The Compositions and Protective Efficacies of the Oral Killed Cholera Vaccines: A Critical Analysis

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ABSTRACT

Two cholera vaccines, sold as Shanchol and Dukoral, are currently available. This review presents a critical analysis of protective efficacies of these vaccines. Children under 5 are very vulnerable to the disease, and account for the highest incidence of cholera cases and more than half of the mortality. Both Shanchol and Dukoral are spaced two dose oral vaccines comprising large numbers of killed cholera bacteria. The former contains *Vibrio cholerae* O1 and O139 cells, and the latter contains *V. cholerae* O1 cells with the recombinant B subunit of cholera toxin. In a field trial in Kolkata (India), Shanchol, the preferred vaccine, offered protection of 45% in all age groups and only 17% in children under 5 during the first year of surveillance. In a field trial in Peru, two spaced doses of Dukoral offered negative protection in children under 5 and little protection (15%) in vaccinees above 6 during the first year of surveillance. Little is known on Dukoral’s long term protective efficacy. Both the vaccines have questionable composition, using *V. cholerae* O1 strains isolated in 1947 that have been inactivated by heat and formalin treatment which may denature protein. Immunological studies revealed Dukoral’s reduced and short-lived efficacy, as measured by several immunological endpoints. Various factors such as necessity for multiple doses, poor protection in children under 5, requirement of cold chain, production cost and complex logistics in vaccine delivery greatly reduce the suitability of either of these vaccines for endemic or epidemic cholera control in resource poor settings.
INTRODUCTION

Cholera is an acute intestinal infection caused by the Gram-negative bacterium *Vibrio cholerae* that colonizes the small intestine without invading the epithelium. Ingestion of food and/or drinking water contaminated with *V. cholerae* can cause the disease that is often mild or asymptomatic, but sometimes can be severe. The disease, only affecting humans, is mediated by cholera toxin (CT) secreted by *V. cholerae* in the intestine which acts upon the mucosal cells of the gut causing a copious, painless, watery diarrhea that can lead to severe dehydration and shock. If untreated, death can occur within hours. Cholera, a social disease arising out of poverty and lack of basic sanitation, currently prevails in parts of Asia, Africa and Latin America. Although cholera outbreaks used to occur in the past in Europe and the U.S.A, the disease has been essentially eradicated there through effective sanitation and public health measures (1).

Although more than 200 serogroups of *V. cholerae* have been identified, the majority of cholera is caused by two serogroups, O1 and O139 (2). *V. cholerae* O1 has two biotypes (classical and El Tor), each of which is further subdivided into two serotypes (Ogawa and Inaba). Both *V. cholerae* O1 and O139 secrete similar CT, but they differ in the composition of surface components as *V. cholerae* O139 produces a polysaccharide capsule (3, 4). Hence, previous exposure to *V. cholerae* O1 does not confer immunity to attacks by *V. cholerae* O139. Outbreaks due to *V. cholerae* O139 occurred first in India in 1992, then in neighbouring countries in the following years but have been rarely reported during the last decade (5, 6).

Antibodies to various cholera antigens such as lipopolysaccharide (LPS), outer membrane proteins, CT and the major subunit of the toxin-coregulated pilus (TcpA) have been detected in sera from individuals immunized with *V. cholerae* O1 or from convalescent patients (7–11). *V. cholerae* O1 infection in cholera patients induces both memory B and T
cell responses (12-14). Although intestinal lavage and human blood have been used to study immune responses, these materials may not correspond to the actual level of immunoglobulins in the gut after an antigenic stimulus (15, 16). Ethical considerations can limit a detailed investigation of the immune responses occurring in the gut of cholera patients. However, a thorough study of immune responses is possible in experimental animals such as rabbits (17, 18). A single-dose intraduodenal inoculation of live \textit{V. cholerae} O1 in rabbits produced antibodies to both somatic (LPS and cell surface proteins) and secreted (cholera toxin and neuraminidase) antigens in various body fluids (sera and bile) and intestinal extracts of rabbits, the latter containing predominantly IgA together with considerable amount of IgG (18). Studies on volunteers from the USA, who were orally immunized and subsequently challenged with live \textit{V. cholerae} O1, demonstrated that cholera infection can induce a high degree of protection for up to 3 years against challenge with either Ogawa or Inaba serotype belonging to the same biotype (19).

\textbf{Cholera vaccines:}

In 1883 Robert Koch identified \textit{V. cholerae} O1 as the aetiological agent of cholera (20). Soon afterward parenteral cholera vaccines were used in humans by the Spanish physician Ferran who also introduced the concept of mass oral immunization with live \textit{V. cholerae} O1 through drinking water supplies (21). Parenteral killed cholera vaccines had been used since then until the 1970s and afterward discarded as these vaccines offered protection for limited duration, lasting up to six months only (22). Since the 1980s, oral cholera vaccines (OCVs) comprising either killed or live cells have been used. Of these, the following oral vaccines have been subjected to large scale field trials: killed whole \textit{V. cholerae} O1 cells (WC), WC with the B subunit of cholera toxin (CTB) isolated from culture superanatant (WC-CTB), WC with the CTB prepared by recombinant DNA technology (WC-rCTB), WC with the killed \textit{V. cholerae} O139 cells and live attenuated \textit{V. cholerae} O1 cells (CVD-103 HgR) (23-30).
Only two vaccines, WC-rCTB and WC with *V. cholerae* O139, sold commercially as Dukoral and Shanchol respectively, are currently available and have been prequalified by the World Health Organization (WHO) (31, 32). As cholera is a public health problem for the poor people of various regions of the world, a critical analysis of the compositions and protective efficacies of the currently available OCVs is essential as many questions related to their compositions and protective efficacies remain unanswered. Of these two vaccines, Shanchol is now preferred for mass vaccination against cholera (33-35) and will be discussed first. There are an estimated 3-5 million cholera cases and 100,000–120,000 deaths worldwide per year with children under 5 accounting for the highest incidence of cholera and more than half of the mortality (36). Results presented in this review demonstrate that neither Shanchol nor Dukoral can offer effective protection against endemic or epidemic cholera, especially among the children under 5, the group most vulnerable to cholera.

