Killed oral cholera vaccines: history, development and implementation challenges

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***Abstract*:** Cholera is still a major global health problem, affecting mainly people living in unsanitary conditions and who are at risk for outbreaks of cholera. During the past decade, outbreaks are increasingly reported from more countries. From the early killed oral cholera vaccine, rapid improvements in vaccine development occurred as a result of a better understanding of the epidemiology of the disease, pathogenesis of cholera infection and immunity. The newer-generation oral killed cholera vaccines have been shown to be safe and effective in field trials conducted in cholera endemic areas. Likewise, they have been shown to be protective when used during outbreak settings. Aside from providing direct protection to vaccinated individuals, recent studies have demonstrated that these killed oral vaccines also confer indirect protection through herd immunity. Although new-generation oral cholera vaccines should not be considered in isolation from other preventive approaches in countries where they are most needed, especially improved water quality and sanitation, these vaccines serve as immediately available public health tools for preventing further morbidity and mortality from cholera. However, despite its availability for more than two decades, use of these vaccines has not been optimized. Although there are limitations of the currently available oral cholera vaccines, recent data show that the vaccines are safe, feasible to use even in difficult circumstances and able to provide protection in various settings. Clear identification of the areas and target population groups who will benefit from the use of the cholera vaccines will be required and strategies to facilitate accessibility and usage of these vaccines in these areas and population groups will need to be developed.

Keywords: cholera, cholera vaccine, oral cholera vaccine, *Vibrio cholerae*

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Introduction

From Sanskrit writings describing an illness similar to the disease, cholera is believed to have been endemic in the Indian subcontinent, particularly in the Ganges region, as far back as 500 BC [Barua, 1992]. The disease was much feared because of its virulence and high mortality rate that religious rites were performed to ward off its effects [Pollitzer, 1954]. Because of its rapidly dehydrating nature, cholera, if left untreated, can easily lead to fatalities and spread fast, especially in naïve populations.

Cholera disease

Cholera is caused by *Vibrio cholerae* and epidemics are primarily caused by serogroups O1 and O139. Serogroup O1 has two biotypes (El Tor and classical) and both biotypes can further be classified into three serotypes: Ogawa, Inaba and Hikojima. *V. cholerae* O1 Hikojima is an unstable form and rarely occurs in nature [Kaper *et al.* 1995].

Apart from the epidemic serogroups, there are at least around 200 recognized *V. cholerae* serogroups collectively known as non-O1 non-O139 which are found in the aquatic environments and rarely associated with outbreaks. *V. cholerae* are noninvasive. Following ingestion of the organism by the human host, vibrios colonize the small intestine by penetrating the mucus layer and rapidly multiplying. Here, *V. cholerae* produce an enterotoxin, cholera toxin (CT), composed of five receptor binding

(B) subunits surrounding a single catalytic (A) subunit. The B subunits bind to GM1 ganglioside receptors in the small intestine and the A subunit is released into the cell where it activates adenylate cyclase. This activation stimulates the processes that lead to massive outpouring of fluid from the small intestine, overcoming the absorptive capacity of the bowels resulting in massive amounts of watery diarrhea [Sack *et al.* 2004].

Treatment requires rapid rehydration and antibiotics for severe disease [WHO, 2005]. If appropriate treatment is provided in a timely manner, mortality can be reduced to less than 1%. Case fatality rates of up to 50% have been reported in vulnerable populations [WHO, 2010b].

Epidemiology

In the past two decades, cholera has increasingly affected more countries and more people, resulting in protracted outbreaks. The epidemic in Haiti that began in October 2010 with 8540 deaths and nearly 700,000 people affected still continues and has spread to neighboring Dominican Republic and other countries [Pan American Health Organization, 2014]. Prior to Haiti, the protracted outbreaks were seen in 2008–2009 in Zimbabwe with more than 98,000 cases including 4000 deaths [WHO, 2010a]; in 2006–2007 in Angola with 85,000 cases and a case fatality rate of 4% and in 2002 in South Africa with 116,000 cases and a case fatality rate of less than 1% [Koenig, 2009]. Since the first cholera pandemic caused by *V. cholerae* O1 classical that began in 1817 and spread from the Indian subcontinent through trade routes to China, Japan, parts of Southeast Asia, the Middle East and southern Russia to the seventh and latest pandemic that has been ongoing since 1961, the disease continues to spread globally. A different biotype of *V. cholerae* serogroup O1 called El Tor was first isolated in 1905 at a quarantine station in El Tor, Egypt from Indonesian pilgrims travelling to Mecca [Barua, 1972]. The seventh cholera pandemic, caused by the El Tor strain, spread rapidly from Indonesia to other countries in Asia, Europe, Africa and finally to Latin America where 400,000 cases and over 4000 deaths were reported in 1991 [WHO, 2013a].

