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A phase I/II study to evaluate safety, tolerability and immunogenicity of Hillchol®, an inactivated single Hikojima strain based oral cholera vaccine, in a sequentially age descending population in Bangladesh

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ABSTRACT

Background: The World Health Organization (WHO) recommends the use of oral cholera vaccines (OCVs) as part of an integrated control program, both in highly endemic settings and during cholera epidemics. The available and internationally recommended WHO-prequalified OCVs (Dukoral, Shanchol, Euvichol) contain multiple heat and formalin-killed *V. cholerae* strains of Inaba and Ogawa serotypes. MSD Wellcome Trust Hilleman Laboratories Pvt. Ltd. in technical collaboration with University of Gothenburg, Sweden has developed a new single strain OCV, Hillchol. This vaccine consists of formaldehyde-inactivated whole cell El Tor *V. cholerae* O1 bacteria engineered into the Hikojima serotype for stable expression of both the Ogawa (AB) and Inaba (AC) LPS antigens on the bacterial surface. We evaluated the safety and immunogenicity of this novel and potentially much less expensive OCV in comparison with Shanchol.

Methods: We conducted a randomized, non-inferiority, age-descending clinical trial of OCV (Hillchol vs. Shanchol) in the Mirpur area of Dhaka city from July 2016 to May 2017. This study was carried out in three different age cohorts (1–<5, 5–17 and ≥18 years old). Two doses of vaccine were given at 14 days intervals to 560 healthy participants.

Findings: No serious adverse events were reported. There were no significant differences in the rates of adverse events between the test vaccine (Hillchol) and the comparator (Shanchol) group. Serum vibriocidal antibody responses in all age groups combined were comparable for all the O1 Ogawa (59% vs. 67%; 90% CI of difference: −14.55, −0.84) and Inaba (70% vs. 71%; 90% CI of difference: −7.24, 5.77) serotypes, showing that the Hillchol vaccine was non-inferior to Shanchol. This new vaccine was also non-inferior to Shanchol in the different age strata.

Conclusion: The safety and immunogenicity profile of the new OCV Hillchol is comparable to Shanchol in persons residing in a cholera-endemic setting. ClinicalTrials.gov number: NCT02823899.

1. Introduction

Cholera continues to be a major cause of morbidity and mortality in endemic low income countries including Bangladesh. It is estimated that there are at least 300,000 severe cholera cases and over 4500 cholera deaths in Bangladesh annually [1]. Since 2010 the WHO recommends the use of oral cholera vaccines

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(OCVs) in association with other control measures both for routine preventive use in high endemic settings and for intervention during cholera outbreaks [2,3]. Consequently, a global stockpile of OCV has been created by WHO since 2013 for use in both epidemic and endemic, as well as humanitarian crisis settings [4]. However, the global demand for OCV far exceeds the present supply for both epidemic and endemic settings [5] prompting the WHO to suggest the need for additional OCV manufacturers worldwide to meet the projected demands. Global OCV demand is expected to increase further as OCV is an essential component of the WHO led Global Task Force on Cholera Control's road map for ending cholera by 2030 [6,7]. According to the Gavi Alliance's Vaccine Investment Strategy, up to 660,000 future deaths and 26 million future cases could be averted in the period 2020–2035 using cholera vaccination campaigns for at risk populations.

Practically all cholera cases globally today are caused by *V. cholerae* of the El Tor biotype and the O1 serogroup with its two main serotypes, Ogawa and Inaba. Cholera caused by El Tor bacteria of the O139 serogroup which accounted for up to 10% of cholera in South-East Asia in the 1990 s are exceedingly rare today. The most important protective antigen for OCV induced protective immunity is the O1 lipopolysaccharide (LPS) antigen of the *V. cholerae* cell wall [5]. The Ogawa and Inaba serotypes differ only by the presence of terminal perosamine (a methoxyl group at position 2 in Ogawa but a hydroxyl group in Inaba) in the Ogawa LPS, which is absent in the Inaba serotype [8]. There is substantial immunological cross-reactivity between the O1 Ogawa and Inaba serotypes, but serotype-specific immunity also contributes to the immune protection. An effective OCV, therefore, should contain both Ogawa and Inaba O1 LPS antigens.

The available WHO prequalified OCVs (Dukoral®, Shanchol™, Euvichol®) [9] contain a combination of the same *V. cholerae* O1 strains of Inaba and Ogawa serotypes, and El Tor and classical biotypes. Dukoral® additionally contains the recombinantly produced B-subunit of cholera toxin (rCTB) which is lacking in Shanchol™ and Euvichol®. The two latter vaccines instead contain an additional *V. cholerae* strain of the O139 serogroup. These OCVs are relatively complex to manufacture due to their multiple strain composition and use of two different methods (heat or formalin) for inactivation of these strains. MSD Wellcome Trust Hilleman Laboratories Pvt. Ltd., in technical collaboration with University of Gothenburg and Gotovax AB, Sweden, have developed a new whole cell (WC) inactivated OCV, Hillchol® [10]. This vaccine consists of a single formaldehyde-inactivated recombinant Hikojima *V. cholerae* strain MS1568 generated from the El Tor *V. cholerae* O1 parent/ancestor Inaba strain 'Phil6973′ (a strain present in all the three current OCVs) [2]. Hillchol® offers significant advantages over the existing OCVs as it is based on a single *V. cholerae* O1 strain stably co-expressing the Inaba and Ogawa serotype LPS antigens, which is inactivated by only one (formalin) rather than two methods (heat and formalin). This single formulation as compared to the five formulations of available inactivated OCVs will translate into a substantially simplified and lower cost manufacturing process.