**A. Shanchol**

Shanchol is the trade name of a candidate OCV comprising large amount of two groups of killed cholera bacteria (*Vibrio cholerae* O1 and O139), manufactured by Shantha Biotechnics of India, a subsidiary of French pharmaceutical company Sanofi-Aventis (28, 29, 32). It is a two-dose oral vaccine to be taken with a minimum interval of two weeks; immunity against cholera is expected to appear 7-10 days after the second dose (28, 32). This vaccine has been primarily developed by a group of scientists from Sweden and South Korea, its initial studies being carried out in Vietnam (37). As the national regulatory authority of Vietnam was not recognized by WHO, the study was continued in India, the latter’s regulatory authority meeting WHO’s requirements for global marketing (28). In 2006 the vaccine was subjected to a large scale field trial in an impoverished cholera endemic area of Kolkata (India) and the...
results of the vaccine’s performance during the subsequent years have been published (23, 28, 29). WHO has prequalified the vaccine on September 29, 2011 (32).

A cholera epidemic caused by *Vibrio cholerae* O1 (El Tor, Ogawa) had struck Haiti in October 2010 with catastrophic consequences claiming in the first 3 years and 5 months (i.e., through March 10, 2014) 8,546 lives and sickening more than 700,541 people (38, 39). Shanchol was used in 2012 in a pilot study to demonstrate the feasibility of mass vaccination in urban and rural Haiti (33, 34). As the study was not aimed to monitor Shanchol’s effectiveness, information on its protective efficacy against cholera in Haitian population remains unknown. Although the future use of this two-dose vaccine has been proposed (33-35), concerns on the operational and logistic challenges regarding its deployment in cholera outbreaks have been raised (40). Several aspects of this vaccine related to its composition and protective efficacy require in-depth examination.

**Composition of Shanchol:**

Shanchol comprises four strains: one *V. cholerae* O139 (4260B) and three *V. cholerae* O1 [2 Classical (Cairo 48, Cairo 50) and 1 El Tor (Phil 6973)] strains. All are killed, some with heat and some with formaldehyde treatment (28, 32, 37, 41; Table 1). The vaccine contained a total of $1.75 \times 10^{11}$ cells [$7.5 \times 10^{10}$ cells from classical O1 strains, $5.0 \times 10^{10}$ cells from El Tor and $5.0 \times 10^{10}$ cells from O139 strain]. The content of *V. cholerae* strains in the vaccine, claimed to be prepared in conformity with WHO standards, has been expressed in ELISA Units of lipopolysaccharide (LPS) (28, 32, 37). The vaccine is reported to contain 300 ELISA Units of LPS from each of the three preparations of classical strains, 600 ELISA Units of LPS from the El Tor strain and 600 ELISA Units of LPS from O139 strain.
Vaccine participants:

This was a cluster randomized, double-blind, placebo-controlled trial carried out in 3933 dwellings with a total population of 107,774 (28). However, about one-third of them (n = 38,027) declined to take part in the program. Two spaced doses of the vaccine were administered to 31,932 persons excluding infants under the age of 1 and pregnant women. A placebo of heat killed *Escherichia coli* K12 was fed to 34,968 persons. Among the two-dose vaccine recipients, 71% were adults including those above 15 years of age, 22% in the age range of 5-15 years and 7% under 5. Not all members of the same dwelling took part in the trial.

Protective efficacy:

The trial only recorded severe cases of cholera requiring medical attention (23, 28). The vaccine’s efficacy against asymptomatic and mild cases was not evaluated. During the first year of surveillance the vaccine offered protection of 45% in all age groups, and only 17% in children under 5, the group most vulnerable to cholera (28, 29; Table 2, Figure 1). However, for unexplained reasons, the vaccine’s protective efficacy rose sharply in the following year to 77% in all age groups and to 81% in children under 5 (29). During the third year of surveillance, the vaccine’s protective efficacy was 65% and 37% in all age groups and in children under 5 respectively (29). In older children between 5-15 years, the vaccine had offered 81%, 92% and 89% protection for surveillance periods of 1, 2 and 3 years respectively (29). During the 4th and 5th year, the vaccine had offered 58% and 80% protection respectively in all ages, separate data for these years in children under 5, between 5-15 and persons above 15 were not reported (23). The vaccine’s cumulative protective efficacy during 5 years of surveillance was 65%, 42%, 68%, and 74% in all age groups, children under 5, between 5-15 and participants above 15 years respectively (23).
Concerns regarding the Shanchol vaccine:

There are several concerns regarding the vaccine’s composition, protective efficacy and reliability of the trial.

A. Vaccine composition:

i) The vaccine’s composition has been described in ELISA Units of LPS without defining what ELISA Units of LPS are and how they were derived from killed *V. cholerae* cells (28, 32, 37, 41). It is well established that the two groups of *V. cholerae* (O1 and O139) have LPS that differ in composition and amounts (42, 43). *V. cholerae* O139 possesses both LPS and capsular polysaccharide (CPS) present in the ratio of 1:2, LPS being the minor component (43). *V. cholerae* O1 does not possess CPS. Although, the definition of an LPS unit in these vaccine strains is unclear, it is an interesting coincidence that 5.0 x10^10 cells of *V. cholerae*, whether from *V. cholerae* O1 El Tor or *V. cholerae* O139, produced the same 600 ELISA Units of LPS (28, 32, 41; Table 1). Without a clear definition of the “ELISA units of LPS” used to quantitate the bacterial strains in the vaccine, it is extremely difficult to understand vaccine composition.

