Oral Vaccines Against Cholera

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The current seventh pandemic of cholera, caused by serogroup O1, El Tor biotype, has now involved almost the entire developing world. The ongoing dynamic epidemiology of cholera, involving evolution of new strains, prolonged and more frequent epidemics, increased antimicrobial resistance, and awareness of the role of climate change upon the global burden has returned cholera to the forefront of global public health discussions. Improved water and sanitation should continue to be the mainstays of cholera-prevention efforts, but major improvements are a far-off goal for much of the cholera-affected developing world. The advent of safe and effective, new-generation oral vaccines against cholera has created renewed interest in the use of vaccines as a tool to control cholera.

Cholera remains an important global health challenge, particularly in resource poor countries in Asia and Africa. This rapidly dehydrating diarrheal disease, caused by O1 and O139 serogroups of the bacterium *Vibrio cholerae*, is transmitted primarily by contaminated water or food and has the ability to spread quickly, with case fatality rates in excess of 20% without appropriate treatment, but with a reduction to <1% with proper rehydration [1]. Modern, licensed vaccines against cholera are given orally. This article reviews currently available oral cholera vaccines (OCVs), as well as candidate vaccines that are in the pipeline.

EPIDEMIOLOGY AND RECENT TRENDS

More than 200 *V. cholerae* serogroups exist, classified by the O antigen. Of these, O1 causes over 98% of cases of cholera globally, with a small percentage of cases in Asia due to O139 [2]. Pathogenic *V. cholerae* have two biotypes—El Tor and classical, which both can be further classified into 2 serotypes—Ogawa and Inaba. Cases due to new variant strains of El Tor, expressing a toxin similar to that produced by classical biotype strains, have recently emerged in Africa and Asia and appear to be more severe. Moreover, an increase in the prevalence of antibiotic resistance has been noted, complicating clinical management [3].

In 2009, the World Health Organization (WHO) reported 221,226 cases and 4946 deaths from 45 countries: 98% of cases and 99% of all deaths were reported in Africa alone (Figure 1) [4]. Considering substantial under-reporting of cholera, especially in Asia, ~3 million cases and 120,000 deaths are estimated to occur annually due to cholera [5]. Although cholera can strike any age group, children <5 years of age are at greatest risk in settings where the disease is endemic [6].

Cholera outbreaks can be devastating, as seen in the Goma refugee camps of Zaire (1994), where 70,000 cases and 12,000 deaths were recorded [7]. The large Zimbabwean outbreak of 2008/2009 was responsible for 30% of the globally reported cases [4]. In 2010, cholera outbreaks in Cameroon, Chad, Niger, Nigeria, and Pakistan accounted for over 40,000 cases and ~2000 deaths; the recent Haitian epidemic added an additional toll of >194,000 cases and 3819 deaths as of 16 January 2011 [8, 9]. With cholera incidence tending to rise in warmer environmental temperatures, global climate change has been suggested as a factor that may increase the global burden of cholera over time [10].

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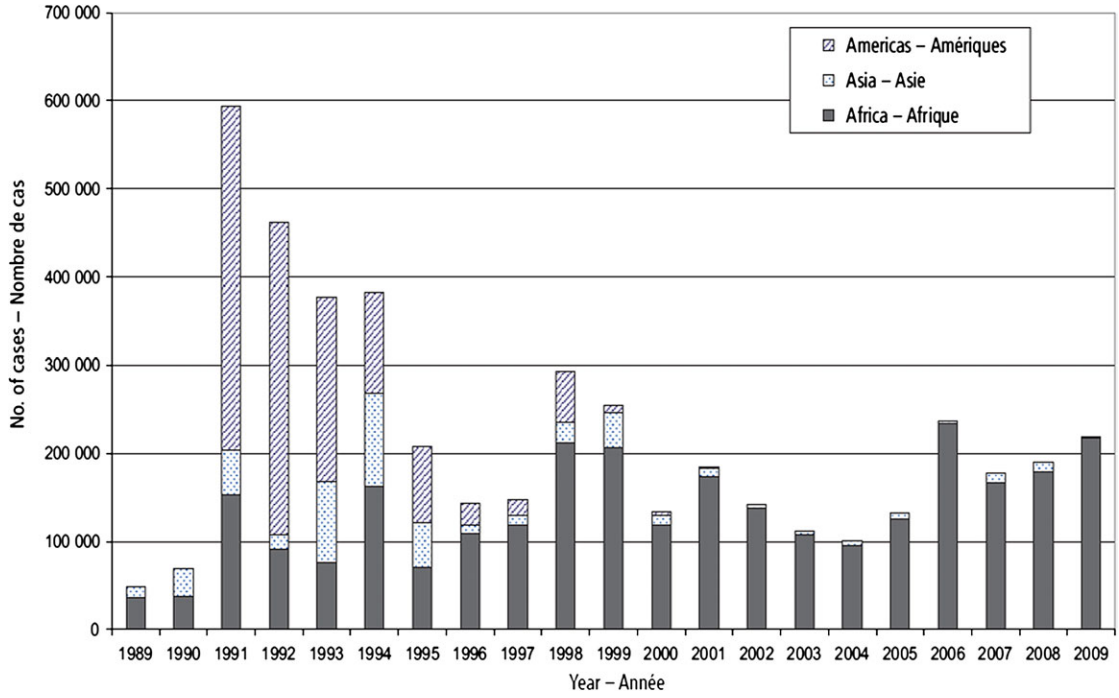