Preclinical oral immunogenicity studies in mice have demonstrated that Hillchol elicits strong intestinal IgA anti-LPS as well as serum vibriocidal and anti-LPS antibody responses against both Inaba and Ogawa that are fully comparable to the responses induced by the Shanchol™ OCV used as a side-by-side tested comparator vaccine [10]. Passive protection studies in infant mice also showed that immune sera raised against the Hikojima vaccine strain protected baby mice against infection with virulent strains of both serotypes [2].

The current phase I/II clinical trial in Bangladesh represents not only the first step of the clinical development of the Hillchol® OCV but also the first ever clinical study of a single strain OCV stably co-expressing Ogawa and Inaba LPS. The study was conducted to evaluate the safety, tolerability and immunogenicity of Hillchol® in sequentially age descending participants of healthy adults and children, in comparison with the WHO prequalified OCV Shanchol™, in an urban slum setting of Bangladesh. Since this was the first study of Hillchol® in humans two formulations of different dosage strengths were tested. The low dose formulation (not less than 600 μg/ml) was found to be inferior in terms of immunogenicity to both Shanchol™ and the high dose formulation (equivalent to the Shanchol™ dose). Thus it was decided to develop the high dose formulation further and this article pertains only to the high dose Hillchol® formulation.

2. Methods

2.1. Study participants and ethical considerations

This was a randomized, open-labeled safety and immunogenicity clinical trial of Hillchol® OCV conducted in Mirpur area of Dhaka, Bangladesh from July 2016 to May in 2017 (registered at ClinicalTrials.gov; registration no. NCT02823899). The WHO prequalified Shanchol™ OCV was used as the comparator. The study comprised in total 560 healthy participants, with 280 individuals receiving Hillchol® (test vaccine) and the other 280 receiving Shanchol™ (comparator vaccine), and was conducted in three age descending cohorts consisting of 240 participants of age 18–45 years (''adults''), 160 participants of age 5–17 years (''older children'') and160 participants of age 1–<5 years (''younger children''). Participants in each age cohort were randomized to receive either test or comparator vaccine. Only healthy non-pregnant individuals, as assessed by the study physician, were included. Individuals with diarrhea, abdominal pain or vomiting in the past 24 hours, or diarrhea lasting for more than 2 weeks in the past 6 months before start of study were excluded, as were individuals who were currently on antibiotic or immunosuppressive therapy and pregnant women (identified by the urine strip test on married women). Moreover, subjects who had ever taken OCV or any other live or killed enteric vaccine in the last 8 weeks or any confirmed cholera, salmonella, shigella and ETEC diarrhea (stool samples from the participants were collected and microbiological culture carried out within 7 days prior to enrollment in the study) were also excluded.

The trial protocol was approved by the research and the ethical review committees of the International Centre for Diarrhoeal Disease Research (icddr,b). An independent data and safety monitoring board reviewed the protocol and monitored the progress of the studies. Written informed consent was obtained from adult participants and from parents/guardians of children less than 18 years of age. Assent was also obtained from 11 to 17 years of age.

2.2. Sample size

Data from prior studies have shown that seroconversion rates, based on $\geq$ 4-fold rises in vibriocidal assay, among comparator vaccine Shanchol™ recipients were 60% in adults, 74% in older children and 84% in toddlers [11]. To establish non-inferiority of the test vaccine Hillchol® to the comparator vaccine Shanchol™, we needed a total of 492 participants to be able to reject the null hypothesis. The sample size was calculated to provide 90% power and significance level of 5%, to show serotype specific non-inferiority with a 20% margin (Hillchol is non-inferior to Shanchol then the 90% lower boundary of the seroconversion difference is not less than −20%) for the seroconversion rate estimated as a $\geq$ 4-fold vibriocidal response against *V. cholerae* O1 serotype. In

addition, to take a margin for an up to 10% attrition rate we enrolled a total of 560 participants (240 adults aged 18 – 45 years, 160 older children aged 5–17 years and 160 younger children 1–<5 years) [12].

2.3. Randomization and blinding

Three separate randomization lists, one for each age cohort, were generated by an independent statistician who was not part of this study. A total of 560 participants were randomized either to receive one dose of the test vaccine or the comparator vaccine per randomization list in ratio of 1:1.