ii) The rationale for strain selection and method of killing (some strains with heat and some with formaldehyde) has not been provided. Importantly, the two classical strains (Cairo 48, Cairo 50) of the vaccine were collected from the Egyptian cholera epidemic of 1947. It is likely that these strains have undergone numerous transfers and variations since then. Further, information on the antigenic analysis of these strains after killing with heat or formaldehyde has not been provided, though it is known that these procedures can impact antigen expression.
Treatment with heat and formalin can denature the cell surface proteins of *V. cholerae* and alter the antigenic mosaic of these cells (44, 45). Formalin, a well known cross-linking agent, can modify cell surface proteins of *V. cholerae* by reacting with primary amino groups to form unstable products that can react further with several other amino acid residues to form stable methylene bridges (46, 47). Besides, formalin treatment of proteins has been reported to constrain antigen presentation to T cells (46). Therefore formalin treatment to produce a vaccine is less than ideal. A WC vaccine obtained by irradiation that only destroyed chromosomal DNA was reported to offer higher protection against challenges in rabbits than that offered by the heat – or formalin killed WC (45).

iii) *The vaccine’s emphasis on cholera due to classical O1 strains:*

The vaccine comprises three preparations of classical O1 strains preparations totaling $7.5 \times 10^{10}$ cells and only one preparation of El Tor strain containing $5.0 \times 10^{10}$ cells (28, 32, 41; Table 1). The cholera cases detected during the Kolkata trial and also in other recent epidemics such as in Haiti and Zimbabwe were due to the El Tor biotype (28, 38, 48). The rationale for enriching the vaccine with classical strain preparations is not apparent, and has not been provided.

iv) *Vibrio cholerae* O139 - *a component of questionable value:*

Although the vaccine contains a large proportion of killed *V. cholerae* O139 cells (29% of the total amount, $5.0 \times 10^{10}$ cells), field trials both in India and Vietnam revealed that *V. cholerae* O139 component of the vaccine induced weak immune responses; only 10% of adults and 27% of children under 18 seroconverted (37, 49). The vaccine’s protective efficacy against cholera caused by *V. cholerae* O139 remains to be ascertained as the cholera in Kolkata was caused by *V. cholerae* O1 [El Tor, Ogawa (28)]. It is worth noting that cholera outbreaks due to *V. cholerae* O139 had occurred mostly in South Asia in the 1990s and have
not been reported during the last decade (5, 6). Therefore, the inclusion of a large amount of killed V. cholerae O139 (1x10^11) cells in two doses of the vaccine is of questionable value.

B. Questions regarding protective efficacy:

More than 80% of people infected with toxigenic V. cholerae do not develop any symptoms (50). A smaller proportion of symptomatic cases develop mild to moderate diarrhea and only a small percentage develop severe cholera. As the Kolkata trial only recorded severe cases of cholera requiring medical attention, the vaccine’s efficacy against asymptomatic and mild cases was not evaluated. Asymptomatic carriers can still shed bacteria and play a vital role in disseminating infection (51). The effect of Shanchol in reducing the incidence of carriers remains unknown.

Evaluating the protective efficacy of a cholera vaccine by carrying out the trial on an already primed adult population in a heavily endemic area produces a biased picture of its efficiency (52). Knowing this, only children aged 0-14 were included in a few cholera vaccine trials in the 1960s in Bangladesh (53). But in the Kolkata trial of Shanchol, carried out in a heavily endemic area, the vast majority of participants (71%) were adults and older children above 15 with a high likelihood of exposure to cholera prior to vaccination (28). Therefore, results coming out of the Kolkata trial may not be applicable to the population of other countries who are still naive and not exposed to cholera antigens.

It is difficult to understand a mechanism by which the protective efficacy of a vaccine, monitored for several years, increases with time. However, in this trial the protective efficacy of Shanchol was very poor (only 17%) in the most vulnerable group (i.e., children under 5) during the first year of surveillance (29). Remarkably, it dramatically climbed to 81% in the following year (29, Table 2, Figure 1). The vaccine’s efficiency also increased from 45% to
In the 1985 oral cholera vaccine trial in Bangladesh, protection offered in children under 5 by the oral killed whole V. cholerae O1 cells (WC) progressively declined with time, protective efficacy being 31%, 24% and 2% during the first, second and third years respectively (26).

Compared to the Shanchol trial, even a single-dose parenteral classical bivalent (Ogawa and Inaba) whole-cell vaccine with aluminum adjuvant produced much better results in children under 5 in the field trials carried out in India and Indonesia in the 1970s (54, 55). In a large-scale field trial in Kolkata in 1975, the parenteral vaccine with aluminum adjuvant had offered 100% protection in children under 5 for 6 months, 89% for 12 months and 92% protection for 18 months (54). The overall protection rate in all age groups during the surveillance period of 1 year was 62%. Thus, the performance of two doses of Shanchol in the same city 30 years later was much inferior to that of the single-dose parenteral vaccine with aluminum adjuvant.

The protective efficacy of Shanchol in the Kolkata trial in older children between 5-15 was very high (above 80% during the three years of surveillance), even higher than those in adults, who due to exposure to cholera antigens in a cholera endemic area were expected to experience the best protection among all age groups (29, Table 2, Figure 1). Despite these highly unusual observations regarding protective efficacy, no credible explanation has been provided.

C. Problems with cost and logistics of delivering the vaccine:

Although the vaccine has been widely propagated as inexpensive (28, 29), it may not be economically feasible to deliver this vaccine to those who need it most considering the fact that cholera is a social disease prevailing in resource poor countries. The vaccine’s negotiated price in 2011 with the manufacturer for bulk purchase (200,000 doses) for use in Haiti for a
two-dose regime was USD 3.70 (33). That did not include the cost involved in actually disseminating and administering it. Further, the vaccine has a cold chain requirement that is difficult to maintain in countries where cholera outbreaks occur. As it is a spaced two-dose vaccine, the vaccination campaign had encountered logistics and cold chain challenges involving substantial planning and has been described as “no small task” by the field workers delivering the vaccine in Haiti (33, 34). It is worthwhile to point out that the rationale for the requirement of the cold chain for Shanchol, a vaccine consisting largely of killed cells, has not been provided.

