Int.J.Curr.Microbiol.App.Sci (2023) *12*(07): 107-118

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 7 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1207.013

Oral Cholera Vaccine (Shanchol) is Effective to Prevent Cholera: A Message Learnt from a Post Vaccination Diarrhoea Surveillance Study

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Keywords

Cholera, *Vibrio cholerae*, diarrhoea, cholera vaccine, rotavirus, sanitation

Article Info

Received:
08 June 2023
Accepted:
04 July 2023
Available Online:
10 July 2023

A B S T R A C T

A diarrhea surveillance study was conducted after a mass vaccination with a new Oral cholera vaccine Shanchol, in India. During the study period between 2011-2013, a total of 4050 vaccinated, non-vaccinated resident and traveler diarrhoea patients were examined for different etiologial agents. Among the pathogens, toxigenic *E. coli* (15.15%), *V. cholera* (3.38%) and rotavirus (29.12%) were found to be main organisms. The isolation frequencies of *V. cholera* in vaccinated diarrhea patients, in non-vaccinated diarrhea patients in vaccinated area, in non-vaccinated diarrhea patients in neighboring vaccinated area and in non-vaccinated traveler diarrhea patients from other states were 0.72% (4 of 550), 2.3% (12 of 517), 3.7% (109 of 2885) and 12.2% (12 of 98) respectively. The isolation of *V. cholerae* from vaccinated diarrhea patients is significantly less than the non-vaccinated traveler diarrhea patients which indicate that reduction of cholera among vaccinated people might be due to immunization with Shanchol. Development of herd immunity may be responsible for less isolation of *V. cholerae* among non-vaccinated residents in vaccinated area compared to non-vaccinated traveler diarrhea patients. It is suggested to vaccinate the vulnerable population using Shanchol to prevent cholera infection in Puri, in other parts of the country and globe.

Introduction

Acute diarrhoeal disease is a major public health threat throughout the world with over two million deaths occurring each year and affecting mostly children under 5 years age in developing countries (Kosek *et al.*, 2003; Bryce *et al.*, 2005). This disease is very common in developing countries due to lack of proper hygiene, sanitation and safe drinking water. The etiological agents causing acute

diarrhoea include a wide range of viruses, bacteria and parasites. Among different diarrhoea causing bacterial pathogens, *V. cholerae* has been recognized as an important etiological agent followed by diarrhea genic *E. coli*, shigella spp, salmonella spp. Aeromonas spp etc. Cholera, a rapidly dehydrating diarrhoeal disease is caused by toxigenic *V. cholera* O1 and O139 serogroups. In recent years variants of *V. cholerae*O1 such as El Tor variant and Haitian variants harboring *ctxB1* and *ctxB7* genes respectively has emerged and causing most of the cholera outbreaks in cholera endemic areas in developing countries.

Diarrhoea has been documented as one of the important health problems in Odisha, a coastal eastern Indian state leading to large morbidity and substantial mortality following natural calamity like cyclone and flood. Several studies have reported toxigenic *V. cholerae* is the important causative agent of diarrhoeal every year followed by other toxigenic bacterial pathogens in Odisha (Chhotray *et al.,* 2002; Pal *et al.,* 2010; Pal *et al.,* 2009; Khuntia *et al.,* 2013; Pal *et al.,* 2017). Puri, a holy city on the coast of Bay of Bengal in Odisha is well known for occurrence of diarrhoeal diseases throughout the year affecting the local residents as well as the travelers. Prehistoric knowledge discerns, cholera continues to pose a major threat in Puri since antiquity particularly during Car Festival of Lord Jagarnath due to huge congregation of devotees. Our previous study has reported cholera is endemic in Puri district which is comprised of 11 administrative Blocks. (Samal *et al.,* 2008).

In order to prevent potential threats of cholera outbreaks, WHO recommends use of available oral cholera vaccine (OCV) in conjunction with other preventive and/or control measures in cholera endemic areas as well as areas at risk of outbreaks (Cholera, 2013). A community based study in Kolkata reported OCV a bivalent cholera vaccine 'Shanchol" gives 65% protection against cholera (Bhattacharya *et al.,* 2013). Prior to implication of mass vaccination; its feasibility, acceptability and cost effect is essential to be determined. In order to evaluate these components, Regional Medical Research Center (RMRC), Bhubaneswar and IVR, Korea conducted a collaborative vaccination campaign in Satyabadi Block in Puri district, during May and June 2011 where 31552 cholera vulnerable people were vaccinated with OCV.