2.4. Vaccines

Each dose of Hillchol® vaccine was composed of formaldehyde-inactivated *V. cholerae* O1 Hikojima bacteria of strain MS1568, containing approximately 1.0 mg total O1 LPS with approximately 50% each of the Ogawa and the Inaba serotypes [10]. This vaccine was developed by MSD Wellcome Trust Hilleman Laboratories Pvt. Ltd. in technical collaboration with University of Gothenburg, Sweden, and the Hillchol® vaccine doses used in the clinical study were manufactured under current Good Manufacturing Practices (cGMP), also meeting the WHO production guidelines at Incepta Vaccine Limited, Bangladesh with technological support from Hilleman Laboratories. Shanchol™ (Shantha Biotechnics-Sanofi Pasteur; lot SCN020A14, SCN010A15), which is licensed in India since 2009 and prequalified by WHO since 2011, contains approximately 1.5×10^{11} inactivated *Vibrio cholerae* O1 bacteria and 5×10^{10} inactivated *V. cholerae* O139 bacteria. It was found to contain an equivalent amount of total O1 LPS per dose (approximately 1.0 mg/ not less than 900 μg/ml, as determined by an inhibition ELISA method) as the Hillchol® OCV [10,13]. Both Shanchol™ and Hillchol® were presented in single-dose vials with a volume of each dose being 1.5 ml. Both vaccines were stored at 2–8 °C until administered.

2.5. Enrollment and vaccine administration

Participants were enrolled in the study in the age de-escalating manner. Study was initiated with the enrollment (day 0) and vaccination of adults (18–45 years of age), and thereafter vaccination safety data of the adult age cohort were compiled and submitted to the icddr,b DSMB for review. After review of the safety data and a go ahead from the DSMB the next age cohort of older children (5–17 years of age) was enrolled and vaccinated. Similarly, after review of the older children safety data by the DSMB and approval, the cohort of younger children (1–<5 years of age) was enrolled and vaccinated. Two doses of either test or comparator vaccine were administered orally at an interval of 2 weeks to every participant. Vaccine vials were shaken before administration to the participants. The participants were offered a cup of water after vaccination.

2.6. Surveillance for adverse events

Participants were observed for 30 min at the study center to monitor for any immediate adverse events (reactogenicity) after each dose of vaccination. Adverse events (AE) graded as detailed below were monitored actively for the first 3 days in all participants and passively up to 14 days after each vaccine dose by trained study staff. All participants were visited daily for three consecutive days to ascertain solicited symptoms (diarrhea, abdominal pain, nausea, weakness, fever, vomiting, itching, rash, cough, vertigo, dryness of mouth) and any other symptoms, using a structured questionnaire after each dose of vaccine. All reported AEs were graded as mild (no interference with daily activity), moderate (some interference with activity not requiring medical intervention) or severe (prevents daily activity and requires medical intervention). The causal relation of every AE to the study vaccines was determined by the study physician during reporting and reassessed by the 'Independent Safety Monitor Team(ISMT)' set up for the study by the icddr,b Institutional Review Board (IRB). This ISMT was consisted of two senior physicians who were not related or involved with the study.

2.7. Blood sample collection

In this study, venous blood samples were withdrawn from the participants prior to first vaccine dose, 14 days after first dose (prior to administration of second dose), and 14 days after administration of the second dose of study vaccine (day 0, day 14, day 28). A window of 3 days was provided for second dose administration and associated blood draw i.e. day 14 (+3 days), and also for blood draw after the second dose on day 28 (±3 days). Sera were separated from these blood samples and stored at −20 °C for immunological analyses (vibriocidal antibody assays) when samples at different time points had been collected.

2.8. Vibriocidal antibody assay

The vibriocidal antibody responses were analyzed at icddr,b by testing serial two-fold dilutions of pre- and postvaccination serum samples, using guinea pig complement (Sigma-Aldrich Chemie GmbH) and *V. cholerae* O1 Ogawa (X-25049) and Inaba (T-19479) as the target organisms using standard operating procedures [14]. The vibriocidal titer was defined as the reciprocal of the highest dilution resulting in $\geq$ 50% reduction in the optical density (reflecting the bacterial growth) compared to that of control wells without serum. Sero-conversion was defined as a $\geq$ 4-fold increase in vibriocidal titer from baseline to day 14 and day 28. To account for any variations, test plates also contained pooled convalescent plasma sample from patients with cholera as a positive control (pooled O1 Ogawa and O1 Inaba convalescent plasma from icddr, b's earlier collection of specimens from cholera patients) [15,16].

2.9. Statistical analyses

Data analyses were performed using R software [R Studio; Version 1.1.447]. Analyses for comparisons of AEs and seroconversion rates (the percentage of individuals who responded with a $\geq$ 4-fold increase in vibriocidal titer from baseline to day 14 and day 28) were performed using the chi-square test with Yates correction or by the Fisher's exact test if the numbers were sparse. For comparisons of vibriocidal titers, the Student's *t*-test for log-transformed titers was performed.

The primary endpoint of immunogenicity included titers of serum vibriocidal antibodies (against *V. cholerae* O1 Inaba and Ogawa serogroup) measured: before the first vaccination; 14 days after the first vaccination; and 14 days after the second vaccination. Based on these titers the following endpoints were computed: (1) Participants with 4-fold or greater rises in titers relative to baseline; and (2) Individual ratio of titers between Visit 2 (Day 14) and baseline (Day 0), and between Visit 3 (Day 28) and baseline (Day 0).