D. Conflict of interest:

Although the field trial was conducted by an institute of the Indian Government (The National Institute of Cholera and Enteric Diseases, Kolkata) with its director being the principal investigator of the program (28, 41), the confidentiality of the codes related to the trial were maintained by the private vaccine company Shantha Biotechnics and the vaccine’s prime developer the International Vaccine Institute (29). This introduced the potential for conflict of interest. This potential for conflict of interest could have been avoided if the trial had been monitored and its confidentiality maintained by an independent and impartial body with no conflict of interest and personal ties to those associated with vaccine.

B. Oral vaccination of a combination of *V. cholerae* O1 components

The killed whole cell-B subunit (WC-CTB) vaccine’s trial in Bangladesh

In 1976 two Swedish researchers reported that a combination of *V. cholerae* O1 antigens such as lipopolysaccharide (LPS) and cholera toxin (CT) or choleraagenoid [now termed as the B subunit of cholera toxin (CTB)] induced in rabbits more than 100-fold higher protection against challenge with live vibrios than did vaccination with either of the two
antigens alone (56). To substantiate this claim, a vaccine (WC-CTB) comprising a combination of killed whole cholera bacteria (WC) and CTB was subjected to a large scale randomized, double blind, placebo controlled field trial in Bangladesh in January 1985 (24).

It is noteworthy that a Dutch study in 1987 with the same WC-CTB vaccine could not reproduce in rabbits the earlier claims made by the Swedish investigators (17). The WC-CTB vaccine comprised four different preparations of three *V. cholerae* O1 strains [Inaba (classical and El Tor) and Ogawa classical] and 1 mg of CTB isolated chemically from the culture supernatant produced by *V. cholerae* 569B (Inaba, classical) strain (Table 3). All strains were killed, some with heat and some with formaldehyde treatment. The trial comprising 63,498 participants included a control WC vaccine without CTB and a placebo of *Escherichia coli* K12. Each dose of the vaccine contained a total of 1x10¹¹ cells (Table 3). Three spaced doses of the vaccine totaling 3x10¹¹ cells were fed orally to each vaccine recipient.

As children are at higher risk for cholera, 62% of trial participants were children aged 2 to 15 years, the remainder comprising only adult females (15 years or older). All adult males and children under 2 were excluded.

Prior to the field trial in Bangladesh, a few healthy adults in the USA were immunized orally with three spaced doses of either WC-CTB or WC vaccine. They were challenged with live *V. cholerae* O1 after 5 weeks of immunization (8). Both the vaccines had a moderate protective efficacy of approximately 60%.

The trial in Bangladesh that had started in January 1985 was followed by a 6-month pre-epidemic period (April-September 1985) during which cholera incidence was low and the WC-CTB vaccine had offered protective efficacy of 85% in all age groups (24, Table 4, Figure 2). With the arrival of a cholera epidemic afterwards, protective efficacy of WC-CTB in all age groups fell to 62% at 1 year (25, 26, Table 4, Figure 2). Upon analysis by age groups, protective efficacy of WC-CTB in children (2-5 years) at 1 year fell
drastically to 38% (25, 26, Table 4, Figure 2). During the third year of surveillance, protective efficacy of WC-CTB fell significantly in all age groups, in participants above 5 and in children (2-5 years) to 17%, 40% and -37% (negative) respectively (26, Table 4, Figure 2). The WC-CTB vaccine offered hardly any protection in vaccinees during the fourth year (57) and is no longer being produced.

Protective efficacy of WC was lower (58%) in comparison to that of WC-CTB (85%) in all age groups during the initial 6 months after vaccination (24, 25, Tables 4 and 5). During the first year, protective efficacy of WC in all ages, in participants above 5 and in children (2-5 years) were 53%, 67% and 31% respectively (26, Table 5). While protective efficacy of WC in participants above 5 years of age was in the range of 62-73% during the 3 years of follow up, it was much lower (2-31%) in children (2-5 years) during that period and was not evident in the third year (26, Table 5). Both the vaccines enriched in V. cholerae O1 of classical biotype offered reduced protection against El Tor infections (25).

Antibody responses after immunization with WC-CTB and WC were evaluated in sera obtained from a number of randomly selected vaccinees (58). Two weeks after immunization geometric mean antitoxin titres were 2.5–4.5 times higher in vaccines receiving the WC-CTB vaccine. The vibriocidal titres were 1.3-2.1 times higher in vaccinees receiving both the vaccines. However this elevated level of vibriocidal titre persisted for only a brief period of time, barely detectable after 7 months even though protection was observed afterward.

The whole cell-recombinant CTB (WC-rCTB) vaccine trial

(a) In South America

As the production of recombinant CTB (rCTB) by DNA technology was first reported in 1989 (59), the WC-CTB vaccine’s CTB unit was substituted in the early 1990s with rCTB prepared from V. cholerae O1(classical) [60, 61]. Subsequently, the WC-rCTB vaccine was
marketed by the trade name Dukoral. A small-scale trial of WC-rCTB (Dukoral), carried out for a short period (18 weeks only) on 1426 military recruits of Peru in 1994, had offered protective efficacy of 86% against cholera (61). The relatively high protective efficacy was due to very few cases of cholera and a reassignment of the military recruits to other bases, which led to the early closure of the trial (62). In 1994, WC-rCTB was subjected to a randomized, double-blind, placebo controlled field trial in Peru where participants (n=17,799) received either two spaced doses of the vaccine (n=9012) or the placebo (n=8787) [30]. During the first year of surveillance, the vaccine failed to offer any protection to the vaccinees of all ages (4%). An analysis by age groups showed that the vaccine had offered negative protection in children under 5 and very little protection (15%) in recipients above 6. Protection level during the 2nd year of surveillance increased only when a third booster dose was administered 10 months after the second one, protection being 61% in all age groups and 51.5% in children (2-5 years). The trial was not continued beyond the second year. A debate took place afterward where the viewpoints of those supporting Dukoral’s 2-dose regimen (63) were rebutted by the scientists associated with the Peruvian trial (62). According to the proponents of the 2-dose regimen, 2 doses of WC-CTB were as good as 3 doses as claimed in the Bangladeshi trial of 1985 (26, 63). However, a recent study on oral cholera vaccines, conducted by the UK’s Cochrane Infectious Diseases Group, has been unable to get access to the data to confirm this finding arising out of the Bangladeshi trial (64).