In 1992, a newly described, non-O1 serogroup of *V. cholerae*, designated O139 Bengal, caused unusual cholera outbreaks in India and Bangladesh [Cholera Working Group, 1993; Ramamurthy *et al.* 1993]. Since then, however, cases of O139 have declined, with only China reporting cases in 2012 [WHO, 2013c]. While the threat of another pandemic due to O139 may have abated, a new strain of *V. cholerae* O1 phenotypically El Tor, but producing classical CT was reported first in Bangladesh and later in other parts of Asia and Africa [Nair *et al.* 2002, 2006; Ansaruzzaman *et al.* 2007]. This hybrid, now the predominant strain in most parts of the world, is responsible for the recent cholera outbreaks in Haiti.

Since John Snow first showed the association of the disease with contaminated drinking water in 1854, improvements in sanitary facilities and development of clean water systems led to the disappearance of cholera in much of the developed world. However, cholera continues to affect many impoverished populations, especially those living in slums with little or no access to sanitation facilities and running water and those affected by man-made or natural disasters.

Cholera is underreported globally. Recent estimates reveal that up to 1.4 billion people are at risk of cholera in endemic countries alone, with 2.8 million cholera cases occurring resulting in around 94,000 deaths [Ali *et al.* 2012]. Diarrheal diseases including cholera are one of the leading causes of mortality worldwide among children younger than 5 years [Walker *et al.* 2013]. However, in 2012, only 245,393 cholera cases were reported to World Health Organization (WHO) with 3034 deaths. While a decline in the number of cases was reported compared with 2011, the death toll due to cholera remains high [WHO, 2013c]. The past decade has seen a rise in the number of cholera outbreaks, leading the World Health Assembly to pass a resolution calling for integrated cholera control efforts, including the use of oral cholera vaccines (OCVs) [WHO, 2011].

Currently, only killed OCVs are available. Live OCVs were previously available on the market but manufacturing has been discontinued. This review is limited to killed OCVs.

Early killed OCV development

Earlier observations by Koch and colleagues in the 1880s revealed that people who had cholera were protected against subsequent infections during the same epidemic, suggesting that some form of naturally acquired immunity had developed [Pollitzer and Burrows, 1955]. The first cholera vaccine was developed by Ferran in 1885 and

used in mass vaccination campaigns in Spain [Pollitzer and Burrows, 1955; Mukerjee, 1963]. Although variations of parenterally administered killed whole cell (WC) bacterial vaccine were previously used, they have never been recommended by the WHO due to their limited efficacy and short duration of protection [WHO, 2010b].

Sawtschenko and Sabolotny developed the first OCV in 1893 when they and their student ingested agar-grown, heat-killed *V. cholerae* ('virulent cholera broth'). They remained asymptomatic after challenge, but despite these results, questions remained about the practicality of oral immunization since multiple high doses were required [Pollitzer and Burrows, 1955].

In the 1920s–1930s, field trials were conducted in India on the use of bilivaccine, a commercially prepared tablet containing 70 billion dried *V. cholerae* organisms. Three doses were given on three successive days. While the bilivaccine was shown to provide protection against cholera, it was deemed inferior to the parenteral vaccine, furthermore the 'difficulty and costliness of preparing oral vaccines' led Pollitzer to state, 'the method of cholera vaccination per os has been given up entirely' [Pollitzer and Burrows, 1955]. But subsequent studies revealed that stimulation of intestinal immune cells through the oral route elicited intestinal antibody responses that might result in superior protection against enteric infections, including cholera [Mukerjee, 196; Freter, 1964; Ganguly et al. 1975]. As increased understanding of the intestinal immunity and cholera evolved, a shift in parenteral vaccine to oral vaccine development in the 1980s occurred.

Immunity and vaccine development

Epidemiologic studies in Bangladesh support the idea that protection occurs after cholera infection [Clemens *et al.* 1991]. An infection severe enough to require treatment in a healthcare facility reduced the risk of subsequent clinical infection by about 90% [Glass *et al.* 1985]. Further supportive data may be gleaned from challenge studies with volunteers. These revealed that an initial infection with classical vibrios of either Inaba or Ogawa serotype resulted in 100% protection against subsequent challenge of classical vibrios of either heterologous or homologous serotypes. It was also protective against shedding. However, an initial infection with El Tor vibrios resulted in only 90% protection against subsequent challenge of El Tor vibrios of either heterologous or homologous serotypes [Levine *et al.* 1983]. Upon rechallenge of previously *V. cholerae* infected volunteers, the same strain could be isolated from the stool of about one-third of volunteers [Kaper *et al.* 1995]. In Bangladesh, studies revealed that infections with El Tor Inaba were protective against subsequent infections with either Inaba or Ogawa serotype; however, El Tor Ogawa infections did not provide protection against subsequent Inaba infections. Furthermore, no cross protection was seen between *V. cholerae* O1 and O139 [Ali *et al.* 2011]. These findings have implications on the strains included in cholera vaccines.