Figure 1. Number of cholera cases reported to the World Health Organization by year and continent, 1989–2009. Used with permission from the World Health Organization [4].

CLINICAL PATHOPHYSIOLOGY, VIRULENCE, AND IMMUNE RESPONSE

Cholera infection requires the colonization of the small intestine by vibrios, and pathogenicity is mediated primarily by the 2-subunit cholera toxin. The B subunit binds the bacteria to the epithelial cell surface, stimulating an immune response, but having no toxic effect. When the A subunit is released, it stimulates a cellular biochemical cascade causing active secretion of water and electrolytes and leading to watery diarrhea, which can result in severe dehydration and death [11].

Humans mount both a systemic and a local mucosal immune response following challenge or infection with cholera vibrios, which is capable of producing immune protection against subsequent disease due to serogroup-homologous organisms [12]. Although both serogroups O1 and O139 can elicit serum antitoxin responses, infection with one serogroup has not been shown to offer cross protection against the other [13]. In areas of endemicity, serum vibriocidal antibody titers have been noted to increase with age and to be inversely related to the risk of developing cholera [14, 15]. Although these antibodies are conventionally used to measure immune responses to cholera vaccines, they are not likely to be immune mediators of protection.

ORAL CHOLERA VACCINES

Injectable, killed whole-cell (WC) cholera vaccines date back virtually to the discovery of the cholera vibrio in the nineteenth century. These vaccines fell from favor in the 1970s because they were found to confer low levels of efficacy of short duration and to have an unfavorable safety profile [16]. Currently, these vaccines are not recommended for use.

Attention shifted from parenteral to oral vaccines against cholera with the recognition that protective immunity against cholera results primarily from local, mucosally secreted intestinal antibodies and that oral presentation of antigens is an efficient method of eliciting intestinal mucosal immune responses. In comparison with parentally delivered vaccines, oral vaccines are easier to administer, more acceptable to recipients, and have a reduced risk of transmitting blood-borne infections [17]. There are 2 major types of oral vaccines against cholera: killed WC-based and genetically attenuated live vaccines.

LICENSED KILLED WHOLE CELL-BASED ORAL VACCINES

Killed Whole Cell Vaccine With Cholera Toxin B Subunit (Dukoral)

[Table 1, 18] A killed WC vaccine with cholera toxin B subunit vaccine (WC-rBS), produced by Crucell/SBL Vaccines since 1991 and sold as Dukoral, consists of a mixture of killed WCs of both the El Tor and classical biotypes and the Ogawa and Inaba serotypes of *V. cholerae* O1, along with recombinant B subunit of cholera toxin. The vaccine is licensed for persons

