5 year efficacy of a bivalent killed whole-cell oral cholera vaccine in Kolkata, India: a cluster-randomised, double-blind, placebo-controlled trial

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Summary

Background Efficacy and safety of a two-dose regimen of bivalent killed whole-cell oral cholera vaccine (Shantha Biotechnics, Hyderabad, India) to 3 years is established, but long-term efficacy is not. We aimed to assess protective efficacy up to 5 years in a slum area of Kolkata, India.

Methods In our double-blind, cluster-randomised, placebo-controlled trial, we assessed incidence of cholera in non-pregnant individuals older than 1 year residing in 3933 dwellings (clusters) in Kolkata, India. We randomly allocated participants, by dwelling, to receive two oral doses of modified killed bivalent whole-cell cholera vaccine or heat-killed Escherichia coli K12 placebo, 14 days apart. Randomisation was done by use of a computer-generated sequence in blocks of four. The primary endpoint was prevention of episodes of culture-confirmed Vibrio cholerae O1 diarrhoea severe enough for patients to seek treatment in a health-care facility. We identified culture-confirmed cholera cases among participants seeking treatment for diarrhoea at a study clinic or government hospital between 14 days and 1825 days after receipt of the second dose. We assessed vaccine protection in a per-protocol population of participants who had completely ingested two doses of assigned study treatment.

Findings 69 of 31932 recipients of vaccine and 219 of 34968 recipients of placebo developed cholera during 5 year follow-up (incidence 2·2 per 1000 in the vaccine group and 6·3 per 1000 in the placebo group). Cumulative protective efficacy of the vaccine at 5 years was 65% (95% CI 52–74; p<0·0001), and point estimates by year of follow-up suggested no evidence of decline in protective efficacy.

Interpretation Sustained protection for 5 years at the level we reported has not been noted previously with other oral cholera vaccines. Established long-term efficacy of this vaccine could assist policy makers formulate rational vaccination strategies to reduce overall cholera burden in endemic settings.

Funding Bill & Melinda Gates Foundation and the governments of South Korea and Sweden.

Introduction Cholera is a serious global public health problem because clean drinking water and sanitation are not universally available, and appropriate case management is not accessible to many patients. In endemic countries alone, about 1·4 billion people are at risk of cholera and an estimated 2·8 million cases and 91000 deaths occur each year. More than half these cases and deaths occur in cholera-endemic countries in Asia and in Africa.1 Furthermore, recent outbreaks in Cuba, Haiti, and Zimbabwe show the ability of this disease to spread rapidly to new areas and to produce outbreaks with substantial morbidity and mortality. Because of their capacity to spread rapidly, cholera outbreaks can overwhelm existing public health infrastructures and require substantial resources. The economic burden of cholera in African countries alone in 2005–07 ranged from US$39 million to $156 million per year, dependent on the estimate of average life expectancy used.3 Additional preventive interventions are needed.

To complement improvements in access to water and sanitation and rehydration therapies, much attention has been given to development of a cholera vaccine. After several studies in the 1960s showed that injectable whole-cell cholera vaccines conferred only modest protection of short duration, often with significant side-effects, researchers focused on oral vaccines that could efficiently stimulate local immunity in the gut. The first oral cholera vaccine to be prequalified by WHO for purchase by UN agencies contains a mixture of killed Vibrio cholerae O1 bacteria and the non-toxic B subunit of cholera toxin, and is marketed under the trade name Dukoral (Crucell, Netherlands). This vaccine was licensed largely on the basis of studies done more than 20 years ago in Bangladesh4 and Peru5 that showed 85% protection for the first 4–6 months and 60% protection for 2 years after a primary regimen of two or three doses. The protection declined substantially in the third year and was evident against V cholerae O1 El Tor only in the first year for individuals younger than 5 years.6 The vaccine is used primarily by people travelling from...
developed countries to cholera-endemic areas and has not been used routinely by these countries in their public health systems. A second killed whole-cell oral cholera vaccine (Shanchol, Shantha Biotechnics, Hyderabad, India), which contained the same *V cholerae* O1 whole-cell strains as Dukoral, at different doses, and killed *V cholerae* O139 bacteria but not the B subunit component, was developed and licensed in India in 2009. Licensure was based in part on results of a placebo-controlled, cluster-randomised trial in which the bivalent killed whole-cell oral cholera vaccine was shown to be safe and conferred 67% efficacy (cumulative) against cholera severe enough to require treatment in a health facility at 2 years and 66% efficacy at 3 years of follow-up.7,8 The vaccine was prequalified by the WHO in 2011. However, use of oral cholera vaccines can be hindered by the perceived short duration of protection that the vaccine confers (compared with recommended vaccines for other diseases). To assess the duration of protection of the vaccine, the results of which also have implications for the requirements for a boosting dose or re-immunisation, we extended the follow-up period to 5 years.