Mucosally secreted immunoglobulin A (IgA) antibodies against *V. cholerae*, primarily directed against lipopolysaccharide (LPS) and CT, are believed to confer immunity against cholera [Svennerholm *et al.* 1984a, 1984b; Clemens *et al.* 2011]. Although anti-CT immunity is believed to be of less importance than antibacterial immunity, it is likely that they work synergistically towards protection against cholera [Clemens *et al.* 2011]. The presence of a strong antibacterial immunity interfering with survival and growth of vibrios in the intestine is apparent from the sporadic isolation of vibrios from stools of rechallenged volunteers [Kaper *et al.* 1995].

No correlate of protection exists for cholera. Similarly, there is no reliable animal model that can be used to measure or predict the potency of OCV in humans. Intestinal secretory IgA is probably the best predictor of protection; however, this is not practical in clinical trials. Serum vibriocidal antibodies were instead used as a proxy of intestinal immune response when the antigen was given through the oral route [WHO, 2004]. Because vibriocidal responses are affected by prior exposure to the disease, higher baseline titers are seen among individuals residing in cholera-endemic areas [Anh *et al.* 2007; Mahalanabis *et al.* 2008]. Therefore, vibriocidal responses cannot be used to predict the level of protection afforded by vaccines. In the absence of a reliable correlate of protection, assessment of new vaccines requires large randomized controlled clinical trials of efficacy.

Killed OCVs

Over the last three decades, considerable efforts have been made for the development of safe and efficacious killed OCVs. The four currently available killed WC vaccines are described in Table 1.

Table 1. Currently available, licensed killed oral cholera vaccines.

	Dukoral	Shanchol	mORC-Vax	Oravacs
Manufacturer	Crucell, Leiden, The Netherlands	Shantha Biotechnics, India	Vabiotech, Hanoi, Vietnam	Shanghai United Cell Biotechnology, China
Composition	Recombinant cholera toxin B subunit 1 mg plus killed whole cells of the following *Vibrio cholerae* 01 organisms: *V. cholerae* 01 Inaba classical biotype (Cairo 48 strain), heat killed, ca. 31.25×10^9 vibrios *V. cholerae* 01 Inaba El Tor (Phil 6973 strain), formalin killed, ca. 31.25×10^9 vibrios *V. cholerae* 01 Ogawa classical biotype (Cairo 50 strain), heat killed, ca. 31.25×10^9 vibrios *V. cholerae* 01 Ogawa classical biotype (Cairo 50 strain), formalin killed, ca. 31.25×10^9 vibrios	Killed whole cells of 01 classical and El Tor biotypes plus 0139: *V. cholerae* Inaba El Tor biotype (Phil 6973 strain), formaldehyde-killed 600 ELISA units (EU) LPS *V. cholerae* Ogawa classical biotype (Cairo 50 strain), heat-killed 300 EU LPS *V. cholerae* Ogawa classical biotype (Cairo 50 strain), formaldehyde-killed 300 EU LPS *V. cholerae* Inaba classical biotype (Cairo 48 strain), heat-killed 300 EU LPS *V. cholerae* 0139 (4260B strain), formaldehyde-killed 600 EU LPS	Killed whole cells of 01 classical and El Tor biotypes plus 0139: *V. cholerae* Inaba El Tor biotype (Phil 6973 strain), formaldehyde-killed 600 ELISA EU LPS V. cholerae Ogawa classical biotype (Cairo 50 strain), heat-killed 300 EU LPS *V. cholerae* Ogawa classical biotype (Cairo 50 strain), formaldehyde-killed 300 EU LPS *V. cholerae* Inaba classical biotype (Cairo 48 strain), heat-killed 300 EU LPS *V. cholerae* 0139 (4260B strain), formaldehyde-killed 600 EU LPS	Recombinant cholera toxin B subunit 1 mg plus 5.0×10^{10} Killed whole cells of *V. cholerae* 01 classical biotype or El Tor biotype
Regimen	Age > 6 years: two doses given at least 1 week apart 2–6 years old: three doses given at least 1 week apart	Age 1 year and older: two doses 14 days apart	Age 2 years and older: two doses 14 days apart	Age 2 years and older: three doses given at days 0, 7 and 28
Booster dose	Age > 6 years: every 2 years 2–6 years old: every 6 months If the patient received the last dose >5 years before, a complete primary immunization (two doses) is recommended to renew the protection	Every 2 years (may be subject to change)		Every year
Administration	Administer with oral buffer (sodium hydrogen carbonate solution) Food and drink must be avoided for 1 h before and 1 h after	No oral buffer required	No oral buffer required	Enteric-coated capsule, no oral buffer required
Licensure	WHO prequalified since October 2001, licensed in ~60 countries [WHO, 2013b]	WHO prequalified since September 2011, licensed in India, Philippines, Nepal, Malaysia and Ivory Coast [WHO, 2010b, 2013b]	Not WHO prequalified, licensed only in Vietnam	Not WHO prequalified, licensed only in China and the Philippines

ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide.