For assessing the non-inferiority of the Hillchol® test OCV in comparison with the Shanchol™ comparator vaccine, this was evaluated by calculating the difference in seroconversion rates between the Hillchol® and Shanchol™ vaccine cohorts from baseline to 14 days after the second dose. We confirmed here non-inferiority using 90% confidence interval (CI) for seroconversion rate difference and geometric mean ratio between test vaccine

and comparator vaccine. The Test vaccine is non-inferior than comparator vaccine if lower boundary of CI is equal to or greater than the margin of −20% and 0.50 for difference and ratio respectively.

3. Result

3.1. Study participants

Participants were enrolled into the study as illustrated in the consort diagram in Fig. 1. The total study cohort consisted of 560 participants comprising healthy adults (n = 240; 18–45 years, median age 30 years), older children (n = 160; 5–17 years, median age 11 years) and younger children (n = 160; 1–<5 years, median age 3 years). In total 193 males (Shanchol: 98; Hillchol: 95) and 367 females (Shanchol: 182; Hillchol: 185) were enrolled in the study. As per protocol 546 participants completed the study. No significant difference was found in age and sex distribution between the test and comparator vaccine groups (Table 1).

3.2. Adverse events

All 560 participants enrolled in the study were included in the safety analysis. The total number of solicited symptoms were similar among test 9.2% (90% CI; 7.3–11.5) and comparator vaccine groups 9.6% (90% CI;7.6–11.9) (Table 2). No serious adverse event was observed throughout the study period. The most common solicited AE reported was weakness in the adult age cohort (4.2% in Shanchol™ recipients and 3.3% in Hillchol® recipients), followed by vertigo (in 3.8% Shanchol™ recipients and 2.9% in Hillchol® recipients); among older children, it was nausea in Shanchol™ recipients (4.5%) and vertigo in Hillchol® recipients (3.8%); and in younger children it was diarrhoea and fever in the Shanchol™ recipients (3.8%) and vomiting and cough in Hillchol® recipients (3.1%). All AEs were mild in severity and resolved completely.

A total of 7 unsolicited AEs were reported during the course of the study; one was acute febrile illness, one acute febrile illness with common cold, one upper respiratory infection, one acute watery diarrhea, and three accidental cut injury cases. The occurrence rate of unsolicited AEs after 1st and 2nd doses among 7 participants (4 of these cases, 1.4%, were in the Shanchol™ group and 3 cases, 1.1%, were in the Hillchol® group) had no statistically significant difference. Based on severity, one acute watery diarrhea in the Hillchol group (defined as passage of three or more loose stool in 24 hrs or one to two or indeterminate number of loose stools with evidence of dehydration according to WHO criteria) and one accidental cut injury in the Hillchol group occurring on day 8 after