**Methods**

**Study design**

Our cluster-randomised, double-blind, placebo-controlled trial was done in a cholera-endemic area in the urban slums of Kolkata, India, with a population of about 109,000 individuals. Before the trial, a census was done to enumerate the population according to their regular or legal residence, map the households residing in the area, assign unique study identification numbers to each individual, and establish household socioeconomic, water-use, sanitation and hygienic characteristics.

Details on the study site, study agents, study procedures, and assembly of participants for this trial have been reported previously.7,8 Residents who were at least 1 year old and not pregnant were eligible to participate in the study. The study protocol was approved by the Drugs Controller General of India, the ethics committee of the National Institute of Cholera and Enteric Diseases, the Health Ministry Screening Committee of India, and the International Vaccine Institute institutional review board. We obtained written informed consent from residents older than 18 years and from the guardians of residents aged 1–17 years. We also obtained written assent from residents aged 12–17 years. An external statistician, who was masked to the identities of the codes, randomly assigned dwellings to the four codes in a 1:1:1:1 ratio within each of the strata.

Randomisation and masking

We used stratified randomisation to preassign all eligible participants in each dwelling to one of the four letter codes that served to identify the vaccine and placebo. The assigned codes were printed in vaccination books for use during dosing. Assignment of each dwelling to one of the codes used six strata, defined by administrative ward and dwelling size (≤20 residents or >20 residents). An external statistician, who was masked to the identities of the codes, randomly assigned dwellings to the four codes in a 1:1:1:1 ratio within each of the strata.

**Procedures**

Each dose of the modified killed bivalent whole-cell vaccine (Shanchol) contained about $1 \times 10^{11}$ inactivated *V cholerae* O1 and $5 \times 10^{10}$ *V cholerae* O139 bacteria dispensed based upon their lipopolysaccharide contents: 600 ELISA units of lipopolysaccharide of formalin-killed *V cholerae* O1 El Tor Inaba (strain Phil 6973); 300 ELISA units of lipopolysaccharide of formalin-killed *V cholerae* O1.

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**Figure 1: Trial profile**

*Entire cluster did not receive allocated vaccine or placebo, was lost to follow-up, or discontinued.* | Present in the study area during the vaccination campaign, but did not attend the vaccination outpost.
units of lipopolysaccharide each of heat-killed *V cholerae* O1 classical Ogawa (strain Cairo 50), formalin-killed *V cholerae* O1 classical Ogawa (strain Cairo 50), and heat-killed *V cholerae* O1 classical Ogawa (strain Cairo 48); and 600 ELISA units of lipopolysaccharide of formalin-killed *V cholerae* O139 (strain 4260B). We used vials containing identical-appearing heat-killed *Escherichia coli* K12 cells as placebo. Single-dose vials were labelled with one of four letter codes, two for vaccine and two for placebo. Project staff and study individuals were unaware of the identities of the codes. Two doses of the assigned agents were given in two rounds: from July 27 to Aug 13, 2006, and from Aug 27 to Sept 10, 2006.