After vaccination, a follow up diarrhea surveillance study was conducted with an aim to document the incidence of *V. cholerae* and spectrum of other etiological agents causing diarrhea among vaccinated and non-vaccinated residents in Satyabadi, residents in other blocks in Puri district and travelers from other states during post vaccination period from July 2011 to March 2013.

Materials and Methods

Study design and sampling

This study was conducted by RMRC in five health facilities in Puri district namely primary health care centers (ie. Alugum and Sukala PHC), SakhigopalAarea Hospital in Satyabadi Block, Infectious Disease Hospital (IDH) and Pediatric ward of District Head Quarter Hospital located near Satyabadi Block in Puri district. The cholera ward in IDH and Pediatric ward in District Head Quarter Hospital has capacity of 33 and 20 beds respectively. The other three health facilities provide healthcares to the local rural communities. Most of the patients who admit in IDH and pediatric ward are from municipality and rural areas of Puri district head quarter and referral cases from other health facilities in the district.

Diarrhoea is defined using the WHO guidelines: the presence of three or more loose, liquid or watery stools or at least one bloody loose stool passed in 24 hours (WHO, 2000). Rectal swabs were collected in Cary-Blair transport (CBT) medium from hospitalized patients with acute diarrhoea of all age groups to detect bacterial pathogens. A batch of separate watery stool samples was collected from hospitalized pediatric diarrhoea patients for identification of rotavirus. Every day samples were

collected by trained healthcare staffs from hospitalized diarrhoea patients before administration of antibiotics and transported at room temperature to microbiology laboratory in RMRC to process within 4 hours of collection. Stool samples were collected after taking consent from parents or legal guardians. Demographic information (age and sex) and clinical symptoms (fever, vomiting and dehydration) were recorded for each patient using a structured questionnaire. Dehydration (severe, moderate, no dehydration) was classified by hospital doctors according to the WHO guidelines. The study protocol was approved by the Institutional Ethics Committee.

Pathogen detection, identification and isolation

Rectal swabs were sub-cultured on MacConkey agar (BD) for the isolation of *E. coli* spp, Hektoen enteric agar (BD) for the isolation of Shigella spp and Salmonella spp. and Aeromonas isolation agar for the isolation of Aeromonas spp. For isolation of *V. cholerae* rectal swabs were initially inoculated directly on thiosulphate-citrate-bile salt sucrose (TCBS) agar (BD, Sparks, Md, USA) followed by enrichment in alkaline peptone water (APW) for 4 to 6 hour at 37^0C and p^H 8.6. Bacterial isolates were identified following the protocol of the World Health Organization manual 1987 (WHO, 1987). Serotyping of *V. cholerae* was done using slide agglutination with polyvalent and mono- valent antisera specific for Ogawa and Inaba (BD, USA) as described elsewhere (Chhotray *et al.,* 2002). *V. choleare* isolates those agglutinated with O1 antisera were bio-typed as described elsewhere (Taneja *et al.,* 2009). Sucrose non-fermenting colonies appeared on TCBS agar were screened for identification of *Vibrio parahemolyticus* and confirmed by standard cultural and biochemical characterization. Toxigenic *E. coli* strains were identified using PCR assays for specific genes in EPEC, ETEC, EHEC and EAggEC following the method as described elsewhere (Chhotray *et al.,* 2002). A 10% fecal suspension of each sample in phosphate-buffered saline was processed for rotavirus antigen detection by enzyme-linked immunosorbent assay (ELISA) using the Ridascreen kit (R-Biofarm, Germany as described elsewhere (Lyde *et al.,* 2013). ELISA was performed according to manufacturer's instruction. Test results were validated with appropriate positive and negative controls.