Table 1. Licensed Oral Cholera Vaccines

Variable	WC-rBS	WC-only	Modified WC-only	CVD 103-HgR
Trade name	Dukoral	ORC-Vax	Shanchol or mORC-Vax	Orochol or Mutacol
Live or killed	Killed	Killed	Killed	Live
Target	O1 classical and El Tor	O1 classical and El-Tor; possibly O139 (no clinical evaluation to date)	O1 classical and El-Tor; possibly O139 (no clinical evaluation to date)	O1 classical and El Tor
Regimen	2 doses given 7–42 days apart (3 doses for children 2–5 years of age)	2 doses given at least 14 days apart	2 doses given 14 days apart	1 dose
Duration of protection	2 years (6 months for children 2–5 years of age)	≥3 years	≥3 years	≥ 6 months (established only in North American volunteers)
Booster dose requirements	Every 2 years (every 6 months for children, 2–5 years of age)	Every 2 years	Every 3 years (may be longer after further evaluation of Kolkata trial)	Unknown
Age range for vaccination	>2 years	≥1 year	Shanchol: ≥1 year; mORC-Vax: ≥2 years	>2 years
Requirement for oral buffer	Yes	No	No	Yes
Storage temperature	2–8°C	2–8°C	2–8°C	2–8°C
Shelf life	3 years	2 years	2 years	2 years
International acceptance	WHO prequalified	Not prequalified by WHO	Pending WHO prequalification	Not prequalified by WHO
Price to the public sector per dose	~$5.25	$0.75	Shanchol: $1.85 or less depending on volume mORC-Vax: ~$0.75	Vaccine not currently available

NOTE. WC, whole cell. Adapted from [18]. WHO, World Health Organization.

≥2 years of age, requiring 2 doses for adults and children ≥6 years of age, and 3 doses for children ≤5 years of age. Because the B subunit is structurally altered by gastric acid, the vaccine requires coadministration with a liquid buffer. Prior to administration, clean water should be added to the buffer and mixed with the liquid vaccine, which needs to be kept cold.

Two earlier versions of WC vaccines, either with or without B subunit produced via chemical extraction, respectively, were tested in Matlab, Bangladesh, in the mid-1980s. In this trial, WC-BS vaccine was found to be safe and provided 85% protection for 4–6 months after vaccination, 62% protection at 1 year, and 58% protection at 2 years [19]. Protection in children <6 years of age was 100% for the first 4–6 months but decreased rapidly thereafter. Detailed analyses suggested that protection by 2 doses of vaccine was equivalent to protection by the complete 3-dose regimen [19]. The vaccine also provided short-term protection against diarrhea due to enterotoxigenic *E. coli* (ETEC) that produce heat-labile enterotoxin [20].

Later, the production technology of WC-BS vaccine was modified: B subunit was prepared by recombinant genetic technology (WC-rBS). This vaccine was tested in multiple clinical trials in Peru in the 1990s. A trial in a cohort of adult military volunteers confirmed that the vaccine confers high-grade protection (86%) against El Tor cholera in the short term [21]. Another trial, performed in the general population, failed to find protection during the year after a 2-dose regimen but observed that a single booster dose given a year after the primary regimen elicited robust protection [22]. Because of methodological problems with the latter trial, a 2-dose regimen of WC-rBS has been licensed internationally on the basis of the other cited trials [23]. A 2-dose regimen of WC-rBS was administered in a mass vaccination program in 2003 and 2004 in Beira, Mozambique, and was found to confer 84% protection to all persons aged ≥2 years and 82% protection to children vaccinated at <5 years of age [24].

Although WC-rBS has been prequalified by the WHO for purchase by the UN, it has mainly been used as a travelers' vaccine, primarily due to the high price of the product.

Vietnamese Killed Whole Cell-Only Vaccine (ORC-Vax)

The Bangladesh trial of killed OCVs, described above, included a group randomized to receive a 3-dose regimen of a vaccine

largely identical to WC-rBS but lacking BS. The trial found that this WC-only vaccine conferred moderate (58%) short-term (4–6-month) protection against cholera but that protection was sustained at ~60% for 2 years and was still present (42%) during the third year of follow-up [19]. Based on these data, the government of Vietnam embarked on local production of an oral WC-only vaccine in the late 1980s for use in its public health programs. In an open labeled trial in Hue, Vietnam, a 2-dose regimen of locally produced WC vaccine provided 66% protection to subjects ≥1 year of age for 8–10 months after vaccination [25]. After adding killed O139 cholera vibrios to the vaccine, the Vietnamese producer, VaBiotech, licensed this vaccine as a 2-dose regimen for persons ≥1 year of age, under the trade name ORC-Vax; a later case-control study observed 50% protection against El Tor cholera for 3–5 years after dosing with this vaccine [26].

The Vietnamese vaccine differs from the WC-rBS vaccine in several important aspects. Because it does not contain cholera toxin B subunit, it does not elicit antitoxic immunity, nor does it require coadministration with buffer. It is also less expensive to manufacture. Approximately 20 million doses of this vaccine have been administered in public health programs in Vietnam. However, for reasons described below, this vaccine has been replaced by a substantially modified, bivalent oral WC-only vaccine called mORC-Vax.