Before vaccination, another census of the study population was done to update the census used for allocation. Surveillance was done in nine community clinics established for the trial and in two hospitals serving the study population. Study physicians completed structured forms to obtain pertinent clinical information, and obtained faecal specimens that were tested for *V cholera*, including identification of O1 and O139 serogroups and Inaba and Ogawa serotypes. Biotype was ascertained for all O1 isolates, and the biotype of the cholera toxin genetically encoded was identified as previously described. A diarrhoeal visit was defined as having, in the 24 h before presentation, at least three loose stools or (if one, two, or an indeterminate number of loose stools were reported) the patient must have shown evidence of some or severe dehydration according to WHO criteria. The onset of a diarrhoeal episode was the day on which the patient first reported loose or liquid stools. Diarrhoeal visits for which the date of onset was less than or equal to 7 days from the date of discharge for the previous visit were grouped into the same diarrhoeal episode. The primary endpoint (a cholera episode) was defined as a diarrhoeal episode in which the diarrhoea was non-bloody, a faecal specimen yielded *V cholerae* O1 or O139, and a domiciliary check confirmed that the individual had visited the treatment centre for diarrhoea on the recorded date of presentation. Demographic surveillance for migrations and deaths among the study population was maintained during the 5 years of follow-up, and verbal autopsies were done for identification of the cause of deaths.

### Statistical analysis

We analysed efficacy in a per-protocol population and according to intention to vaccinate. In the per-protocol analysis, we investigated vaccine protection in participants who had completely ingested two doses of assigned study treatment and assessed cholera episodes that occurred between 14 days and 1825 days after receipt of the second dose. In the intent-to-vaccinate analysis, we included all individuals who received at least one dose of study treatment, and analysed the data on the basis of the assigned treatment irrespective of the amount swallowed. Episodes of cholera were included in the intent-to-vaccinate analysis if they had onset between 1 day and 1825 days after the first dose. In both analyses, we analysed only the first episodes of cholera. Before analysis, data were frozen, and the analytic plan was approved by the data and safety monitoring board. In our study, the zero time (day 0) for per-protocol and intent to vaccinate analyses was the date of second dose and first dose, respectively. Individuals who were present (ie, number at risk) at day 0 were assessed in the year 1 analysis.

We did survival analyses to calculate vaccine protective efficacy with measurements of the time to the first episode of cholera, censoring the follow-up of individuals who died or migrated out. In descriptive analyses, we fitted Kaplan-Meier curves. We fitted unadjusted and adjusted Cox proportional hazards regression models, after verifying that the proportionality assumptions were fulfilled for all independent variables, and estimated the hazard ratios by exponentiation of the coefficient for the vaccine variable in these models. We calculated percentage vaccine protective efficacy as (1–hazard ratio)×100. We used robust sandwich variance estimates to account for

### Figure 2

Incidence of laboratory-confirmed cholera cases by study year in Kolkata, India

<table>
<thead>
<tr>
<th>Year</th>
<th>Overall population</th>
<th>Vaccine recipients</th>
<th>Placebo recipients</th>
<th>Non-participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.0</td>
<td>5.4</td>
<td>3.6</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>6.8</td>
<td>4.2</td>
<td>2.6</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>5.7</td>
<td>3.9</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>4.7</td>
<td>3.7</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>3.8</td>
<td>2.9</td>
<td>0.9</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Table 1: Overall occurrence of cholera episodes and cumulative protective efficacy of the oral cholera vaccine

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccine protective efficacy (95% CI)</th>
<th>Adjusted protective efficacy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66% (53–75; p=0.0001)</td>
<td>65% (52–74; p=0.0001)</td>
</tr>
<tr>
<td>2</td>
<td>62% (49–72; p=0.0001)</td>
<td>60% (46–71; p=0.0001)</td>
</tr>
</tbody>
</table>