Detection of genotypes of *V. cholerae* by PCR assay

Fifty representative strains of *V. cholerae* O1were grown in Luria-Bertani broth (Difco) at 37^0 C and template DNA was extracted in LB bacterial suspension as described previously (15) followed by storage at -20^0C. *V. cholerae* O1strains were screened for the presence of various genes including genes encoding cholera toxin sub-unit A (*ctxA*), somatic antigen (*wbe*), toxin co-regulated pilli El Tor/classical (*tcpA* El Tor/ classical), toxin regulatorR (*ToxR*), zonnulaocluden toxin (*zot*), and accessory cholera entero-toxin (ace) (Khuntia *et al.,* 2008; Khuntia *et al.,* 2000). Mismatch amplification mutation assay (MAMA) PCR was performed to detect the *ctxB* classical and/or El Tor harbored by *V. cholerae* O1 serogroups using specific *ctxB* primer pairs as described earlier (Morita *et al.,* 2008). Standard *V. cholerae* O1 classical (569B) *V. cholerae* O1 El Tor (VC20) were used as positive control provided by National Institute of Cholera and Enteric Diseases (NICED) Kolkata, India. Double-mismatch-amplification mutation assay (DMAMA) PCR-based assay was employed to discriminate the classical, El Tor, and Haitian types of *ctxB* alleles (CTB genotype7) using primers (*ctxB*-F3/Rv-cla) as described elsewhere (Naha *et al.,* 2012).

Results and Discussion

A total of 4050 rectal swabs were analyzed for detection of various enteropathogens during the study period. Of the total 4050 studied diarrhea patients, 3952 were residents of Puri district and 98 were travelers from other state in India. Out of these total 3952 diarrhoea patient, 550 were vaccinated diarrhea patients (VDP), belonged to Satyabady

block. The remaining were non- vaccinated diarrhea patients (NVDP) include 517 from the same Satyabadi block and 2885 from other blocks in Puri district. All 98 traveler were non-vaccinated diarrhea patients from other states. The age distribution of 4050 diarrhea patients were such that 990 (24.4%) patients were younger than 1 year of age, 803 (19.82%) were between 1 to 5 year of age, 74(1.82%) between 5 to 10 years, 85 (2.1%) between 10 to 15 years and 2098 (51.8%) patients were older than 15 years of age with nearly same proportion of males and females. (2.1: 1.8).

The incidence of enteropathogenic bacteria in total patients comprising of vaccinated and non-vaccinated people is summarized in Table1. Of the total samples, 776 swabs collected from pediatric diarrhea patients (<15years) were analyzed for both bacterial entero-pathogens and rotavirus. One or more bacterial pathogens were detected in 877 (21.65%) cases in 4050 diarrhoea patients (Table1&2). Analysis revealed that diarrhoeogenic *E. coli* were predominantly isolated followed by *V. cholerae* O1 serogroup.

Genes indicating *E. coli* pathogroups were found in 618 (15.25%) patients followed by *V. cholerae* 137 (3.38%), Shigellaspp 72(1.7%), Salmonella spp 3(0.07%), *V. parahaemolyticus* 7 (0.17%), *V. cholerae* non-O1 and non-O139 16 (0.39%) and *Aeromonas hydrophila* 24 (0.59%). Of the *E. coli* pathotypes detected, the most frequent isolate was ETEC 243 (6%) followed by EPEC 188 (4.64%), EHEC 125 (3%), EAggEC 62 (1.5%) in all age groups. Of the 72 Shigella isolates 41 were *S. flexenery*, 18 were *S. boydii* and 13 were *S. Sonnei*. All the three Salmonella spp were *Salmonella typhimurium*. The frequency of bacterial pathogens among vaccinated and 3 non-vaccinated groups was more or less close to each other Table1.

Age wise distribution of enteropathogenic bacteria in diarrhoea patients is presented in Table2. Although no significant difference was observed in the incidence of total bacterial enteropathogens in different age groups however significant variations in frequency of isolation rate of some organisms were detected. Higher frequencies of EPEC strains were significantly associated with <1 year's children compared to other elder patients except 1-10 years children ($P<0.005$). Although not significant, however higher frequencies of *V. cholerae* were observed in patients of above 5 years age.

The incidence of total bacterial mix infection in those patients from whom the enteropathogens were isolated was significantly higher for patients aged 1 to 5 years of age (21.63%; 37 of 171), 10 to 15 year (12.5%; 3 of 24) and >15 years (12.13%; 50 of 412) than for patients younger than 1 year of age (4.38%; 11 of 251) ($P< 0.05$) (Table3). The most frequent pattern of mixed infection was the combination of ETEC with EPEC strains (48; 5.6%).

Analysis of 776 samples from children revealed the incidence of total rotavirus was 226 (29.12%) out of which rotavirus as a single pathogen was 111 (14.3%) and mix infection of rotavirus and bacterial pathogens was 115 (14.8%) (Table4). The age wise incidence of rotavirus revealed 26.94% (118 of 438) were from patients younger than 1 year of age, 34.51% (107 of 310) were from those of aged 1 to 5 years of age and 3.44% (1 of 28) belonged to 5-10 years age group.