Recombinant B-subunit WC (Dukoral) vaccine

Dukoral (Crucell, Leiden, The Netherlands) was the first killed WC oral vaccine to be licensed internationally in 1991. It contains CT B subunit (CTB) in addition to heat- or formalin-killed WCs of *V. cholerae* O1 serogroup (a mixture of classical and El Tor biotypes, including both Ogawa and Inaba serotypes). The vaccine requires administration with a sodium bicarbonate buffer to protect the acid labile CTB component from degradation by gastric acid.

The vaccine was developed in Sweden in the 1980s and originally contained native B subunit purified from CT produced by a wild-type strain. Immunogenicity and safety of the B-subunit WC (Bs-WC) vaccine was shown in trials that included healthy adults and children coming from endemic [Svennerholm *et al.* 1984b; Clemens *et al.* 1987] and nonendemic areas [Jertborn *et al.* 1984, 1986]. Since 1991, the native CTB used in vaccine production was replaced by recombinant CTB (rCTB), which was easier and less expensive to produce. Studies showed that the rCTB cholera vaccine produced by recombinant DNA technology induced comparable immune responses and had a similar safety profile to the original CTB vaccine formulation [Sanchez and Holmgren, 1989; Jertborn *et al.* 1992, Concha *et al.* 1995].

An efficacy trial of the original Bs-WC formulation was conducted in 89,596 people aged 2–15 years and women over the age of 15 years in Bangladesh [Clemens *et al.* 1986, 1988, 1990; Van Loon *et al.* 1996]. In this trial, study participants were randomly allocated to receive three doses of the Bs-WC, a vaccine containing the killed WC constituents only, or placebo (Table 2). Although protective efficacy (PE) was sustained for 2 years in adults and children above 5 years of age, protection lasted for only 6 months in children 2–5 years of age, suggesting more frequent dosing were required for this age group. Efficacy was noted to be substantially higher against classical cholera than against El Tor cholera. This study also suggested that a two-dose regimen was as effective as a three-dose regimen given at 6-week intervals. Challenge studies with the Bs-WC vaccine among healthy North American volunteers aged 18–35 years in Baltimore showed 64% protection against El Tor Inaba 4 weeks after receipt of three oral doses of the vaccine at 2-week intervals [Black *et al.* 1987].

Subsequently, the efficacy of a two-dose regimen of the rBs-WC was evaluated in two placebo-controlled, randomized trials conducted in Peru [Sanchez *et al.* 1994; Taylor *et al.* 2000]. In the first trial, among Peruvian military recruits, PE was determined to be 86%, similar to the short-term protection with the chemically extracted Bs-WC vaccine in Bangladesh. The second large efficacy trial of a two-dose regimen of the rBs-WC failed to demonstrate protection after two doses [Taylor *et al.* 2000]. After a third (booster) dose of the vaccine given 10 months later, the reported vaccine PE was 61%. The vaccine was observed to be more efficacious in individuals older than 15 years of age and in preventing illness requiring hospitalization, implying that three doses may be more effective in younger children and in preventing more severe cholera. The apparent lack of efficacy following the two-dose regimen was later attributed to methodological reasons [Clemens *et al.* 2001].

Because of the lower efficacy of the Bs-WC vaccine in children in Bangladesh aged 2–5 years, studies were conducted on the use of vitamin A and zinc to improve the immune response to the vaccine. Zinc supplementation resulted in a 43% increase in the number of children with vibriocidal immune responses after just one dose of the vaccine [Albert *et al.* 2003]. Whether these enhanced immune responses with zinc supplementation to OCV will result in increased protection against cholera remains to be seen.

Post-licensure studies of the rBs-WC vaccine have confirmed its safety and immunogenicity, including in HIV-infected individuals [Lewis *et al.* 1994]. A randomized controlled trial to assess vaccine efficacy among HIV-infected individuals has not been conducted. In Beira, Mozambique, a city with 20–30% seroprevalence of HIV infection, vaccine effectiveness measured using a case–control design was 78% against cholera detected in cholera treatment centers and 89% against cholera with severe dehydration during an outbreak that lasted for 5 months after a mass vaccination campaign [Lucas *et al.* 2005]. Thus, the vaccine is likely safe and protective against cholera in HIV-infected individuals.

Herd protection. In addition to providing direct protection to vaccine recipients, these vaccines conferred indirect (herd) protection to neighboring nonvaccinated individuals [Ali *et al.* 2005]. Reanalysis of the 1-year follow-up dataset from the Bangladeshi efficacy trial [Clemens *et al.* 1988], using a geographic information systems (GIS)

Table 2. Efficacy and effectiveness studies of killed whole cell oral cholera vaccines.