*Estimated after adjustment for variables used to stratify for randomisation (age group and ward of residence) and household expenditure per head higher than median expenditure, and longer than median distance from the household to the nearest water body.*
the design effect of cluster randomisation, allowing inferences for vaccine efficacy at the individual level.\textsuperscript{14}

To calculate vaccine protective efficacy adjusting for putative confounders, we included stratification variables (cluster size and ward of residence) in the model irrespective of the level of statistical significance. In addition, baseline variables that were significantly associated with time to event at \(p<0.10\) in bivariate analyses were candidates as independent variables in the model. To avoid overfitting the models, we used a backward elimination algorithm to select independent model variables at \(p<0.10\). The list of baseline variables is described elsewhere.\textsuperscript{8}

We assessed vaccine efficacy in predefined subgroups. We assessed heterogeneity of vaccine protection by levels of baseline variables in these subgroups by analysing two-way interaction terms in the models. To test whether vaccine protection declined in the 5 years of follow-up, we tested the proportional hazards assumption for the vaccine variable. To assess cases prevented from vaccination by age groups, we calculated the prevented number of cases for every 1000 vaccinated individuals (\(Pr\)) as:

\[
Pr = \left( \frac{Cp}{Np} - \frac{Cv}{Nv} \right) \times 1000
\]

Where \(Cv\) is the number of cases in the vaccine group, \(Cp\) is the number of cases in the placebo group, \(Nv\) is number of individuals in the vaccine group, and \(Np\) is the number of individuals in the placebo group.

Although our study protocol called for one-tailed analyses of protective efficacy, we present \(p\) values and 95% CIs for protective efficacy in a more conservative two-tailed fashion to assist interpretation of our data. The study extended the 3 year analysis by including 5 years of follow-up, and for this secondary analysis the threshold of significance was \(p<0.05\) with corresponding two-sided 95% CI. All statistical analyses were done with SAS version 9.3.

This trial was registered at ClinicalTrials.gov number, NCT00289224.

Role of the funding source
The funding agencies of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
We included 31932 participants in 1721 clusters in the vaccine group and 34968 participants in 1757 clusters in the placebo group in the per-protocol population (figure 1). In the intent-to-vaccinate analysis, we included 1727 clusters with 33127 recipients of vaccine and 1768 clusters with 36201 recipients of placebo. Individual-level and cluster-level baseline characteristics were comparable for recipients of the vaccine and placebo, as reported previously.\textsuperscript{7} We noted no substantive imbalances in baseline variables in participants who were excluded or lost to follow-up in each group.

In the 5 years of follow-up of the per-protocol population, there were 69 episodes of cholera in the vaccine group and 219 in the placebo group. For the intent-to-vaccinate population, there were 82 episodes of cholera in the vaccine group and 234 episodes of cholera in the placebo group. All detected cholera episodes were attributable to \(V\) cholerae O1 El Tor biotype, and 95% of the isolates were Ogawa serotype. One death in the vaccine group and two deaths in the placebo group were attributed to diarrhoea or gastroenteritis of presumed infectious origin (international classification of diseases 10 code A09). None of these three deaths or any other deaths could be attributed to \(V\) cholerae infection or to administration of the vaccine. Incidence of laboratory-confirmed cholera among all residents, whether or not they participated in the trial, varied by year after the...
vaccination period (figure 2). However, vaccine protection was evident in the 5 years of follow-up, as shown by the decreased incidence in recipients of vaccine compared with recipients of placebo.