The incidence of co-infection of rotavirus and bacterial pathogens among the specimen was 14.81% (115 of 776). The most frequent pattern of mix infection was combination of rotavirus with EPEC 6% (46 of 776), followed by ETEC 3.8% (28 of 776) and EHEC 3.4% (27 of 776). In mix infection category no *V. cholerae* association was detected with rotavirus.

Of the total 111 rotavirus as a single viral pathogen, 16.4% (72 of 438), 12.6% (38 of 310) and 3.44% (1 of 28) were encountered in children <1 year, 1-5 year and 5-10 years age group respectively. Rotavirus was rarely detected (3.44%) in the age group of 5-10 years while no rotavirus was detected above 10 years old children and adult diarrhea patients. Although higher incidence of rotavirus as a

single viral pathogen was detected among the children of < 1year than 1-5 year age, however no significant difference was observed.

The incidence of *V. cholerae* O1 from VDP and NVDP is shown in the Table1.The analysis revealed, 137(3.38%) samples were positive for *V. cholera* O1, Ogawa, El Tor biotype. All the strains of *V. cholerae* were detected with *ctxA, tcpA* El Tor biotype, *zot* and *ace* genes which confirmed their toxigenic capacity and epidemic potential and the presence of *wbe* provided their molecular evidence of O1 serogroup. Of the total 50 representative tested *V. cholerae* O1, 12 were El Tor variant as these were positive for VP, chicken cell agglutination test and resistant to Polymyxin B and carried *ctxB,* classical gene (*ctxB1*) while remaining 38 *V. cholerae* O1 strains were prototype El Tor biotype. Haitian variants of *V. cholerae* O1 harboring *ctxB7* were not isolated during the study period.

The incidence of *V. cholerae* among VDP and NVDP in Satyabadi block, NVDP in other 10 blocks in Puri district and NVDP travelers was 4 (0.72%), 12 (2.3%), 109 (3.77%) and 12 (12.24%) respectively. Although no significant difference was observed in isolation rate of *V. cholera* in vaccinated and two groups of non vaccinated dirrhoea patients of Puri district, however higher isolation rate of *V. cholerae* was observed in non vaccinated groups (2.3% and 3.77%) compared to vaccinated group (0.72%) Table1. The isolation of *V. cholerae* 12.24% (12/98) in traveler diarrhea patients coming from other states is significantly higher than the vaccinated (0.72%), non vaccinated patients (2.3%) from Satyabadi block and non-vaccinated patients (3.77%) from other blocks in Puri district ($P<0.05$).

Diarrhoea continues to occur frequently in Puri with large morbidity and substantial mortality that peaks in rainy season. The IDH and other hospitals in Puri have rare diagnostic laboratory facilities to identify the etiological agents of diarrhea. Report of enteropathogens associated with diarrhoeal disease in Puri, a tourist hub has high significance for implementation of appropriate control measures that will aid to restrict the spread of pathogens to other part of country and to other countries through travelers. In our previous study conducted in three hospitals in Odisha including IDH in Puri, we have reported *V. cholerae*, DEC, Sigellaspp, Salmonella spp, and Aeromonasspp are the causative agents of diarrhea in Odisha (Samal *et al.,* 2008).

However this is the first comprehensive surveillance study in Puri which was designed to detect new additional pathogens such as VP, NAG and rotavirus in addition to the previously reported bacterial enteropathogens and among these findings rotavirus was frequently detected in children causing acute diarrhea. At least one bacterial pathogen was encountered among 21.65% of all diarrhea patients in our study. We found different bacterial pathogens including DEC, *V. cholerae*, Shigellaspp, Salmonella spp, Aeromonasspp, VP and NAG. Additionally for the first time rotavirus and its co-infection with bacterial pathogens were most frequently detected supporting the well-documented role of rotavirus in childhood diarrhoeal disease in Puri as in some other developing countries (Bonkoungou *et al.,* 2013; Nitiema *et al.,* 2011; Benhafid *et al.,* 2006). Moreover the effect of OCV on incidence of *V. cholerae* among the vaccinated and non-vaccinated population was highlighted during this study period.