(b) In East Africa

In a field trial of short duration in 2009 that lasted only for 14 months, 23,921 individuals (above 2 years of age) of Zanzibar, East Africa were fed two spaced doses of WC-rCTB (65). The trial had several drawbacks. Instead of being a randomized, double blind, placebo controlled study, the vaccine recipients were volunteers who had opted to receive the vaccine.
Controls, who did not receive the vaccine, differed in many ways from vaccine recipients. There were more males among non-recipients who were older, drank more tap water, lived in more densely populated areas with lower neighborhood-level vaccine coverage and less willing to take the vaccine. After 14 months of surveillance, the vaccine was reported to offer 79% protection against cholera in all participants. No data were presented on the vaccine’s efficacy in children under the age of 5, the group most vulnerable to cholera. Moreover the incidence of cholera was extremely low. Interestingly, the vaccine increased significantly the risk of non-cholera diarrhea among the recipients, although an earlier study had suggested that the WC-CTB vaccine protected against enterotoxigenic *E. coli* diarrhea (66). The causes of non-cholera diarrhea were not identified.

In a case-control trial of WC-rCTB in Mozambique in 2004, 2-doses of the vaccine were reported to offer 82 and 67% protection respectively for recipients below 5 and above 5 years of age respectively (67). Unfortunately, the trial was conducted only for a period of 6 months, severely limiting any conclusions about Dukoral’s protective efficacy.

**Lack of synergy between WC and CTB/rCTB**

Controlled trials of the WC-CTB/rCTB vaccine carried out in Bangladesh, Peru and the USA (8, 24, 26, 30) failed to demonstrate any synergy between WC and CTB as claimed earlier (56). The high protective efficacy of 85–86% offered by the WC-CTB/rCTB vaccine in Bangladesh and Peru, tacitly attributed to CTB/rCTB, was observed during the first 6 months and 18 weeks respectively when cholera cases were few. No differences in protective efficacy between the WC-CTB and WC vaccines were observed when one year’s follow-up results in the Bangladeshi trial are considered, suggesting that antitoxic immunity played a short term role and antibacterial immunity had a greater role in conferring longer protection. While protective efficacy offered by these two vaccines in Bangladesh were moderate and similar during the second year of surveillance, protective efficacy of the
WC-CTB vaccine was inferior to that of the WC vaccine in the third year, offering negative protection in children (2-5 years) and even making them more susceptible to cholera (26, 68, Figure 2). It is worthwhile to point out that no long term controlled field trial of WC-rCTB has been carried out, the Peruvian trial being the longest one lasting for 2 years only.

Concerns regarding the killed oral combination vaccine (WC-CTB/rCTB)

A. Strain selection and killing procedure

The *V. cholerae* O1 strains used for WC-CTB/rCTB vaccines are identical to those present in Shanchol (Table 3). These strains were isolated from the cholera epidemic in Egypt in 1947 and possibly underwent numerous transfers and variations since then. To produce the vaccine, some of the bacteria were killed with heat, some with formaldehyde and antigenic analysis of these strains after such treatments has not been provided.

b) Requirement of large amount of cells

A two-dose WC-rCTB vaccine used in field trials contained 2.00x10^{11} cells (30, 65) which is several fold more than that received by a vaccinee immunized with the now discarded killed parenteral WC vaccine containing 0.08x10^{11} cells/ml (68). This amount present in WC-rCTB is the equivalent of *V. cholerae* O1 growth from a confluently streaked Petri dish (68). As of late 2011, the bacterial content of WC-rCTB has been further increased by 25% to 1.25x10^{11} cells/dose without providing a rationale for the increase (69).
c) Lack of information for controlling WC-rCTB

At present, there is no in vitro test to evaluate and compare the potencies of different lots of killed whole cell oral cholera vaccines (68, 70). This could account for the variation of results of WC-rCTB observed in different field trials (30, 65, 67).

e) B subunit of cholera toxin in WC-CTB/rCTB

The B subunit of cholera toxin, incorporated in WC-CTB/rCTB vaccine, is derived from a strain of classical biotype (CT-1) that does not adequately protect against toxin produced by El Tor vibrios. This choice of toxin B-subunit is unfortunate, as almost all cholera is now caused by El Tor strains that produce CT-2, a B-subunit related, but not structurally or immunologically, identical to CT-1 (71).

f) Limitations on the use of WC-rCTB

i) WC-CTB/rCTB is a spaced two dose vaccine with an interval of at least one week between doses. Immunity usually does not develop until one week after the second dose (60). The period between initial vaccination and protective immunity would decrease efficacy during epidemics.

ii) It cannot be given to children under the age of 2 despite the fact that they may be vulnerable to cholera (30, 60).

iii) The vaccine’s protective efficacy is short. For continuous protection against cholera a single booster dose is recommended within 2 years for adults and children from 6 years of age, and after 6 months for children aged 2 to 6 years (72). If more than 2 years have elapsed since the last vaccination, the primary vaccination course should be repeated (72).
iv) The vaccine delivery system is inconvenient, requiring stomach acid neutralization, which can be problematic for people with stomach ailments (73).

v) The vaccine requires large quantity of safe water as the product is very voluminous (150 ml/dose, 60), 30 times more than other usual vaccine, thus limiting its application in recent epidemics (74, 75).

vi) The vaccine’s strict requirement of cold chain and very high production cost make it unsuitable for use in many resource poor countries where cholera prevails (73).

vi) As stated by the manufacturer, formaldehyde used during manufacturing process can be present in the final product thereby acting as a potential allergen to those who are sensitive to it (76). Adverse reactions such as diarrhea, stomach cramps, vomiting and fever have also been reported (77).

vii) Cholera prevails in areas of Africa and Latin America, also hit by the HIV/AIDS epidemic. But the vaccine is not recommended for use in HIV-infected subjects as it was reported to increase HIV viral load (from 2 to 60 fold) in the plasma of patients (72, 78).