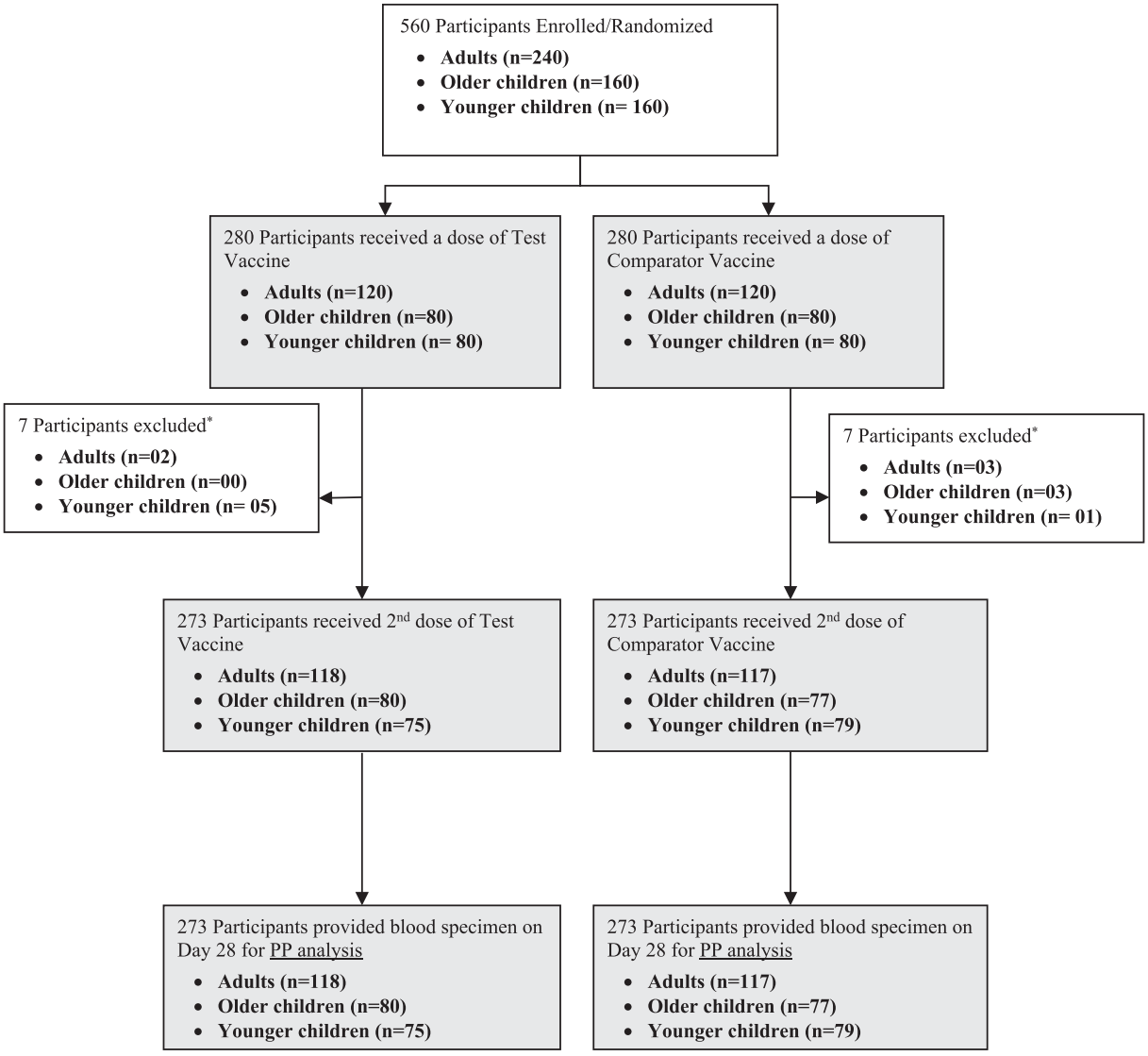

Fig. 1. Flow diagram of participant disposition (Consort Flow Diagram). * participant withdraw themselves or became pregnant before 2nd dose.

Table 1
Demographic characteristics of study participants.

Characteristics Overall		Test Vaccine (Hillchol) Group n = 280	Comparator Vaccine (Shanchol) Group n = 280	Total n = 560	p-value
Gender	Male (%)	95 (33.9)	98 (35.0)	193 (34.5)	0.859
	Female (%)	185 (66.1)	182 (65.0)	367 (65.5)	
Age (years)	Mean (SD)	16.9 ± 13.4	15.8 ± 12.8	16.9 ± 3.0	0.851
	Median (min, max)	12(1.44)	14(1,44)	13(1,44)	
Adult cohort		**n = 120**	**n = 120**	**n = 240**	
Gender	Male (%)	28 (23.3)	20 (16.7)	48 (20.0)	0.197
	Female (%)	92 (76.7)	100 (83.3)	192 (80.0)	
Age (years)	Mean (SD)	31.0 ± 6.9	30.0 ± 6.1	30.5 ± 6.5	0.268
	Median (min, max)	32(18,44)	30(18,44)	31(18,44)	
Older Children cohort		**n = 80**	**n = 80**	**n = 160**	
Gender	Male (%)	29 (36.3)	37 (46.3)	66 (41.2)	0.199
	Female (%)	51 (63.7)	43 (53.7)	94 (58.8)	
Age (years)	Mean (SD)	10.4 ± 2.9	11.2 ± 3.3	10.8 ± 3.1	0.094
	Median (min, max)	10(5,16)	11(5,17)	11(5,17)	
Younger Children cohort		**n = 80**	**n = 80**	**n = 160**	
Gender	Male (%)	38 (47.5)	41 (51.3)	79 (49.4)	0.365
	Female (%)	42 (52.5)	39 (48.7)	81 (50.6)	
Age (years)	Mean (SD)	2.7 ± 1.1	2.5 ± 1.0	2.6 ± 1.0	0.350
	Median (min, max)	3(1,4)	3(1,4)	3(1,4)	

Table 2
Solicited adverse events among the study participants for the Shanchol and Hillchol oral cholera vaccines.

Solicited AE(Within 3 days after 1st and 2nd dose)	Vaccine	N	Incidence	Percentage (%)	90% CI	p-value(χ^2-test)
All	Shanchol	553	53	9.6	7.6–11.9	0.858
	Hillchol	553	51	9.2	7.3–11.5	
Adults	Shanchol	237	23	9.7	6.7–13.5	0.454
	Hillchol	238	18	7.6	5.0–11.1	
Older Children	Shanchol	157	15	9.5	6.0–14.3	0.301
	Hillchol	160	22	13.7	9.5–19.1	
Younger Children	Shanchol	159	15	9.4	5.9–14.2	0.489
	Hillchol	155	11	7.1	4.0–11.5	

vaccination were considered as "severe" and one acute febrile illness, one acute febrile illness with common cold, and one upper respiratory infection, were considered as "moderate", and the remaining two cut injury events were graded as "mild". None of the AEs, whether solicited or unsolicited, were found to be related to the study vaccines by the study investigator.