Among different types of pathogens associated with diarrhoeal infection, DEC is known to be the commonest reported etiology in India (Rathaur *et al.,* 2014). DEC is more frequent next to rotavirus causing diarrhea among 30 to 40% of all diarrhoeal episodes among children below five years age in developing countries (O'Ryan *et al.,* 2005; Clarke, 2001; Nair *et al.,* 2010) while DEC-mediated diarrhoeal infection is recognized next to cholera in all age groups. In a previous study DEC was reported to be responsible for 25% of all diarrhoeal diseases in developing countries (Niyogi *et al.,* 1994).

Table.1 Distribution of bacterial pathogens in vaccinated and non-vaccinated patients in studied patients

Bacterial pathogens	Vaccinated patients in SB N=550 (%)	Non-Vaccinated patients in SB N=517 (%)	Non vaccinated patients in other blocks, n=2885 (%)	Non-vaccinated Travellers N=98 (%)	Total N=4050 (%)
V. cholerae	4 (0.72)	12 (2.3)	109 (3.77)	12(12.24)	137(3.38)
***Vibrio cholera* non-O1 & non-O139**	3(0.54)	8(0.77)	5 (0.27)	-	16 (0.39)
VP	1 (0.18)	1 (0.19)	4 (0.13)	1(1.03)	7(0.17)
ETEC	29 (5.3)	33 (6.38)	178 (6.1)	3(3.09)	243(6)
EPEC	26 (4.7)	25 (4.8)	135 (4.6)	2(2.06)	188 (4.64)
EHEC	21 (3.8)	17 (3.2)	87 (3)	-	125 (3)
EAggEC	11 (2)	8 (1.54)	43 (1.5)	-	62(1.5)
Shigellaspp	6 (1.1)	6(1.16)	56 (1.94)	4(4.12)	72(1.7)
Salmonella spp	-	1(0.19)	2 (0.07)	-	3(0.07)
Aeromonasspp	3 (0.54)	3 (0.58)	17 (0.58)	1(1.03)	24((0.59)
Total	104(18.9)	114(22)	636 (22)	23 (23.46)	877 (21.65)

SB-Satyabadi block

Table.2 Incidence and Age wise distribution of bacterial enteropathogens

(n=3919)

Bacterial pathogen	<1 year (n=990)	1-5 Year (n=803)	5-10year n=74	10-15years N=85	>15year (n=2098)	P value	Total (n=4050)
V. cholerae	9 (0.9%)	14 (1.74%)	4(5.4)	8(9.4)	102(4.86%)		136 (3.35%)
ETEC	41(4.1%)	40(5%)	3 (4)	4(4.7)	155(7.38%)		243 (6%)
EPEC	101(10.2%)	31(3.8%)	5(6.7)	2(2.35)	49(2.3%)		188 (4.6%)
EHEC	41 (4.1%)	51(6.3%)	2(2.7)	3(3.52)	28 (1.33%)		125 (3%)
EAggEC	29(2.9%)	21 (2.6%)	1(2.7)	2(3.52)	9 (0.42%)		62 (1.53%)
Shigellaspp	14(1.4%)	8 (0.1%)	2(2.7)	4(4.7)	44(2.09%)		72 (1.77)
Salmonella spp	-	-	-	-	3 (0.0.14%)		3 (0.0.07)
Aeromonasspp	13 (1.3%)	5(0.62%)	1(1.3)	1(1.17)	4 (0.19%)		24 (0.59%)
VP	-	-	-		7 (0.33%)		7 (0.17%)
NAG	3(0.1)	1(0.12)	-		12(0.57%)		16 (0.39%)
Total	251(25.35)	171(21.3)	18(25.7)	24(29.4)	413 (19.68%)		876 (21.62%)

Table.3 Patients with bacterial mix-infection from a single specimen in Puri district

Bacterial pathogens	< 1 year (n=251)	1-5 Year (n=171)	5-10 Year n=18	10-15Year n=24	>15year (n=412)	Total (n=876) (%)
Vibrio+ EPEC	2	4	-	1	5	12(1.47)
Vibrio+ ETEC	1	3	-	1	6	11(2.44)
Shigellaspp+EPEC	2	1	1	-	5	9(1.22)
Shigellaspp+ETEC	1	3	-	1	12	17(2)
EPEC+ETEC	4	25	1		18	48(5.6)
NAG+ EPEC	-	-	-		1	1 (0.12)
Aero+EPEC	1	1	-		1	3 (0.24)
Aeromonas+ETEC	-	-	-		2	2 (0.24)
Total	11 (4.38%)	37 (21.63%)	2 (11.1%)	3 (12.5%)	50 (12.13%)	103 (11.75%)

Table.4 Patients with rotavirus and its mix infection with bacterial pathogens isolated from a single specimen in children below 10 years age.

