







Variants of *ctxB* alleles of *Vibrio cholerae* O1 caused sequential cholera outbreaks in the tribal areas of Odisha, India

Bibhuti Bhusan Pal , Smruti Ranjan Nayak , Ashish Kumar Nayak , Dipti Ranjan Behera , Swatishree Pany  and Sanghamitra Pati 

Microbiology Division, ICMR-Regional Medical Research Centre, Chandrasekharpur, Bhubaneswar 751023, Odisha, India

*Corresponding author. E-mail: bbpal_rmrc@yahoo.co.in

 BBP, 0000-0001-6338-4425; SRN, 0000-0002-2807-6037; AKN, 0000-0002-9639-8820; DRB, 0000-0001-7605-3342; SP, 0000-0002-4146-6064; SP, 0000-0002-7717-5592

ABSTRACT

Cholera localized outbreaks/epidemics accounting for high morbidity and mortality have been reported in different years both from the coastal and tribal districts of Odisha. In the present study, the emergence and spread of two sequential cholera outbreaks reported in July to October 2012 from Rayagada and Kalahandi districts of Odisha was investigated. Environmental water samples from different sources and rectal swabs from diarrhoea patients were analysed for identification, antibiogram profiles and molecular studies using DMAMA-PCR assays. The pulsed field gel electrophoresis (PFGE) was done on some selected *Vibrio cholerae* O1 strains isolated from these cholera outbreak areas. Results showed 42% of rectal swabs and 2.3% of water samples collected from both the districts were positive for *Vibrio cholerae* O1 Ogawa biotype El Tor carrying both *ctxB1* and *ctxB7* genotypes. The common resistance profile of *V. cholerae* O1 strains was ampicillin, nalidixic acid, furazolidone and co-trimoxazole. The PFGE analysis on selected *V. cholerae* O1 strains of *ctxB1* and *ctxB7* genotypes showed three pulsotypes with 96% similarity matrix exhibiting the relationship with their respective water sources. Hence, continuous surveillance is highly essential to monitor the antibiogram profile and changing pattern of *ctxB* genotypes of *V. cholerae* O1 in this region.

Key words: antibiogram profiles, *ctxB* genotypes, pulsotypes, *Vibrio cholerae*

HIGHLIGHTS

- The study reports the presence of *V. cholerae* O1 Ogawa biotype El Tor in 42% of rectal swabs and 2.3% of environmental water samples respectively.
- The DMAMA-PCR assay revealed that the cholera outbreak in Rayagada district was predominantly due to *ctxB1* allele; whereas it was due to *ctxB7* allele of *V. cholerae* O1 El Tor variant strains reported in Kalahandi district.

INTRODUCTION

Vibrio cholerae is an autochthonous environmental species existing in zooplankton, phytoplankton, shrimps, crabs, etc. It infects human beings causing cholera resulting in high morbidity and mortality in developing countries (Lacey 1995). *V. cholerae* has >200 serogroups based on the somatic O antigens, of which, only O1 and O139 have caused outbreaks and epidemics throughout the world. The *V. cholerae* O1 has two biotypes, namely, classical and El Tor. Several variants of El Tor biotypes of *V. cholerae* have been identified based on *ctxB* genotypes which differed by a few non-random point mutations that yielded various *ctxB* alleles from *ctxB1* to *ctxB9* (Faruque *et al.* 1998; Safa *et al.* 2010; Keymer & Boehm 2011). The current seventh pandemic of cholera that started in Indonesia during 1961 was due to El Tor biotype. This biotype spread to Africa in 1971 and the Americas in 1991. Subsequently, the altered El Tor biotypes of *V. cholerae* O1 that carried the classical *ctxB1* allele emerged in 2002 and first reported from Bangladesh (Nair *et al.* 2006). Moreover, a new variant of *ctxB* genotype (*ctxB7*) of *V. cholerae* O1 was reported after the disastrous cholera outbreak in Haiti in 2010 (Piarroux *et al.* 2011). The appearance and the gradual dissemination of the *ctxB7* allele of *V. cholerae* O1 were reported from Kolkata in 2006 (Naha *et al.* 2012). Later, the El Tor variant strains carrying *ctxB7* allele of *V. cholerae* O1 were also reported from Nepal during 2012, eastern Africa (Kenya, Tanzania and Uganda) during 2015, and the ongoing cholera epidemic in

This is an Open Access article distributed under the terms of the Creative Commons Attribution Licence (CC BY 4.0), which permits copying, adaptation and redistribution, provided the original work is properly cited (<http://creativecommons.org/licenses/by/4.0/>).