In the per-protocol analysis, the adjusted cumulative 5 year protective efficacy of this vaccine for individuals aged 1 year or older (at the time of receipt of the first dose of vaccine) was 65% (95% CI 52–74%, p<0·0001) and in the intent-to-vaccinate analysis the protective efficacy was 60% (46–71%, p<0·0001; table 1). In the per-protocol analysis, Inaba serotype was detected in only four episodes in the vaccine group and ten episodes in the placebo group during follow-up (unadjusted protective efficacy 56%, 95% CI 37 to 86; p<0·016). Event-free survival for patients favoured vaccine over placebo (figure 3). When we stratified cumulative 5 year protection by age, protective efficacies did not differ significantly for either the per-protocol or the intent-to-vaccinate analyses (table 2). Nonetheless, the point estimates suggested reduced efficacy in individuals younger than 5·0 years at vaccination (table 2). The point estimate for children aged 1·0–2·0 years was also low at 31% (95% CI –58 to 70; p=0·37) with eight cases in 349 recipients of vaccine and 16 cases in 439 recipients of placebo. However, more cases seemed to be prevented by vaccination (10·5 per 1000) for children aged 1·0–5·0 years than were prevented in individuals aged 5·0–15·0 years (5·5 per 1000) and aged at least 15·0 years (3·1 per 1000; table 2). We noted no significant differences in the protective efficacies for the trial population for study years 1–5 during follow-up (table 3). Occurrence of cholera episodes and protective efficacy of the oral cholera vaccine by age at vaccination and year of follow-up in per-protocol population are shown in the appendix.

**Discussion**

During 5 years of follow-up, a two dose regimen of the cholera vaccine reduced the incidence of clinically
significant cholera by about two-thirds in the study population of individuals vaccinated at the age of 1 year or older. Although 95% of the cholera cases identified in the study were due to *V cholerae* O1 Ogawa, point estimates of protection against El Tor Inaba and Ogawa were much the same. The absence of cases attributable to *V cholerae* O139 precluded assessment of efficacy against this serogroup. Overall protection was sustained for 5 years follow-up. This level of sustained protection has not been reported for any previous cholera vaccine (panel).

We did not detect significant differences in the cumulative 5 year vaccine protection among different age groups at vaccination. However, our sample size was not calculated with adequate power to assess efficacy by age group. Nevertheless, the seemingly low efficacy in individuals aged 1·0–5·0 years and subsets of those aged 1·0–2·0 years (who might have been immunologically naive at vaccination) suggests that vaccine might not protect children younger than 5 years for 5 years. Results from a previous trial of a different killed oral cholera vaccine in Bangladesh are consistent with low levels of protection in young children. The Bangladeshi study reported that children vaccinated at 2·0–5·0 years of age had reduced levels of protection against *El Tor* cholera in the first year of observation after vaccination. However, even if low levels of protection were afforded to young children, our results suggest a potential reduction in morbidity because of the higher cholera incidence in the youngest age groups. Postlicensure assessments of the use of the vaccine studied in our trial in cholera-endemic populations should be done to address this issue more definitively.

Although we noted variation in efficacy by year of follow-up, including a substantial increase in protection in follow-up year 5 (perhaps due a boosting of immunity from the large outbreak during March and April, 2010), the variations in vaccine efficacy by year of follow-up did not differ significantly. However, analysis by year of follow-up was not planned and the sample size was not calculated with adequate power to assess such trends. Thus, we cannot conclude that the differences in protection by length of protection do not exist.

Beyond limitations resulting from the limited power of our trial to address interactions of vaccine protection with baseline variables and to assess trends in protection, our assessment has additional important limitations. First, because the trial was done in a population that has endemic cholera at high rates every year, the protection that we noted reflects protection from vaccine-induced immunity, together with the immunity induced by natural cholera infections occurring after baseline in recipients of vaccine and placebo. Second, individuals in our study population probably had previous exposure to cholera and some level of pre-existing natural immunity at baseline. Thus, our trial did not assess the protective efficacy of this vaccine in immunologically naive individuals who might be at risk in some cholera epidemics, such as that in Haiti. Third, we did not assess whether use of this vaccine prevents asymptomatic or mild disease but only whether vaccination prevents disease that is severe enough to seek treatment. These asymptomatic or mild cases could contribute to transmission.