**Immunological studies of the immune response to WC-rCTB:**

Recent humoral and cellular immunological studies on the immune responses to *V. cholerae* O1 antigens in adults and children in Bangladesh comparing clinical infection and vaccination have provided a number of important observations (79-82). Vibriocidal antibodies, predominantly directed against LPS, are regarded as a measure of immunity (83). Children under 5 receiving two spaced doses of Dukoral (WC-rCTB) had (i) lower levels of antibodies to LPS, (ii) lower vibriocidal titres and (iii) no memory B cell (MBC) response to LPS as compared to children of the same age group with natural cholera infection (81). Adult vaccine recipients, while having anti-CTB and anti-LPS antibodies comparable to
those detected in adult cholera patients, showed (i) weaker vibriocidal responses and (ii) no
IgA or IgG memory B cell responses to LPS (79). Studies on the antigen-specific memory T
cell responses showed that cholera patients developed significant levels of toxin-specific
memory T (T EM) cells and cytokines characteristic Th1, Th2 and Th17 cell responses (80). In
contrast, younger children (2-6 years) receiving Dukoral neither developed T EM nor
showed an increase in Th1 cells. But they showed a decrease in Th17 cells and an increase
in Treg cells indicating diminished T cell memory responses required for the subsequent
development of memory B cell responses. These findings may account for the lower
protective efficacy afforded by Dukoral in children under 5.

On the questionable biological similarity between CTB and rCTB:

The isolation of rCTB by recombinant DNA technology involves procedures that
produce pure rCTB that is not contaminated with intact CT. Results with CTB prepared
from two different sources may not necessarily be similar. CTB obtained by
chemical purification of the cell free supernatant may contain traces of CT, a very powerful
adjuvant (84), that may escape detection by quality control assays but be sufficient to exert
some of its biological functions. The substitution of rCTB for CTB can, potentially, weaken
the vaccine. Some reports have correctly distinguished between WC-CTB and WC-rCTB
(85, 86), however, the purity of chemically isolated products can vary from lot to lot, further
confusing comparisons. Although a rise in serum antitoxin and vibriocidal antibody
titres after oral immunization with WC-rCTB and WC-CTB was detected on a few
Swedish volunteers, the mean vibriocidal antibody titre increase was higher in those
receiving WC-CTB (87). The WC-CTB vaccine used in the Swedish study was not from
the same lot as that was used in the Bangladeshi trial of 1985 (24). Volunteers in the Swedish
study were not challengee with live V. cholerae O1, thereby providing no information on the
comparative protective efficacies of these two vaccines (87, 88). To date, there have been no
field trials simultaneously comparing the protective efficacies of WC-CTB and WC-rCTB. Therefore, the “practical” similarity between CTB and rCTB, as reported in the literature, is not supported by data (89, 90).

The cholera vaccine literature is replete with statements that the WC-rCTB vaccine (Dukoral) was subjected to a large scale field trial in Bangladesh in 1985 offering 80-90% protection during the first 6 months after vaccination (91-96). For example, the manufacturer of Dukoral (SBL Vaccines AB Sweden) has used this statement for commercial purposes, describing the vaccine’s protective efficacy to be 85% against cholera without specifying the period of duration (97). There is no record of a field trial of a vaccine containing WC-rCTB in Bangladesh in 1985. Further, since the production of rCTB by recombinant DNA technology was first reported in 1989 (59), reference to a field trial of WC-rCTB in 1985 is inaccurate. The Bangladeshi trial of 1985 used WC with CTB, the latter isolated biochemically from cell free supernatants; it was not produced using recombinant technology (24). As stated earlier, assumptions that rCTB and CTB are essentially equivalent when incorporated into vaccines are not supported by data, and it is critical to distinguish between vaccines that contain these different components. Consequently, using results from the 1985 Bangladeshi trial of WC-CTB to justify usage of a vaccine containing WC-rCTB is inappropriate and should be avoided. In the Bangladeshi trial, the WC-CTB vaccine had offered protective efficacy of 85% to a large number of vaccinees of all ages (n= 21,141) during the initial 6 months (24). The WC-rCTB vaccine in Peru had offered protective efficacy of 86% to a much smaller number of participants comprising healthy military recruits (n= 1426) for a period of 18 weeks only when cholera cases were few (61). In a subsequent large scale field trial in Peru (n= 9012), the 2-dose WC-rCTB vaccine had failed to offer any protection to the vaccinees (protective efficacy -4%) during the first year of surveillance (30). Moderate over all protection of 61% was only achieved after a booster
dose delivered 10 month after the 2nd dose. In brief, the results of the Bangladeshi field trial in 1985 have been inappropriately used in a number of publications to justify Dukoral’s high protective efficacy of 85% in the early period after vaccination.

Concluding remarks on the killed oral cholera vaccines Shanchol and Dukoral

A comparative evaluation of different field trials of the killed oral cholera vaccines (WC-CTB, WC-rCTB, WC, Shanchol) during the first year after vaccination is shown in Table 6.

Between the two vaccines (Shanchol and Dukoral), Shanchol is preferred because it offers a few operational advantages such as not requiring a buffer solution for administration, requiring less cold chain volume, being applicable to children from 1 year of age (compared to 2 years of age for Dukoral) and being comparatively less expensive to produce (35). However, neither Shanchol nor Dukoral appears suitable for cholera control whether it is epidemic or endemic. Biased results of protective efficacy have come out of the trials of these vaccines conducted in heavily endemic areas where the adult population were already primed to natural cholera antigens. Children under 5 represent the group most vulnerable to cholera (36), an observation confirmed in a survey during the recent cholera epidemic in Haiti in which diarrheal diseases in children under 5 was a major contributor to paediatric hospitalizations and mortality (98). Shanchol, the preferred vaccine, showed very poor protective efficacy of 17% in children under 5; participants of all ages receiving a modest protection of 45% during the first year of surveillance in the Kolkata trial of 2006 (28, 29). Little information is available on the long term protective efficacy of Dukoral. While a short term (6 month) case-control trial of 2-doses of Dukoral in Mozambique in 2004 demonstrated 82% protection in children under 5 (67), a placebo-controlled double-blind large scale 2-dose trial of Dukoral in Peru in 1994 produced negative protection during the first year of surveillance (30). The protective efficacy of Dukoral in the Zanzibar trial of 2009...
in children under 5 was not reported (65). Because of its poor protective efficacy in children under 5, a single booster dose of the vaccine is recommended after every 6 months for continuous protection (72).