Yemen (Dixit *et al.* 2014; Weill *et al.* 2019). The altered El Tor vibrios were also reported from cholera outbreaks from tribal areas of Odisha during 2007 and 2010 (Pal *et al.* 2010; Kar *et al.* 2015), whereas the *ctxB7* alleles of *V. cholerae* O1 were first reported from Odisha during 1999 and caused a cholera outbreak in 2014 in the tribal areas of Odisha (Pal *et al.* 2017). In between, two sequential cholera outbreaks were reported in July to October 2012 accounting for high morbidity from Rayagada and Kalahandi districts of Odisha. However, the same cannot be reported to date due to the delay in molecular analysis and pulsed field gel electrophoresis (PFGE) profiling of isolated *V. cholerae* O1 strains from these outbreak areas. Thus, the present study was undertaken to find the antibiogram profiles and genomic diversities of the *V. cholerae* O1 strains isolated from the above cholera outbreaks reported in the tribal areas of Odisha.

MATERIALS AND METHODS

Study area and demography

Rayagada district is divided into 11 blocks with a population of 967,911 that consists mainly of tribes like the Kondhas and the Sauras. Kalahandi with a population of 1,576,869 is the neighbouring district bordered to the south-east by the Rayagada district (Figure 1). Its major tribes are Shabar, Kondhas and Gond. Kalahandi district has been sub-divided into 13 blocks under two sub-divisions, i.e., Bhawanipatna and Dharmagarh. The tribal population mostly lives in inaccessible hilly and mountainous areas on the forest outskirts. These tribes are mostly illiterate, poor and lack properly treated water supplies, roads and sanitation facilities. Open defecation practices are common in these areas which favour diarrhoeal pathogens to spread and cause the outbreaks reported in different time periods. Cholera-affected blocks of Rayagada district, namely, Kashipur, Kalyansingpur, Gudari, Gunupur (Jagannathpur village) and Rayagada were studied along with Jaipatna, Junagarh and Kalampur blocks from Kalahandi district.

Determination of index case

The diarrhoea survey work was carried out in eight affected blocks of Rayagada and Kalahandi districts during the onset of disease in July until the disease subsided in October 2012. Line listing of all diarrhoea cases from hospital records, index