Our findings confirm that this vaccine is efficacious in cholera-endemic settings, and could be adopted as part of a wider cholera control effort. Now prequalified by WHO, this vaccine does not require a buffer (unlike Dukoral), making it easier to provide in areas where access to clean water is restricted. In 2010, WHO issued a position paper supporting the use of cholera vaccines in conjunction with other preventive and control strategies in areas where the disease is endemic and should be considered in areas at risk for outbreaks. Supported by a World Health Assembly resolution to

Panel: Research in context

**Systematic review**

In 2011, a Cochrane review of randomised or quasi-randomised studies of oral cholera vaccines was published, assessing seven efficacy studies with five variations of the killed whole-cell oral cholera vaccine. The overall vaccine efficacy during the first year was 52% and during the second year was 62%. Protective efficacy was lower in children younger than 5 years (38%) than in individuals older than 5 years (66%). The review noted that for the present vaccine formulation, no data existed for protection beyond 2 years, but it was unlikely to last beyond 3 years. To supplement this review, we searched PubMed without date or language restriction with the terms “killed oral cholera vaccine” and “efficacy” or “effectiveness”, which revealed five other publications not included in the Cochrane review. Four were case-control studies; two of these studies were done in Africa and showed 78% and 79% effectiveness against cholera with the recombinant B-subunit containing whole-cell oral cholera vaccine, up to 6 months and 15 months after vaccination, respectively, after which no cases of cholera were detected. In one of these studies, effectiveness did not differ between individuals younger than 5 years of age and in an older age group. Two case-control studies with a previous version of the bivalent killed whole-cell oral cholera vaccine available only in Vietnam showed 76% effectiveness when used during an outbreak and 50% effectiveness up to 3–5 years after vaccination. Vaccine effectiveness by age group was not assessed separately in these studies. The fifth study was the report on 3 years of follow-up of our trial. At 3 years of follow-up, the modified bivalent killed whole-cell oral cholera vaccine afforded 66% efficacy in all age groups; however, efficacy was somewhat reduced in children aged 1·0–5·0 years at 43%.

**Interpretation**

After 5 years of follow-up, we noted that the bivalent killed whole-cell oral cholera vaccine remained 65% protective in a cholera-endemic area in Kolkata, India. This level of sustained protection for 5 years has not been reported previously with other oral cholera vaccines. Although statistical comparisons were not significant, vaccine efficacy seemed to vary by age group and by year of follow-up. Notably, a lower point estimate of efficacy that we noted in young individuals (1·0–5·0 years) than in older individuals suggests that the vaccine might not provide equivalent levels of protection to younger children. In view of the increased cholera incidence in children younger than 5 years of age, and despite the possibility of lower levels of protection among young children, our study suggests an improved morbidity reduction in this age group. Our findings support the use of oral cholera vaccine as a key component in public health programmes to protect against life-threatening and economically devastating cholera infections.

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**Articles**

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expand cholera control efforts, a WHO Technical Working Group on Creation of an Oral Cholera Vaccine Stockpile issued a report in 2012 recommending that an oral cholera vaccine stockpile of 2 million doses should be maintained and used in response to cholera outbreaks, especially in low-income countries. Funding has been created to obtain and operate the stockpile.

Cholera vaccines should be used in combination with health services that provide rapid detection and treatment of cholera cases with rehydration solutions and antibiotics, and increase access to safe water and sanitation, promote personal hygiene, and improve health education and community mobilisation.

Cholera is now endemic in more than 50 countries and causes substantial mortality and high economic costs in some of the world’s poorest nations. Our findings support the use of oral cholera vaccine as a key component in an integrated public health programme to protect against life-threatening and economically devastating cholera infections.

Contributors
SKB, DS, MA, SK, BM, JLD, RC, AD, ALL, GBN, and JDC conceived and designed the study. MA, YAA, BM, JKP, MHP, DRK, AD, TFW, and JDC collected the data. BSah, JLD, JH, AD, GBN, ALL, TFW, and JDC wrote the paper.

Conflicts of interest
MSD is an employee of Shantha Biotechs. All other authors declare that they have no conflicts of interest.

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