Study attributes	Results
Bs-WC or rBs-WC (Dukoral)	
Bangladesh, 1986–1991 [Clemens *et al.* 1986, 1988, 1990; Van Loon *et al.* 1996] • Randomized, controlled trial • Three doses of either Bs-WC or WC only or *Escherichia coli* K12 placebo • Subjects: children 2–15 years and female patients 15+ years • No. of subjects: 89,586 received at least one dose; three-dose recipients: 62,285 • Follow up: 5 years	Safety: no significant differences in adverse events Efficacy: 4–6 months: Bs-WC with PE = 85%; WC PE = 58% 1 year: BS WC PE = 62% while WC was 53%; Bs-WC PE decreased from 79% at 4 months to ~60%, whereas WC PE was similar throughout the seasons 3 years: cumulative PE for Bs-WC was 50% and for WC 52%; PE on the 3rd year was sustained for older individuals, older than 5 years at 40% and 62% for Bs-WC and WC respectively 5 years: no protection seen after 3 years
Peru, 1994 [Sanchez *et al.* 1994] • Randomized, controlled trial • Two doses of rBs-WC or *E. coli* K12 placebo • Subjects: military recruits aged 16–45 years • No. of subjects: 1426 • Follow up: 3–6 months	Cholera cases occurred 2 weeks after second dose; PE = 86%, protection maintained until end of study period; PE against asymptomatic cholera = 42%
Peru, 1993–1995 [Taylor *et al.* 2000] • Randomized controlled trial • Two doses of rBs-WC or *E. coli* K12 placebo with a booster dose after 10 months • Subjects: nonpregnant individuals aged 2–65 years • No. of subjects: 21,924 received the first dose, 17,799 received the second dose, 14,997 received the third dose • Follow up: 2 years	Two-dose PE = –4% (95%CI –88 to 43); three-dose PE = 61% (95% CI 28–79)
Mozambique [Lucas *et al.* 2005] • Case control with bias indicator study • two doses of rBs-WC • Target population: nonpregnant individuals 2 years and older • No. vaccinated: 14,164 received one dose and 11,070 received two doses • No. of cases and controls: 43 cholera cases and 172 controls • Follow up: 6 months	Receipt of one or more doses of rBS-WC vaccine was associated with 78% protection (95% CI 39–92%; p = 0.004), bias-indicator study showed no protection against noncholera diarrhea
• Zanzibar, Tanzania, 2009–2010 [Khatib *et al.* 2012] • Cohort study • Two doses of rBs-WC • Target population: nonpregnant individuals 2 years and older • No. vaccinated: 23,921 received two doses (50% of target population) • Follow up: 15 months	For two-dose recipients PE = 71%; for one-dose recipients: PE = 46% Indirect protection = 75% in the higher coverage group compared with the lower coverage group
WC only vaccine (ORC-Vax)	
Hue, Vietnam, 1992 [Trach *et al.* 1997] • Open-label study • Two doses of monovalent killed whole cell (O1) OCV • Target population: nonpregnant individuals 1 year and older • No. of subjects: 67,395 vaccine group (77% of targeted age-eligible individuals) and 67,068 nonvaccine group • Follow up: 10 months	All age group, PE = 66%; efficacy in <5 years: 68% Against severe cholera, PE = 76% Impact of vaccine against cholera admission: 60%
Hue, Vietnam, 1998–2003 [Thiem *et al.* 2006] • Case control study • Target population: all nonpregnant residents, ≥2 years old • No. vaccinated: received vaccine in 1998: 149,557, 84% received one dose, 79% received two doses; received vaccine in 2000: 137,082, 76% received one dose, 75% received two doses • No. of cases and controls: 69 cases and 276 controls • Follow up: 5 years	VE = 50% (95% CI 9–63%, p = 0.02) effectiveness if vaccinated either in 1998 or 2000, If vaccinated in 1998, VE = 51% (95% CI –16% to 80%), If vaccinated in 2000, VE = 54% (95% CI –7% to 81%); Indicator study showed no VE against noncholera diarrhea

(*continued*)

Table 2. (continued)

Study attributes	Results
Hanoi, Vietnam, 2008 [Anh *et al.* 2011] • Hospital-based case control study • Target population: 370,000 age-eligible (older than 10 years) individuals • No. vaccinated: 80% received one or two doses of vaccine spaced 1 week apart • No. of cases and controls: 126 matched pairs	VE = 76% (95% CI 5–94%, $p = 0.042$)
WC only vaccine (mORC-Vax/Shanchol)	
Kolkata, India, 2006–2010 [Sur *et al.* 2009, 2011; Bhattacharya *et al.* 2013] • Cluster-randomized trial • Two doses of killed whole cell bivalent (O1 and O139) OCV • No. of subjects: nonpregnant individuals aged 1 year and older: 66,900 • Follow up: 5 years	2 years: all age groups: PE = 67% 1 to <5 years old: PE = 49%; 5 to < 15 years old: PE = 87%; 15+ years old: PE = 63% 3 years: cumulative PE = 66%; 1 to <5 years, cumulative PE = 43%; PE on the third year was 65% for all ages 5 years: cumulative PE = 65%; 1 to <5 years, cumulative PE = 42% ($p = 0.07$ for difference in PE in age groups)
Forecariah and Boffa prefectures in Guinea, 2012 [Luquero *et al.* 2014] • Case control study • Target population: > 12 months of age presenting at vaccination sites in the target rural prefectures of Boffa (163,086 people) and Forecariah (46,008 people) • No. vaccinated: 90% coverage with at least one dose, and 76% coverage with two doses • Follow up: 6 months	Two dose VE = 86.6 % (95% CI 56.7 to 96%; $p = 0.001$) against rapid test-confirmed cholera cases and two dose VE = 91.6% (95% CI 58.6 to 98.3; $p = 0.002$) against culture and PCR-confirmed cholera cases