Modified Killed Whole Cell-Only Vaccines (mORC-Vax; Shanchol)

With the goal of accelerating the global use of low-cost killed OCVs, the International Vaccine Institute, in cooperation with VaBiotech, has made substantial modifications to the Vietnamese WC vaccine. Revision of constituent strains and production methods were necessitated by several issues noted with the earlier version of the Vietnamese vaccine (ORC-Vax): 1) production methods were not adaptable to international Good Manufacturing Practices, 2) standardization tests were not in compliance with WHO recommendations, and 3) the vaccine was found to contain residual cholera toxin. To address these issues, a new bivalent (O1/O139) vaccine has been created in which a high toxin-producing strain (classical Inaba 569B) has been replaced by 2 alternative strains: heat-killed classical Inaba Cairo 48 and formalin-killed classical Ogawa Cairo 50. LPS content has been doubled, and modern quality control and release assays are used, including one to verify the absence of cholera toxin in the final product [27].

Several trials have evaluated a 2-dose regimen of this modified WC vaccine. The vaccine was shown to be safe and highly immunogenic against *V. cholerae* O1, with seroconversion rates of vibriocidal antibodies of 91% among adults in Vietnam [28], 53% among adults in Kolkata, and 80% among children aged ≥1 year of age in Kolkata, where high background immunity exists [29].

A phase III trial of the vaccine among ~70,000 adults and children ≥1 year of age in slum areas of Kolkata, India, found that, during 2 years of follow-up, the vaccine conferred 67% protection against treated episodes of El Tor cholera [30]. Protection was sustained at this level during the third year, and surveillance continues. Interestingly, all cholera isolates detected in this trial exhibited the features of newly emergent modified El Tor cholera described earlier. Protection against O139 cholera was not evaluable.

On the basis of the results of the clinical trials cited above, the modified WC vaccine was licensed in Vietnam in early 2009 (mORC-Vax). However, the Vietnamese national regulatory authority (NRA) has not been recognized by the WHO, and therefore the vaccine could not be considered for international use.

To facilitate its acceptance in developing countries and enable its purchase by United Nations (UN) agencies, an emerging producer (Shantha Biotechnics) in India—a country with a WHO-approved NRA—was selected to be the recipient of the technology to produce the vaccine. This vaccine was licensed in India in February 2009 as a 2-dose vaccine for persons ≥1 year of age and is sold under the trade name Shanchol. It is expected that this vaccine may be WHO-prequalified in early 2011, which would enable purchase by UN agencies and wider implementation.

LIVE ORAL VACCINES

CVD 103-HgR (Orochol; Mutacol)

CVD 103-HgR, derived from the originally virulent classical O1 Inaba strain 569B via deletion of the gene for cholera toxin A subunit and insertion of a gene for mercury resistance, was the first live-attenuated OCV candidate to be licensed (Table 1). It was studied in multiple phase I and II trials involving >7000 subjects in Asia, Latin America, Africa, Europe, and North America that showed it to be consistently safe and immunogenic [31]. Several experimental challenge studies involving North American adult volunteers found a single dose of this vaccine to be protective [32]. These studies paved the way for an efficacy trial of a single-dose regimen in a cohort of 67,508 children and adults in a cholera-endemic setting in Indonesia. During 4 years of follow-up, however, no protection against cholera was detectable [33]. In contrast, a subsequent observational study of mass vaccination with this vaccine in a cholera outbreak setting in Micronesia found that vaccination was associated with 79% protection [34]. Because of the negative result in the phase III trial, the vaccine has never been licensed for use in settings of endemicity. However, the safety profile and protection observed in challenge studies led to its licensure under the trade names Orochol and Mutacol in 1993 as a travelers' vaccine. Production of this vaccine has since been suspended, but a US-based company has recently considered recommercialization of this product.

ORAL CHOLERA VACCINES UNDER DEVELOPMENT

Several experimental live oral candidate vaccines are under development. Peru 15 is a genetically attenuated *V. cholerae* O1 El Tor Inaba strain, originally isolated in Peru in 1991. A single-dose regimen of Peru 15 has been shown to be safe and immunogenic in the US volunteers, as well as in adults and toddlers in Bangladesh [35].

V. cholerae 638 is an attenuated O1 El Tor Ogawa strain that is being developed in Cuba. A single-dose regimen was shown to be immunogenic and protective in an experimental cholera challenge study in Cuban adults [36]. *V. cholerae* IEM 101 is an O1 El Tor Ogawa strain from China that naturally lacks the gene for cholera toxin and several other virulence factors. In human studies, it was found to be immunogenic with no adverse effects. Two additional derivatives—IEM 108 and 109—are also promising candidates, but no human data have been reported to date [37, 38].