3.3. Vibriocidal antibody responses

A total of 560 participants received at least one vaccine dose and provided the blood sample for estimation of baseline vibriocidal antibody titers. A second dose of vaccine was given to 546 participants, from whom blood samples were collected for estimation of immune response after first or second dose vaccine. The immune response was measured by a vibriocidal antibody assay, and was further characterized as seroconversion rates (as defined earlier) and for different cohorts also as Geometric Mean Titers (GMTs) at each visit. The seroconversion rates among all participants irrespective of age group in the different study arms Hillchol® vs. Shanchol™ for O1 Ogawa (59% vs. 67%; lower bound 90% CI of difference: −14.55) (Table 3) and Inaba (70% vs. 71%, lower bound 90% CI of difference: −7.24) serotypes, i.e. the primary endpoints of the study, showed that the overall immunogenicity of the Hillchol® OCV was non-inferior to that of Shanchol™ (Table 4).

The vibriocidal antibody responses in the three age cohorts following two doses of Hillchol® were also similar to those of Shanchol™. Seroconversion rates for Hillchol® and Shanchol™ recipients to *V. cholerae* O1 Ogawa in adults they were 51% vs. 67% (lower bound 90% CI of difference: −26.42), in older children 63% vs. 66% (lower bound 90% CI: −16.49) and in younger children 69% vs. 68% (lower bound 90% CI : −11.7) (Table 3) and to *V. cholerae* O1 Inaba were in adults 64% vs. 69% (lower bound 90% CI: −15.11), in older children 76% vs. 75% (lower bound 90% CI: −10.59), and in younger children 72% vs. 68% (lower bound 90% CI: −8.54) (Table 4). All seroconversion rates for Hillchol® were non-inferior to Shanchol™ except the O1 Ogawa for adults.

There was no statistically significant difference in the baseline vibriocidal titers to Ogawa (48.27 vs. 43.78 to Ogawa; lower bound 90% CI of ratio 0.86) and to Inaba (36.51 vs. 31.91; lower bound 90% CI of ratio 0.90) in terms of GMTs in participants receiving Hillchol® or Shanchol™ vaccines (Table 5 and 6). After either vaccine administration, the vibriocidal GMTs were also similar in both groups.

GMTs after receiving two doses of test or comparator vaccine were 241.41 and 275.48 against Ogawa and 284.00 and 255.28 against Inaba. The ratio of GMTs after two doses being 0.88 (lower bound 90% CI of ratio; 0.7) against Ogawa and 1.11 (lower bound 90% CI of ratio; 0.9) against Inaba (Table 5 and 6). When GMTs of vibriocidal antibodies were compared on an age cohort basis, those elicited by Hillchol® were found to be non-inferior to the GMTs induced by Shanchol™ as noted against both Ogawa and Inaba (Table 5 and 6).

4. Discussion

This is the first ever report of clinical testing of an OCV which is based on an inactivated *V. cholerae* O1 single strain (Hikojima 1568) co-expressing the Inaba and Ogawa O1 antigens. The study was conducted in a high-risk cholera endemic setting in urban Bangladesh. This new vaccine Hillchol® was tested among the par-

Table 3
Vibriocidal antibody seroconversion rates to *V.choleare* O1 Ogawa with Shanchol and Hillchol OCV for all age groups.

	Post first vaccine dose (day 14)				Post second vaccine dose (day 28)			
	n	Seroconversion rate n(%)	Difference (90% CI)	p-value	n	Seroconversion rate n(%)	Difference (90% CI)	p-value
All age								
Shanchol	273	206(75.46)	Ref	–	273	183(67.03)	Ref	–
Hillchol	273	186(68.13)	−7.33(-13.82,-0.83)	0.064	273	162(59.34)	−7.69(-14.55,-0.84)	0.065
Adult								
Shanchol	117	93(79.49)	Ref	–	117	78(66.67)	Ref	–
Hillchol	118	78(66.1)	−13.39(−23.18,-3.59)	0.025	118	60(50.85)	−15.82(-26.42,-5.22)	0.014
Older Children								
Shanchol	77	60(77.92)	Ref	–	77	51(66.23)	Ref	–
Hillchol	80	65(81.25)	3.33(-7.8,14.46)	0.622	80	50(62.5)	−3.73(-16.49,9.02)	0.629
Younger Children								
Shanchol	79	53(67.09)	Ref	–	79	54(68.35)	Ref	–
Hillchol	75	43(57.33)	−9.76(−22.83,3.32)	0.219	75	52(69.33)	0.98(-11.7,13.66)	0.899

Note: Non-inferiority of Test vaccine is confirmed if the lower limit of two-tailed 90% CI of the difference of seroconversion rates between Test vaccine and Comparator vaccine is equal to or greater than the non-inferiority margin of −20%

Table 4
Vibriocidal antibody seroconversion rates to *V.choleare* O1 Inaba with Shanchol and Hillchol OCV for all age groups.