Bs-WC, B-subunit whole cell; CI, confidence interval; PE, protective efficacy; rBs-WC, recombinant B-subunit whole cell; VE, vaccine effectiveness.

approach, showed that high levels of cholera vaccine coverage in a neighborhood were associated with a reduced risk of cholera in nonvaccinated residents of that neighborhood. The findings suggest that increasing use of cholera vaccines would have a major impact on the burden of cholera in endemic settings by providing both direct and indirect protection to vaccine recipients as well as indirect protection in nonvaccinated individuals.

Based on these results, a study was conducted to confirm if similar herd protection would be found in different populations. Both direct and indirect vaccine effectiveness after mass oral cholera vaccination was demonstrated in Zanzibar, an archipelago located off the eastern coast of Africa that has experienced several outbreaks of cholera [Khatib *et al.* 2012]. In this high-risk population, the vaccine was found to confer herd protection as shown by decreasing risk for cholera among nonvaccinated residents in association with increasing vaccine coverage.

Herd protection occurs presumably due to decreased transmission of cholera in the community as more individuals are protected by the vaccine. Longini and coworkers used the same vaccine trial dataset from Bangladesh [Clemens *et al.* 1988] to construct a simulation model of varying vaccine coverage levels and cholera illness [Longini *et al.* 2007]. Their results showed that 50% OCV coverage in this population with high levels of immunity would lead to an 89% reduction in cholera cases even among the unvaccinated and a 93% reduction overall in the entire population.

Dukoral has not been shown to protect against cholera caused by *V. cholerae* serogroup O139 or other species of *Vibrio*. In addition, it has not been routinely adopted for public health use due to its high cost, limited duration of protection and logistic issues with vaccine administration.

Killed WC (monovalent and bivalent) vaccine ORC-vax

Encouraged by the positive results of the cholera vaccine trial in Bangladesh, Vietnamese scientists worked on developing a similar vaccine for use in Vietnam. Following technology transfer from Swedish scientists, scientists at Vietnam's National

Institute of Hygiene and Epidemiology developed a killed OCV containing *V. cholerae* O1 cells without the B subunit. This vaccine underwent field testing in an open trial in Hue. During an outbreak of *V. cholerae* O1 El Tor Ogawa that occurred 8–10 months after vaccination, the vaccine was shown to have an efficacy of 66% in individuals older than 1 year. No difference in protection in younger age groups was detected [Trach *et al.* 1997]. In 1992, following the emergence of O139 in India and Bangladesh, the vaccine was modified to include killed *V. cholerae* O139 cells. The bivalent vaccine was shown to be safe and immunogenic in children and adults in Vietnam [Trach *et al.* 2002] and to confer 50% protection 3–5 years after vaccination [Thiem *et al.* 2006].

Used widely in Vietnam since 1997, the vaccine is part of the national Expanded Programme for Immunization, according to which the vaccine is given to children aged 2–5 years residing in high-risk cholera areas. Furthermore, the vaccine has also been used as a preventive measure during cholera outbreaks [Khiem *et al.* 2003; Anh *et al.* 2011]. During the 2007–2008 cholera outbreaks in Vietnam, the vaccine was given to residents of two districts in Hanoi. The vaccine conferred 76% protection against cholera in this case–control study, suggesting that the vaccine may be protective even after an outbreak has begun.

Licensed and produced locally in Hanoi as ORC-vax by the Company for Vaccine and Biological Production (VaBiotech, Hanoi, Vietnam), this vaccine offered several advantages. Since the bivalent OCV, containing killed WCs of both *V. cholerae* O1 and O139 serogroups, does not include the rCTB, coadministration of oral buffer is not required, making it easier to administer. Furthermore, its manufacturing technology is simple enough that it can easily be transferred to other developing country vaccine manufacturers. WHO does not recognize Vietnam's National Regulatory Agency (NRA), therefore vaccines produced in Vietnam cannot be WHO prequalified. WHO prequalification is required for vaccines to be purchased by United Nations agencies such as the United Nations Children's Fund (UNICEF). Thus, to enable WHO prequalification, the vaccine was modified to ensure compliance with international standards [Anh *et al.* 2007] and its manufacturing technology was transferred to a developing country manufacturer, Shantha Biotechnics (Hyderabad, India; acquired by Sanofiaventis in 2009).