Another interesting *V. cholerae* O1 candidate is the VA1.3 from India, which is a recombinant strain able to produce CTB but which is otherwise devoid of cholera toxin. This vaccine was found to be safe and immunogenic in adults in Kolkata [39]. Two recombinant live attenuated *Vibrio* O139 candidate vaccines, CVD 112 and Bengal 15, have been evaluated in volunteer trials, and they provided ~80% protection against challenge with wild-type O139 strains [40, 41].

PUBLIC HEALTH CONSIDERATIONS

When considering use of modern cholera vaccines, a distinction is made between endemic and epidemic cholera. Endemic cholera refers to cholera resulting from cholera vibrios that normally reside in the local environment. It tends to be predictably recurrent in time and space. In contrast, epidemic cholera denotes cholera that requires exogenous introduction of cholera vibrios into a population and is not recurrent in time and place. Although useful conceptually, these represent 2 extremes, and in practice, large outbreaks termed epidemics may occur in populations with endemic cholera.

For the control of epidemic cholera, selective vaccination of populations at a definably high risk of an epidemic or reactive vaccination shortly after the onset of the epidemic can be considered [42]. Because populations experiencing epidemic cholera often have limited background natural immunity to cholera, vaccines for epidemic cholera must be effective in immunologically naive individuals and should target all age groups, because the risk of epidemic cholera tends to be age-independent.

For endemic cholera, vaccines should be able to immunize in the face of the background natural immunity to cholera that develops in recurrently exposed populations. Vaccination may target pre-school and school-aged children rather than adults in view of the higher risk of cholera in younger age groups in settings of endemicity. Long-term protection is more critical than is early onset of protection after initiation of dosing for vaccines against endemic cholera. In contrast, early onset of protection after the first dose of vaccine would be of greater importance for vaccines used reactively in epidemic situations, and duration of protection would be of lesser importance [43].

A common fallacy has been the assertion that the vaccine protective efficacy must be very high in order for vaccines to be useful against cholera. For currently available killed WC-based OCVs, significant vaccine herd protection of nonvaccinated individuals has been demonstrated, even in areas of modest vaccine coverage [44]. Mathematical models based on these data suggest that, when vaccinating over half of the population in an area of cholera endemicity, incidence can be reduced by 93% due to the vaccine's ability to induce herd protection [45].

Logistical feasibility and cost-effectiveness are additional factors that must be considered in decisions regarding the use of OCVs. Although there are operational challenges in implementing a vaccination campaign requiring a 2-dose vaccine regimen, demonstration studies have shown that it is feasible to use these vaccines in settings of endemicity in Vietnam and Mozambique [46, 47], as well as in refugee camps and during complex emergencies [48, 49]. A number of cost-effectiveness analyses of the use of oral cholera vaccines have been conducted for populations in both endemic and nonendemic areas. Analysis of the preemptive use of WC-rBS vaccine in refugee settings, for example, found the net cost per disability-adjusted life years averted to be US $269, which was considered to be "very cost effective" by the World Bank [50]. Additional studies of the modified WC-only OCV found the vaccine to be very cost effective in urban Kolkata, India, and in Beira, Mozambique, but not in low-incidence populations, according to the same criteria [51]. The modified killed WC vaccine addresses many logistical constraints that are major barriers to the use of cholera vaccine in resource-constrained areas.

Although the provision of clean water and adequate sanitation remain mainstays of cholera control, in view of the current availability of OCVs that are safe and effective, the WHO has issued an updated recommendation that states that vaccination should be used as a tool to help control endemic cholera and shall be considered for use in epidemics [52]. Development of a global stockpile of cholera vaccine offers an attractive mechanism for introduction of OCVs following these new recommendations [53].

CONCLUSION

The WHO's recent, strengthened recommendation on the use of OCVs provides an important impetus for greater use of these

vaccines in the control of endemic and epidemic cholera. The availability of a safe, effective, feasibly delivered, and affordable oral vaccine that can be used in resource- limited regions, together with a pipeline of newer candidate vaccines that may be deployable in single-dose regimens in the future, should facilitate the use of vaccines in the public health armamentarium against cholera. Although much has happened to narrow the gap of accessibility to a cholera vaccine for the world's poorest people, an international concerted effort is now needed to make the promise of such a vaccine a reality.

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