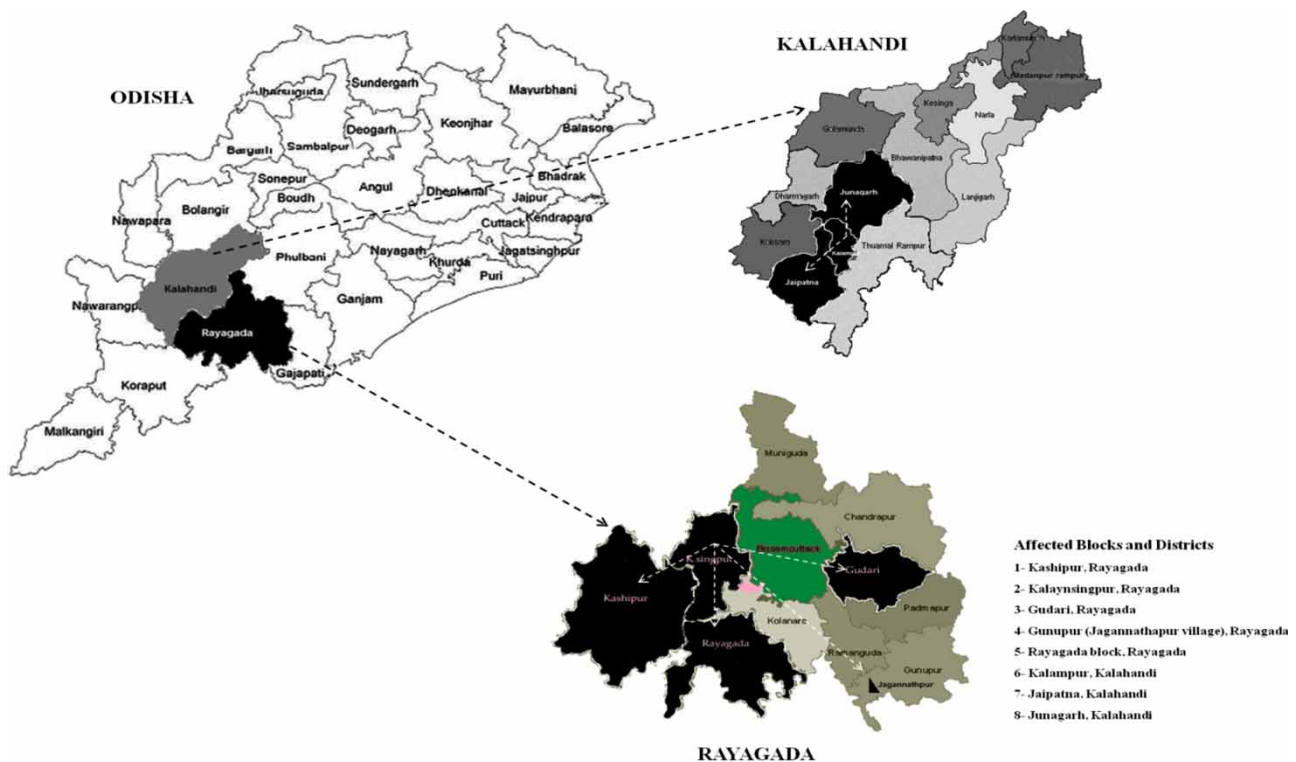


Figure 1 | Map of Odisha showing severe diarrhoea-affected blocks of Rayagada and Kalahandi districts.

cases, sources of drinking water, possible cause and spread of the disease, hygienic condition of the houses, social stigmas and misbeliefs existing among the villagers of each affected block were recorded.

Sample collection

The rectal swabs were collected from diarrhoea patients admitted to the Primary Health Centres (PHCs) and Community Health Centres (CHCs), and also from villages of the eight affected blocks of Rayagada and Kalahandi districts after obtaining the informed consent either from the patient or his/her attendant before the administration of any antibiotics. The rectal swabs from the diarrhoea patients were collected in Cary-Blair transport medium and transported to the laboratory for further analysis. Patients were subdivided into four age groups, i.e., >0–5 years, >5–14 years, >14–40 years and >40 years. Simultaneously, environmental water samples were also collected from the stream, river, chua (small pond in paddy field/pit on the river or stream bed) and nala (small stream) that were used by the local people and also the household water from different affected villages of Rayagada and Kalahandi districts.

Bacteriology

The rectal swabs and environmental water samples were enriched in alkaline peptone water (APW) and streaked on thiosulfate-citrate-bile salts-sucrose (TCBS) agar (BD, USA) followed by 24 hr incubation at 37 °C for selective isolation of *V. cholerae*. Sucrose fermenting yellow colonies were selected from TCBS plates and tested using standard biochemical tests. Serological confirmation was performed using *V. cholerae* specific polyvalent O1 and monovalent Ogawa and Inaba antisera (WHO 1983; Pal *et al.* 2010). The sensitivity and resistance patterns of *V. cholerae* O1 strains were tested using antibiotic impregnated commercial discs (BD, USA) (Bauer *et al.* 1966). The antibiotics used were ampicillin (AMP, 10 µg), co-trimoxazole (COT, 25 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), furazolidone (FR, 50 µg), gentamicin (GEN, 10 µg), nalidixic acid (NA, 30 µg), norfloxacin (NX, 10 µg), streptomycin (S, 10 µg), tetracycline (TE, 30 µg), neomycin (N, 30 µg), azithromycin (AZM, 15 µg), ofloxacin (OF, 5 µg), doxycycline (DO, 30 µg) and erythromycin (E, 15 µg).