N=776

	Number of patients in	following age groups			
Pathogens	<1 year (n=438)	1-5 year (n=310)	5-10 (n=28)	P Value	Total (n=776)
Total rotavirus	118 (26.94%)	107 (34.51%)	1 (3.44%)		226(29.12%)
Rotavirus as single pathogen	72 (16.4%)	38 (12.6%)	1		111(14.30%)
Rotavirus plus followings			-		
ETEC	7 (1.6%)	21(6.8%)	-		28 (3.7%)
EPEC	21 (4.8%)	25 (8%)	-		46 (6.1%)
EHEC	11 (2.5%)	16 (5.1%)	-		27 (3.6%)
EAggEC	2 (0.46%)	4 (1.3%)	-		6(0.8%)
Shigella	3 (0.7%)	2 (0.6%)	-		5 (0.5%)
Aeromonas	2 (0.46%)	1 (0.3%)	-		3(0.4%)
Total mix infection	46(10.5%)	69(22.25%)			115(14.81%)

The predominant pathogen detected in our study was DEC 15.25% (618/4050) in all age groups which is more than the reports from other developing countries (Ochoa *et al.*, 2009; Estrada-Garcia *et al.*, 2009). Although other bacterial enteropathogens contribute for causing diarrhea however in the present study these pathogens have less significance.

Group-A rotavirus is most commonly associated with childhood diarrhea and death with worldwide distribution (Kawai *et al.*, 2012). Rotavirus has been associated with approximately one quarter of all diarrhoea in South Africa (Steele *et al.*, 2003; Mohanty *et al.*, 2017). Three previous studies in Odisha reported the prevalence of rotavirus was

26%, 54% and 30% in diverse area (Shrivastava *et al.*, 2017; Mohanty *et al.*, 2017; Shrivastava *et al.*, 2019). In the present study we found 29.12% rotavirus was associated with childhood diarrhea in Puri. Similar to other studies conducted in Odisha (Shrivastava *et al.*, 2017) our study also revealed high rotavirus incidence in 1-5 years children, while very negligible rotavirus was encountered among children of >5 years (3.4%).

Combinations of enteropathogens causing mix infection in acute diarrhea have been reported in many studies (Nitiema *et al.*, 2011; Shrivastava *et al.*, 2017; Yamashiro *et al.*, 1998; Albert *et al.*, 1999; Li *et al.*, 2016). Some studies from Africa have reported that almost one-third of studied diarrhea cases were infected with bacterial pathogens and about ten percent of them were co-infection (Bonkoungou *et al.*, 2013; Nitiema *et al.*, 2011). In our study we observed that among total bacterial infection, 13.4% were bacterial co-infection where EPEC with ETEC and Vibrio with ETEC were more frequently isolated. Rotavirus co-infection with other bacterial pathogen and protozoa has been reported previously (Zhang *et al.*, 2016). A recent study from China reported viral–bacterial co-infection in <5-yearold children (Li *et al.*, 2016). In our study we observed that among the total rotavirus infection 14% was encountered as a single pathogen and 15% have mix infection with bacterial pathogens. Among these mix infection, rotavirus with EPEC was detected more frequently than ETEC and EHEC. Very interestingly no mix infection of *V. cholerae* and rotavirus was observed. This signifies, probably *V.cholerae* was dominating the active infection inhibiting the survival of rotavirus. The report of mix-infection of enteropathogen associated with acute diarrhea in many studies is a subject of debate regarding which pathogen is causing diarrhea. It might also that multiple micro-organisms might be acting synergistically to produce episode of diarrhoea.