Reformulated killed bivalent WC vaccine mORC-Vax/Shanchol

The reformulated vaccine underwent safety and immunogenicity trials among adults aged 18–40 years in SonLa, Vietnam and was found to be safe and elicited significant serum vibriocidal titers to O1 and O139 among 91% and 11% of vaccine recipients respectively [Anh *et al.* 2007]. The lower than expected rise in titers may have been due to the higher baseline titers as a result of exposure to bacteria with cross-reacting antigens to O139, methodological differences in the assay used for O139 or due to the presence of capsular polysaccharides in *V. cholerae* O139 that may interfere with the immune response and detection of serum vibriocidal antibodies. Finally, while vibriocidal antibodies have been used as a predictor of protection against *V. cholerae* O1, their utility in O139 is debated [Anh *et al.* 2007]. This vaccine underwent phase II and III trials in Kolkata, India (see below) and was subsequently licensed in February 2009 as mORC-Vax.

To internationalize the reformulated killed bivalent WC vaccine, clinical trials were conducted in India to obtain licensure from the Indian NRA, the Drugs Controller General of India, which is recognized by WHO. Licensure in India will allow the vaccine to be WHO prequalified. Phase II trials in India confirmed the vaccine's safety and immunogenicity among adults and children, eliciting significant vibriocidal responses in 53% and 80% of adults and children respectively. Similar to Vietnam, less pronounced immune responses were elicited against O139.

This paved the way for the conduct of a large cluster-randomized trial in Kolkata, India with a trial population of 107,774 people aged 1 year and older living in 3933 clusters [Sur *et al.* 2009, 2011; Bhattacharya *et al.* 2013]. Efficacy following a two-dose regimen given 14 days apart was measured by determining protection against culture-confirmed *V. cholerae* O1 diarrhea severe enough to require treatment in a healthcare facility. The vaccine was shown to provide 67% protection in all age groups at 2 years, which remained at 65% up to 5 years following vaccination. Although statistically not significant, PE was shown to vary by age group, with lower protection noted in children aged 1 to less than 5 years (PE = 42%) compared with those older than 5 years (PE = 68% in those aged 5–15 years and PE = 74% in those older than 15 years). While this suggests that the vaccine might not provide equivalent levels of protection

to younger children, the study was not powered to assess these differences in protection by age group. Further studies to confirm this finding needs to be conducted.

In February 2009, this vaccine was licensed in India as Shanchol (Shantha Biotechnics, Hyderabad, India) and WHO prequalification was attained in November 2011. Further safety and immunogenicity studies were conducted in Bangladesh that showed comparable results to those seen in Kolkata. Plasma IgA antibody responses to O1 Inaba LPS were detected among vaccinees in all age groups [Saha *et al.* 2011]. A large community-based study is currently being conducted in urban Dhaka with a target population of around 172,754 comparing the effectiveness of vaccine, vaccine plus safe water and hand-washing practice, and no intervention [Khan *et al.* 2013]. The results from this study are expected to be released this year.

The current recommendation for administration of the bivalent killed OCV is as a two-dose regimen for individuals aged 1 year and older, and does not require an oral buffer. At present, repeat vaccination is recommended every 2 years, but data obtained after 5 years of follow up showing longer-term efficacy suggest a longer term interval may be sufficient, at least among those aged 5 years and older [Bhattacharya *et al.* 2013].

Herd protection. Similar to Dukoral, this vaccine was shown to provide indirect protection 3 years after vaccination in the same trial in Kolkata, India. Using GIS, indirect (herd) protection of the bivalent killed OCV was demonstrated among residents in Kolkata, India by evaluating the association between two-dose vaccine coverage among populations residing within 250 m of the household and the occurrence of cholera in the defined population. The total (direct and indirect) vaccine protection was 66% and overall vaccine protection among all individuals living in the cluster was 49%. In neighborhoods with higher coverage level, herd protection was observed, presumably due to reduced transmission in the area, leading to a low incidence in the unimmunized segment of the population [Ali *et al.* 2013].

Oravacs

Licensed only in China and the Philippines, Oravacs (Shanghai United Cell Biotechnology, Shanghai, China) comes in an enteric coated capsule. Its contents include rBs as well as killed WCs of *V. cholerae* O1, almost chemically identical to Dukoral [Frew *et al.* 2009] (Table 1). Animal models [Liu *et al.* 1994] and safety and immunogenicity data [Chen *et al.* 1996; Xiong *et al.* 1998] are available in the Chinese literature, however no efficacy assessment is currently available.