CtxB genotyping by DMAMA-PCR assay

DMAMA (double mismatch amplification mutation assay) PCR assay was performed for the differentiation between classical *ctxB* and Haitian *ctxB* alleles of *V. cholerae* O1 strains using primers (*ctxB*-F3/ Rv-cla) for the Haitian *ctxB* allele and (*ctxB*-F4/Rv-cla) for the classical *ctxB* allele (Naha *et al.* 2012).

Pulsed-field gel electrophoresis (PFGE)

PFGE analysis of *NotI*-digested genomic DNA from representative isolates of *V. cholerae* O1 from both the districts was performed for subtyping the *V. cholerae* by the CHEF Mapper System (Bio-Rad, USA) according to the PulseNet standardized PFGE protocol (1% PFGE grade agarose in 0.5× TBE buffer). Run condition was generated by an auto-algorithm mode of the CHEF Mapper and the PFGE system was comparable to a size range of 20–300 kb markers for *V. cholerae* strains. The gel was stained in 10 µg/mL ethidium bromide solution for 30 min, de-stained in distilled water for 15 min and photographed under UV light in an Alpha Imager (Alpha InfoTech Corporation, USA). The analysis of the PFGE profiles of *V. cholerae* O1 isolates was compared using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). The similarities between the isolates were evaluated using the Dice coefficient method. Cluster analysis was carried out using the unweighted-pair group method using average linkages (UPGMA) (Cooper *et al.* 2006).

RESULTS

Index case

The cholera outbreak information was collected from villagers of the cholera-affected villages and, after analysing the hospital records, the index cases were traced back to Kalayansingpur block of Rayagada district, where the diarrhoeal outbreak started. One 65-year-old male from Singari village of Kalyansingpur block went to the paddy field on 20 July 2012 in the early morning, drank water from the small stream and returned home in the afternoon of the same day and suffered from loose motions and vomiting with fever. Gradually the cases increased in that block and the last case was noted on 6 October 2012. Then the disease spread to adjacent blocks, namely, Kashipur, Jagannathpur village of Gunupur block, Rayagada block with the outbreak subsiding in Gudari block on 15 September 2012. These blocks accounted for 263 cases (total population of five blocks was 351,532) with no deaths; the incidence rate was 0.07% with zero case fatality rate.

Similarly, three blocks of Kalahandi district, namely, Kalampur, Jaipatna and Junagarh were affected by diarrhoea. By analysing the hospital records and discussions among the villagers it was noted that one 50-year-old female from Jampara village of Kalampur block went to the field to work on the morning of 22 July 2012 and worked there up to the afternoon, drank water from the nearby river and suffered from loose motions and vomiting. Then, she washed her dirty clothes in the nearby river, which might have been the source and spread of infection. Gradually the diarrhoea cases spread to Jaipatna block on 28 July 2012 and then cases were reported from Junagarh block until 28 August 2012, accounting for 378 cases (total population of three blocks was 384,115) with two deaths. The incidence rate was 0.09% and the case fatality rate was 0.5%. The line listing of all diarrhoea cases is presented in Figure 2.

The line listing of all diarrhoea cases reported in both the districts indicated that the number of cases started increasing from the month of July and declined in October. The highest cases were reported on 12 September 2012 from Rayagada and on 9 August 2012 from Kalahandi district. The age-wise distribution of all cholera cases of both districts indicated that the >15–60 years age group was more affected than those of <5 years of age. The gender-wise distribution of cholera cases indicated that females were more infected than males in the ratio 1.3:1 (Figure 3).