Both the Shanchol and Dukoral vaccines have uncertain composition. While Shanchol’s composition has been inaccurately described in terms of undefined ELISA Units, Dukoral includes CTB derived from a classical instead of an El Tor strain. Both the vaccines are inactivated by heat and formalin treatment, potentially denaturing bacterial protein components (44–47) and reducing their T cell immunogenicity (46). Both Shanchol and Dukoral are spaced two-dose vaccines with immunity developing at least one week after the last vaccination, reducing their efficacy once an epidemic occurs as happened recently in Iraq and Zimbabwe (74, 75). Moreover, though manufacturers of both vaccines claim that they are inexpensive, the cost may be prohibitive in economically strapped regions at risk for cholera. Both the vaccines comprise formalin inactivated strains with the possibility of formalin’s presence in the final product acting as an allergen to formalin sensitive people. In summary, factors such as short term efficacy, poor protection in children under 5, necessity for multiple doses, requirement of cold chain, production cost and complex logistics in vaccine delivery greatly reduce the suitability of either of these vaccines for endemic or epidemic cholera control in resource poor settings.

Immunological studies comparing immune responses induced by WC-rCTB and natural cholera have revealed the reduced and short-lived efficacy of WC-rCTB (79-82). Although these studies were carried out with Dukoral (WC-rCTB), it is likely that similar observations may emerge with the other killed oral vaccine Shanchol as protective immunity against cholera is predominantly antibacterial (86, 99). The major difference between Dukoral and Shanchol is that the former contains additional rCTB (60) and the latter has additional V. cholerae O139 cells (32). During cholera infection V. cholerae O1 strains, apart from
cholera toxin, secrete several biologically active products such as neuraminidase, mucinase, collagenase, lipase and proteinase (100, 101), a few of them being considered as vaccine candidates (102, 103). A killed cholera vaccine cannot present these factors to the host and hence produce a less broad immune response than that induced by natural cholera.

The outcome of a vaccine trial is of great importance for the welfare of the cholera suffering people. The efficacy of a candidate cholera vaccine should be determined by a randomized, double-blind, placebo-controlled trial rather than by less reliable means that are neither placebo-controlled nor double-blind as was carried out with the field testing of Dukoral in Zanzibar (65) and Mozambique (67). The trial should include adequate number of children under 5 as they represent the group most vulnerable to cholera. Further the vaccine trials should be supervised by independent and impartial monitors with no conflict of interest.

A few laboratories are working on the development of attenuated live V. cholerae O1 strains as oral vaccine candidates. This communication has presented an in-depth analysis of the currently available killed oral cholera vaccines. A detailed discussion of the live oral attenuated cholera vaccines, which are not currently available for use and at different stages of development, is not considered here. In spite of extensive research over 100 years, an effective vaccine against cholera has not yet been obtained (73). A single dose economical vaccine offering a high degree of protection in all age groups in general, and in children under 5 in particular is still needed.
References:


41. Anonymous. 2008. A randomized controlled trial of the bivalent killed whole cell oral cholera vaccine in eastern Kolkata, West Bengal, India. protocol/version 3.0 section 6.1 study agents (vaccine and placebo)


of formaldehyde-induced modifications in proteins: reactions with model peptides.

J Biol Chem. 279:6235-6243.


55. Saroso JS, Bahrawi W, Witjaksono H, Budiarsro RL, Brotowasisto, Bencić Z, Dewitt WE, Gomez CZ. 1978. A controlled field trial of plain and aluminium hydroxide-


production of inactivated oral cholera vaccines. p 130-149.


Table 1. Composition of the Killed Whole-cell Oral Cholera Vaccine Shanchol

Each oral dose of 1.5 mL contains:

<table>
<thead>
<tr>
<th>Cell numbers</th>
<th>Quantity</th>
<th>Inactivation</th>
<th>Strain/Serotype</th>
<th>Biotype</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5x10^10</td>
<td>300 EU of LPS</td>
<td>Heat</td>
<td>O1/Inaba</td>
<td>Classic</td>
<td>Cairo 48</td>
</tr>
<tr>
<td>5.0x10^10</td>
<td>600 EU of LPS</td>
<td>Formaldehyde</td>
<td>O1/Inaba</td>
<td>El Tor</td>
<td>Phil 6973</td>
</tr>
<tr>
<td>2.5x10^10</td>
<td>300 EU of LPS</td>
<td>Heat</td>
<td>O1/Ogawa</td>
<td>Classic</td>
<td>Cairo 50</td>
</tr>
<tr>
<td>2.5x10^10</td>
<td>300 EU of LPS</td>
<td>Formaldehyde</td>
<td>O1/Ogawa</td>
<td>Classic</td>
<td>Cairo 50</td>
</tr>
<tr>
<td>5.0x10^10</td>
<td>600 EU of LPS</td>
<td>Formaldehyde</td>
<td>O139</td>
<td></td>
<td>4260B</td>
</tr>
</tbody>
</table>

Total number of Vibrio cells: O1 1.25x10^11; O139 5x10^10
Thiomersal I.P. Not more than 0.02% (w/v); EU is the abbreviation of ELISA Units
Buffer q.s. to 1.5 mL

Table 2: Protective efficacy (%) of the OCV Shanchol by age and year of follow-up

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>17</td>
<td>81</td>
<td>37</td>
</tr>
<tr>
<td>5-15</td>
<td>81</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td>15+</td>
<td>66</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>All ages</td>
<td>45</td>
<td>77</td>
<td>65</td>
</tr>
</tbody>
</table>