Our diarrhea surveillance study conducted in IDH reveals that the pe-vaccination incidence rate of *V. cholerae* was 13%, 12%, 18%, 13% and 14% during the years 2005-2006 (Samal *et al.*, 2008) and 2007-2009 respectively (unpublished data). In the present study the post-vaccination incidence rate of *V. cholerae* was found to be 0.7%, 2.1%, 3.9% and 12.24% among VDP, NVDP in Satyabadi block, NVDP in adjacent blocks in Puri district and NVDP traveler respectively. Average of 5 years (2005-09) pre-vaccination incidence rate of *V. cholerae* (14%) in IDH, Puri is significantly higher than the incidence rate in vaccinated diarrhea patients in Satyabady. Similarly the incidence rate of *V. cholerae* in NVDP traveler is significantly higher than the VDP. A case control study after vaccination in vaccinated area demonstrated that the efficacy of vaccine was 69% (Wiezba *et al.*, 2015). The significant lower isolation of *V. cholerae* from VDP compared to isolation from NVDP during pre-vaccination (2005-09) period and NVDP traveler during post-vaccination period again corroborate the effectiveness of OCV for decline of cholera cases in vaccination population.

In the present environmental water sample analysis study conducted during the same study period isolated *V. cholerae* from surface water in study area (unpublished data). On the other hand people in study area are taking bath in the community pond and using water for cooking, drinking and other domestic use from the same pond without having any evidence of cholera. This results highlight that although *V. cholerae* is present in the aquatic environment which could have been transmitted to human intestine by drinking contaminated water but very few cases and no outbreak was reported during this period. Altogether the results highlight that although *V. cholerae* is present in aquatic environment no cholera occurred which could be due to the effect of OCV.

Development of herd immunity in local unvaccinated residents belonging to the adjacent vaccinated community can protect from cholera infection has been variously discussed (Khatib *et al.*, 2012; Ali *et al.*, 2013). In this respect, the observation in our study reveals that the isolation rate of *V. cholerae* among unvaccinated community

in Puri district is significantly less than the previous year's (2005-09) incidence rate and the incidence in traveler diarrhoea patients. Thus it can be hypothesize that decrease of cholera among vaccinated population could be due to vaccine effect while among unvaccinated population in Satyabadi and adjacent Blocks, herd-immunity might have attributed for reduced isolation of *V. cholerae*. This can be presumed that in vaccinated Block non-vaccinated people also got less infection because of heard immunity and hence restricted the transmission of cholera to other non-vaccinated residents in the neighboring Blocks. This is also evident from the significant difference noted with the non-vaccinated area with regard to cholera incidence. The effect of vaccine is more evident when compared the isolation of *V. cholerae* from travelers who came from out of the state and have not vaccinated. However more detailed study is required to conclude the action of herd-immunity in protecting cholera occurrence in connection to the unvaccinated people.

This study illustrates the spectrum of major enteropathogens causing diarrhea in all age groups of hospitalized acute diarrhea patients in Puri district. Rotavirus was identified as the potential etiological agent causing diarrhea among children and considered as an important public health threat in Puri. Most importantly the study observed that OCV (Shanchol) has direct and indirect influence on vaccinated and neighboring non-vaccinated residents in reduction of cholera incidence.

Despite some weakness of the study, this data can strengthen the knowledge of the health authority for development and implementation of diarrhea control and prevention program in Puri. However further long term study is required to document long-term trends and population based disease profiles to acquire in-depth knowledge on diarrhea illness and assess any intervention program effectively.

Cholera vaccine (Shanchol) and rotavirus vaccine have already been established their potentiality to prevent the diarrhea. It is hence suggested to introduce vaccination program to immunize the people in Puri, in other parts of the country and globe to prevent from cholera.

Funding

No funding was received from any funding agencies to carry out the study

Data availability

The data used to support the findings of this study are available with the corresponding author upon request.

Conflict of Interest

The authors declare that there exists no conflict of interest regarding the publication of the manuscript.

Acknowledgement

The authors are thankfully acknowledged the doctors and paramedical staffs of the hospitals for their whole heated cooperation during sample collection in hospital. We are also extending thanks to the Director General, ICMR, New Delhi for his full support to carry out this study.

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How to cite this article:

Hemant Kumar Khuntia, Bhagyalaxmi Biswal, Shantanu Kumar Kar, Bhagirathi Dwibedi, Jyostnamayee Sabat, Prasanta Kumar Bramha, Sanghamitra Pati and Anna Salomi Kerketta. 2023. Oral Cholera Vaccine (Shanchol) is Effective to Prevent Cholera: A Message Learnt From A Post Vaccination Diarrhoea Surveillance Study. *Int.J.Curr.Microbiol.App.Sci.* 12(07): 107-118.

doi: https://doi.org/10.20546/ijcmas.2023.1207.013