	Post first vaccine dose (day 14)				Post second vaccine dose (day 28)			
	n	Seroconversion rate n(%)	Difference (90% CI)	p-value	n	Seroconversion rate n(%)	Difference (90% CI)	p-value
All age								
Shanchol	273	218(79.85)	Ref	–	273	193(70.7)	Ref	–
Hillchol	273	215(78.75)	−1.1(-6.89,4.7)	0.755	273	191(69.96)	−0.73(-7.24,5.77)	0.853
Adult								
Shanchol	117	99(84.62)	Ref	–	117	81(69.23)	Ref	–
Hillchol	118	91(77.12)	−7.5(-15.98,0.99)	0.146	118	76(64.41)	−4.82(-15.11,5.46)	0.44
Older Children								
Shanchol	77	64(83.12)	Ref	–	77	58(75.32)	Ref	–
Hillchol	80	69(86.25)	3.13(-6.64,12.91)	0.597	80	61(76.25)	0.93(-10.59,12.44)	0.895
Younger Children								
Shanchol	79	55(69.62)	Ref	–	79	54(68.35)	Ref	–
Hillchol	75	55(73.33)	3.71(-8.57,15.99)	0.618	75	54(72)	3.65(-8.54,15.84)	0.622

Note: Non-inferiority of Test vaccine will be confirmed if the lower limit of two-tailed 90% CI of the difference of seroconversion rates between Test vaccine and Comparator vaccine is equal to or greater than the non-inferiority margin of −20%

Table 5
Geometric mean titers (GMT) of vibriocidal antibodies to *V.choleare* O1 Ogawa for all age groups.

	Day 0		Day 14		Day 28	
	GMT	GMR (90% CI)	GMT	GMR (90% CI)	GMT	GMR (90% CI)
All age						
Shanchol	43.78	Ref	388.11	Ref	275.48	Ref
Hillchol	48.27	1.1(0.86,1.41)	350.63	0.9(0.71,1.15)	241.41	0.88(0.71,1.08)
Adult						
Shanchol	84.88	Ref	733.43	Ref	456.59	Ref
Hillchol	115.83	1.36(0.98,1.9)	758.86	1.03(0.81,1.32)	474.32	1.04(0.83,1.3)
Older Children						
Shanchol	50.98	Ref	606.35	Ref	347	Ref
Hillchol	43.24	0.85(0.55,1.31)	422.24	0.7(0.49,0.99)	228.24	0.66(0.48,0.91)
Younger Children						
Shanchol	14.08	Ref	97.89	Ref	104.09	Ref
Hillchol	13.69	0.97(0.67,1.42)	85.35	0.87(0.53,1.44)	88.56	0.85(0.54,1.33)

Note: Non-inferiority of Test vaccine will be confirmed if the lower limit of two-tailed 90% CI of the ratio of GMTs between Test vaccine and Comparator vaccine is equal to or greater than the non-inferiority margin of 0.5

ticipants in an age descending order and was found to be as safe in all age groups (adults 18–45 years, older children 6–17 years and younger children 1–5 years) as the WHO prequalified comparator vaccine Shanchol™ whose safety has been established previously in endemic settings [11,17,18]. No serious adverse events related to either of the study vaccines were observed during the entire study period, but a few non-serious adverse events were reported. Overall the symptoms were found to be similar in both vaccine study groups. The excellent safety profile of the new vaccine in different age groups being similar to that of Shanchol™ supports its potential for use in individuals above 1 years of age.

The immunogenicity of the Hillchol® as compared to that of the Shanchol™ vaccine in the study participants was determined by measuring the serum vibriocidal antibody responses, which is an

Table 6
Geometric mean titers (GMT) of vibriocidal antibodies to *V.choleare* O1 Inaba for all age groups.

	Day 0		Day 14		Day 28	
	GMT	GMR (90% CI)	GMT	GMR (90% CI)	GMT	GMR (90% CI)
All age						
Shanchol	31.91	Ref	402.15	Ref	255.28	Ref
Hillchol	36.51	1.14(0.9,1.46)	424.18	1.05(0.83,1.34)	284	1.11(0.9,1.37)
Adult						
Shanchol	64.25	Ref	764.48	Ref	440.64	Ref
Hillchol	87.37	1.36(0.95,1.94)	853.46	1.12(0.87,1.43)	524.13	1.19(0.94,1.5)
Older Children						
Shanchol	33.64	Ref	657.52	Ref	356.5	Ref
Hillchol	28.53	0.85(0.55,1.3)	480.84	0.73(0.52,1.03)	278.58	0.78(0.58,1.06)
Younger Children						
Shanchol	10.73	Ref	96.19	Ref	82.13	Ref
Hillchol	12.03	1.12(0.76,1.65)	123.52	1.28(0.74,2.22)	110.55	1.35(0.83,2.19)

Note: Non-inferiority of Test vaccine will be confirmed if the lower limit of two-tailed 90% CI of the ratio of GMTs between Test vaccine and Comparator vaccine is equal to or greater than the non-inferiority margin of 0.5

indirect surrogate measure for cholera immune protection [19,20]. The baseline vibriocidal titers were found to be similar for Hillchol® and Shanchol™ in all age groups except in the Hillchol adult group, where the baseline titer was higher than that seen in the 'Shanchol' group in Ogawa serotype . As in previous epidemiologic studies unrelated to vaccination and vaccination studies conducted in the endemic settings using Shanchol™ [21], the baseline vibriocidal titers were highest among the adults and lowest in the younger children, probably reflecting the progressive environmental exposure to *V. cholerae* O1 over time and for the youngest children possibly also a lesser ability to respond to a B cell independent carbohydrate antigen such as the LPS O antigen [22,23].