Data based on the information presented in reference 29.
Table 3. Composition of the whole cell oral cholera vaccine (WC-CTB/rCTB) used in field trials

(a) Whole cell (WC) per dose

<table>
<thead>
<tr>
<th>Cell Numbers</th>
<th>Inactivation</th>
<th>Serotype</th>
<th>Biotype</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 x10^10</td>
<td>Heat</td>
<td>O1/Inaba</td>
<td>Classic</td>
<td>Cairo 48</td>
</tr>
<tr>
<td>2.5 x10^10</td>
<td>Formaldehyde</td>
<td>O1/Inaba</td>
<td>El Tor</td>
<td>Phil 6973</td>
</tr>
<tr>
<td>2.5 x10^10</td>
<td>Heat</td>
<td>O1/Ogawa</td>
<td>Classic</td>
<td>Cairo 50</td>
</tr>
<tr>
<td>2.5 x10^10</td>
<td>Formaldehyde</td>
<td>O1/Ogawa</td>
<td>Classic</td>
<td>Cairo 50</td>
</tr>
</tbody>
</table>

(Data obtained from references 24, 30)

(b) The B subunit of cholera toxin per dose: 1 mg obtained either from the culture supernatant (CTB) or by recombinant technology (rCTB) from *V. cholerae* O1 (Inaba, classical). The vaccine was administered with a sodium bicarbonate buffer (24, 30).

Table 4. Protective efficacy of the WC-CTB vaccine during the 3 years of surveillance in Bangladesh in various age groups

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Age group (year)</th>
<th>6 months</th>
<th>First year (%)</th>
<th>Second year (%)</th>
<th>Third year (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC-CTB</td>
<td>2-5</td>
<td>100</td>
<td>38</td>
<td>47</td>
<td>– 37</td>
</tr>
<tr>
<td></td>
<td>&gt;5</td>
<td>76</td>
<td>78</td>
<td>61</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>All ages</td>
<td>85</td>
<td>62</td>
<td>57</td>
<td>17</td>
</tr>
</tbody>
</table>

Data based on the publications cited in references [24-26, 57]

Table 5. Protective efficacy of the WC vaccine during the 3 years of surveillance in Bangladesh in various age groups

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Age group (year)</th>
<th>6 months</th>
<th>First year (%)</th>
<th>Second year (%)</th>
<th>Third year (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>2-5 yr</td>
<td>35</td>
<td>31</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;5 yr</td>
<td>71</td>
<td>67</td>
<td>73</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>All ages</td>
<td>58</td>
<td>53</td>
<td>57</td>
<td>43</td>
</tr>
</tbody>
</table>

Data based on the publications cited in references [24-26, 57]
### Table 6. Comparative Evaluation of Field Trials of Killed Oral Cholera Vaccines

<table>
<thead>
<tr>
<th>Factors</th>
<th>WC-CTB</th>
<th>WC-rCTB</th>
<th>WC-rCTB</th>
<th>WC</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial place</td>
<td>Bangladesh</td>
<td>Peru</td>
<td>Zanzibar</td>
<td>Bangladesh</td>
<td>India</td>
</tr>
<tr>
<td>Trial methods</td>
<td>RCT, DB, PC</td>
<td>RCT, DB, PC</td>
<td>Non- (RCT, DB, PC)</td>
<td>RCT, DB, PC</td>
<td>CRT, DB, PC</td>
</tr>
<tr>
<td>Vaccine type</td>
<td>Killed V. cholerae O1</td>
<td>Killed V. cholerae O1</td>
<td>Killed V. cholerae O1</td>
<td>Killed V. cholerae O1</td>
<td>Killed V. cholerae O1 + V. cholerae O139</td>
</tr>
<tr>
<td>Composition/dose</td>
<td>1x10^{11} cells + CTB (1 mg)</td>
<td>1x10^{11} cells + rCTB (1 mg)</td>
<td>1x10^{11} cells + rCTB (1 mg)</td>
<td>1x10^{11} cells</td>
<td>1.75x10^{11} cells</td>
</tr>
<tr>
<td>Placebo</td>
<td>Killed E. Coli K12</td>
<td>Killed E. Coli K12</td>
<td>No placebo</td>
<td>Killed E. Coli K12</td>
<td>Killed E. Coli K12</td>
</tr>
<tr>
<td>No of dose</td>
<td>3</td>
<td>2(^a)</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Vaccinees (n)</td>
<td>21 141</td>
<td>10 592</td>
<td>23 921</td>
<td>21 137</td>
<td>31 932</td>
</tr>
<tr>
<td>Male (%)</td>
<td>31</td>
<td>46</td>
<td>44</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>Female (%)</td>
<td>69</td>
<td>54</td>
<td>56</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>1-year PE (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>38</td>
<td>Negative</td>
<td>Not reported</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>All ages</td>
<td>62</td>
<td>Negative</td>
<td>Not reported</td>
<td>79</td>
<td>53</td>
</tr>
<tr>
<td>References</td>
<td>24-26</td>
<td>30</td>
<td>65</td>
<td>24-26</td>
<td>28, 29</td>
</tr>
</tbody>
</table>

RCT, randomized controlled trial; DB, double blind; PC, placebo controlled; CRT, cluster-randomized trial; PE, protective efficacy.

\(^a\) A third dose of the vaccine was administered after the first two doses were given. In the year of surveillance following administration of the third dose, the PE in <5 years and in all ages were 51 and 61% respectively (30).
Figure 1: Title: Performance of the OCV Shanchol by age and year of follow up  
Legend: Kinetics of protective efficacy offered by the oral killed whole cell cholera vaccine Shanchol among two dose recipients during the 3 years after the 2nd dose in various age groups (reference 29).

Figure 2: Title: Performance of WC-CTB by age and year of follow up  
Legend: Kinetics of protective efficacy offered by the oral killed whole cell cholera vaccine (WC) with the cholera toxin B subunit (CTB, non-recombinant) among three dose recipients in Bangladesh during the 3 years after the third dose in various age groups (references 24-26, 57).