Current use of killed OCVs

OCV has been available for more than two decades but its use has largely been confined to travelers from developed countries going to cholera at-risk areas. Aside from Vietnam, populations who are at most at risk do not have the option to be vaccinated under their countries' public health system. For example, during the large cholera outbreak in Haiti, various issues and obstacles were used to defend a decision not to vaccinate soon after the outbreak started [Date *et al.* 2011] but not without some dissent [Ivers *et al.* 2012; Von Seidlein and Deen, 2012]. Some of the challenges identified in the use of killed OCVs in public health settings in developing countries are listed in Table 3.

The arguments used against vaccination are not insurmountable, including the use of OCV in times of outbreaks. Indeed, Doctors without Borders (MSF) conducted another campaign in refugee camps and communities in South Sudan with around 132,000 people using Shanchol [MSF, 2013] and the Haitian Ministry of Health vaccinated around 80,000 people [Vicari *et al.* 2013]. Although these developments are welcome, there is limited capacity for vaccine production. Currently, there are only two WHO prequalified vaccines. Vietnam's NRA need to be recognized by WHO before Vabiotech can apply for WHO prequalification. It is unclear if Oravacs will be licensed elsewhere, but currently this vaccine caters for the travel market and has been procured by the Chinese government following the earthquake in western China in 2008 [Frew *et al.* 2009]. New manufacturers may be needed to keep up with expected demand as a cholera vaccine stockpile has been established. Following the WHO resolution for strengthened cholera surveillance and control efforts, this cholera vaccine stockpile holds 2 million doses and may be accessed by countries primarily as an epidemic response [WHO, 2012] together with established measures for cholera control. This stockpile is managed by the OCV International Coordinating Group for vaccine provision and is composed of

Table 3. Some challenges identified with the public health use of oral cholera vaccines (OCVs) in developing countries.

Challenges identified in the use of OCVs in developing countries	Comments
Dukoral is expensive for public health use in developing countries	There is another OCV, Shanchol priced at $1.85 per dose if procured through United Nations agencies
OCV is perceived to confer only modest protection that is short lived.	OCV provides indirect protection to those who have not been vaccinated in the community, provided that high enough vaccine coverage is achieved [Ali *et al.* 2005, 2013; Khatib *et al.* 2012]. Recent data shows the vaccine is protective up to 5 years following vaccination [Bhattacharya *et al.* 2013]
Administration of OCV is complex: • Dukoral requires a buffer • Two-dose schedule requires that the same individuals have to be vaccinated twice. This may be difficult to achieve in a mobile population	• Shanchol and mORC-Vax do not require buffer, simplifying administration. • Experience in Guinea [Ciglenecki *et al.* 2013; Luquero *et al.* 2013] and Haiti [Ivers *et al.* 2013] showed >79% two-dose vaccine coverage and compliance in the communities
Huge volume required for cold chain: • Vaccine comes in single dose vials that use up space	• New vaccines included in the Expanded Programme on Immunization also come in single-dose preparations that require space • Efforts are being made to develop more efficient containers that are easier to use in the field and require less space
Some public health workers expressed fears that the vaccination will interfere with treatment of the sick, water, sanitation and hygiene (WASH), traditional and long-term strategies used to control cholera and other diarrheal diseases	Mass campaigns in Haiti [Ivers *et al.* 2013; Rouzier *et al.* 2013] and Guinea [Ciglenecki *et al.* 2013] documented that WASH initiatives can be enhanced during vaccination
The relatively long interval between intake of vaccines and onset of protection makes OCV a less viable protective strategy	Studies showed that a reduction in cholera cases and deaths due to cholera might be averted if vaccines were used once a cholera outbreak has begun [Anh *et al.* 2011; Reyburn *et al.* 2011]. It was feasible to mount a campaign in Guinea in a short time after cholera cases appear with >75% two-dose vaccine coverage [Ciglenecki *et al.* 2013; Luquero *et al.* 2013, 2014].

representatives from MSF, the International Federation of Red Cross and Red Crescent Societies, UNICEF and WHO.

Finally, while a less expensive yet effective cholera vaccine is now available, a single dose formulation of Shanchol that provides protection is important, especially for outbreak control. Based on immunogenicity studies conducted in Kolkata, it is feasible that in cholera-endemic areas, a single dose of Shanchol may be protective against the disease [Kanungo *et al.* 2009]. This study, assessing the protection conferred by a single dose of Shanchol, is expected to commence soon in Bangladesh.

Conclusion

The past decade has seen an increase in the number and magnitude of cholera outbreaks. The addition of cholera vaccines to the established armamentarium against this ancient disease will be important to reduce cholera morbidity and mortality. Recent data show that the vaccine is safe, feasible to use even in difficult circumstances and provides protection in various settings. Countries with ongoing cholera transmission should consider its use in their public health programs, identifying areas and target populations who will benefit from the use of the vaccines. Strategies that will facilitate accessibility and usage of these vaccines will need to be developed.

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Conflict of interest statement

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