In this study, serum vibriocidal antibody responses against *V. cholerae* O1 Inaba and Ogawa organisms after two doses were comparable for both vaccines in all three age cohorts. The overall frequencies of vibriocidal antibody responses to *V. cholerae* O1 Inaba and Ogawa induced by Hillchol® was 70% and 59% as compared to 71% and 67% by Shanchol™. These seroconversion rates are similar to those previously reported in response to the Shanchol™ comparator vaccine in other clinical trials in different countries. In Bangladesh, the previously reported seroconversion rates induced by two doses of Shanchol™ OCV against *V.cholerae O1* Inaba and Ogawa respectively were 73% and 75%; in Kolkata, India, 69% and 56%; and in Ethiopia 70% and 65% [11,24,25].

However, only the Ogawa results for adults were inferior to the comparator vaccine in the seroconversion analysis. We noticed that in the 'Hillchol' adult group, the base line titer was higher than that seen in the 'Shanchol' group. This can have arisen due to chance and also probably a larger sample size would have been useful to understand this better.

The fold rises of GMTs observed here in response to Hillchol® and Shanchol™ were also comparable to those previously achieved in endemic settings for cholera with the Shanchol™ OCV in the countries mentioned above [11,24,25].

The serum vibriocidal responses to both Hillchol® and Shanchol™ decreased after second dose as compared to responses generated after the first dose. Similar findings have been reported previously where high vibriocidal titers obtained after the first dose were found to suppress the immune response to the second dose [1,11,26]. These findings might be attributed to a blocking function of the activated immune system in the gut on antigen uptake after the first dose thereby resulting in decreased serum vibriocidal titers in response to the second dose as well as to the fact that the vibriocidal antibodies to the largest extent is of the IgM isotype [17]; in contrast, the intestinal IgA antibody response usually increases substantially after the second as compared to the first dose [27,28] as does the protective efficacy [29].

The new vaccine Hillchol® is composed of a single Hikojima strain co-expressing the Ogawa and Inaba serotypes and as such, and based on our preceding preclinical studies [10], was expected to elicit a potent immune response (vibriocidal response) against these two serotypes. The generation of the observed strong immune responses by Hillchol® against these two serotypes in this study are particularly encouraging considering the fact that that either of the two serotypes can cause severe cholera in epidemics and cholera outbreaks. Furthermore, in Bangladesh and elsewhere the seasonal shift between these serotypes is very common.

This new generation of killed whole cell OCV represented by Hillchol® would be more affordable among the poorer population in both the endemic and epidemic settings, since its manufacturing process is much simpler and cost effective while conferring similar levels of protection as generated by the currently licensed OCVs. In the latter OCVs the use of several strains and two inactivation methods makes their manufacture complex and relatively expensive. Although the comparator vaccine, Shanchol™, has reduced its price 1.96 $/dose [30] compared to its first-generation vaccine, the Hillchol® vaccine could cost well below 1USD/dose which has been determined by the vaccine manufacturing company.

Recently, WHO has focused on the use of OCVs to control cholera in both endemic "hotspots" and in ongoing or predicted outbreaks where rapid action is required [31]. The Global Task Force on Cholera Control (GTFCC) in 2017 launched a road-map to reduce cholera deaths by at least 90% and to eliminate cholera in at least 20 countries by 2030 in which the wide use of OCV is a cornerstone in the strategy [6]. These recommendations may be more easily and expeditiously implemented with this less expensive OCV. From the initiation of the stockpile in 2013 through 2019, more than 50 million doses of OCV were deployed through campaigns in some 25 different countries [32]. However, the global demand for the vaccine far exceeds the supply for both epidemic and endemic settings [33]. Globally, the OCV is in short supply and will not be able to meet the projected demands. In order to increase the supply of OCV, more manufacturers are needed and initiatives are being planned to increase the global supply of OCVs. Based on the current and emerging OCV supply needs, the development of the inexpensive, single-strain and single-formulation Hillchol® OCV, and the highly promising results of this clinical study are an important step towards filling some of the global OCV supply gap. In this trial, Hillchol® was found to be as safe and immunogenic as Shanchol™ which is an important step towards getting Hillchol® licensed and WHO prequalified. With the recent licensing of Hillchol® manufacturing technology to Bharat Biotech International Limited, a major Indian vaccine manufacturer and planned expanded Phase 3 safety and immunogenicity study, Hillchol®

should become available to meet the growing OCV demand of the lower income countries where cholera remains as a major cause of disease and deaths.

The findings of this phase I/II study suggest that the formalin-killed single Hikojima strain MS1568 in Hillchol® is as safe and immunogenic as the mixture of strains and formulations in currently licensed OCVs. Overall, the presented results suggest that this novel vaccine Hillchol® will be able to elicit comparable levels of antibody titers against prevalent serotypes of cholera in the target population(s) to at least the same extent as achieved with the currently licensed inactivated whole-cell OCVs. As such and given its predicted very low cost and very large-scale manufacturing potential it represents a novel, effective and affordable cholera vaccine fully accessible to the people who need it the most.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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