serogroup O1, serotype Inaba and a classic biotype strain in which the toxigenic A1 (ADP-ribosylated) subunit of CT has been removed and only non-toxic and immunogenic CT subunit is synthesized. Vaxchora™ is indicated for active immunization against cholera caused by V. cholerae serogroup O1 bacteria. The vaccine is approved for use in patients aged 18-64 years who visit to areas that infected with cholera. This vaccine has not been shown to the protection against O139 serogroups or other non-O1 serogroups (Chen *et al.*, 2016). Vaxchora™ is approved for oral administration only. A single dose should be given at least 10 days before possible exposure to cholera or traveling to cholera endemic areas. The CVD 103-HgR formulation containing ~5 × 108 cfu (colony-forming units) was originally licensed and commercialized as Orochol® and Mutacol® by Swiss Serum and Vaccine Institute, Berne, Switzerland for travelers' protection from high-income countries (HICs) (Levine *et al.*, 2017). Vaxchora™ is supplied as a single-dose foil pack containing buffer (buffer component) and a single-dose foil pack with lyophilized CVD 103-HgR (active component). Other ingredients in active component package are sucrose, sodium chloride, ascorbic acid, dried lactose, and Hy Case SF (hydrolyzed casein). The buffer component pack contains sodium bicarbonate, sodium carbonate, ascorbic acid and lactose (Saluja *et al.*, 2020). The buffer component is added to 100 mL of cold water and followed by active component. Vaxchora™ should be taken within 15 minutes of recovery. The patient should avoid eating and drinking within one hour before and after oral vaccination, both active component and buffer component should be stored frozen (between -25°C and -15°C) and do not require for thawing prior before the reconstitution (Mosley *et al.*, 2017). Vaxchora™ works by inducing SVA, serum anti-CTB antibodies, serum anti-LPS antibodies, and protecting against cholera infection in humans. Since it is an attenuated oral strain, it is also expected to induce local mucosal immune responses in small intestine with similar way to wild-type V. cholerae infection (Kollaritsch *et al.*, 2000). Several tudies have shown that Vaxchora is highly effective against V. cholerae up to 90 days after vaccination (Mosley *et al.*, 2017).

CONCLUSION

Cholera which is caused by the bacterium Vibrio cholera is still very common in Africa and South Asian countries. The effort to prevent cholera is conducted the administration of a vaccine. many scientists remain to conduct research and development on the vaccine until now. Several oral cholera vaccines are currently licensed or under development such as the monovalent O1 serogroup containing

completely killed bacteria, dukoral, and vaccine containing attenuated bacteria called Vaxchora. In addition, there are bivalent O1 and O139 serogroup vaccines containing completely killed bacteria such as Shanchol, Euvichol, mORC-Vax and Cholvax.

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