Bacteriological analysis

Out of 221 rectal swabs collected from diarrhoea patients of Rayagada and Kalahandi districts, 93 (42%) were confirmed as *V. cholerae* O1 Ogawa. Thirteen (2.3%) out of 567 water samples collected from different contaminated sources from both the districts were also positive for *V. cholerae* O1 Ogawa, biotype El Tor (Table 1).

Antibiotic susceptibility assay

The results of antimicrobial susceptibility testing exhibited different resistance profiles of *V. cholerae* O1 Ogawa isolated from these two districts. The *V. cholerae* isolates obtained from cholera-affected blocks of Rayagada exhibited minor variations in antibiotic resistance profiles as noticed from different blocks. The common resistance profile of *V. cholerae* O1 in both the districts was ampicillin, nalidixic acid, furazolidone, co-trimoxazole, streptomycin and chloramphenicol. The common sensitivity profile to different antibiotics was ofloxacin, ciprofloxacin, norfloxacin, neomycin, gentamicin, azithromycin, tetracycline, and doxycycline with minor changes reported in Rayagada district. Details of the antibiogram resistance profiles are presented in Table 1.

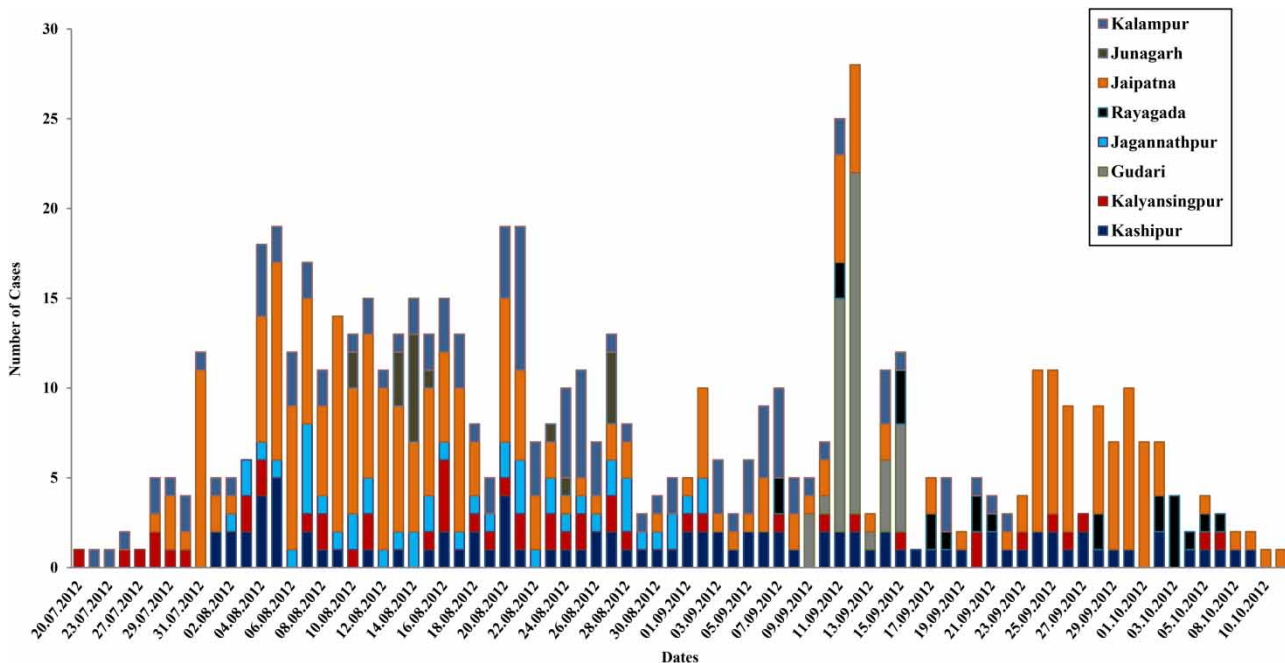


Figure 2 | Date-wise incidence of diarrhoea cases in both Rayagada and Kalahandi districts: July to October 2012.

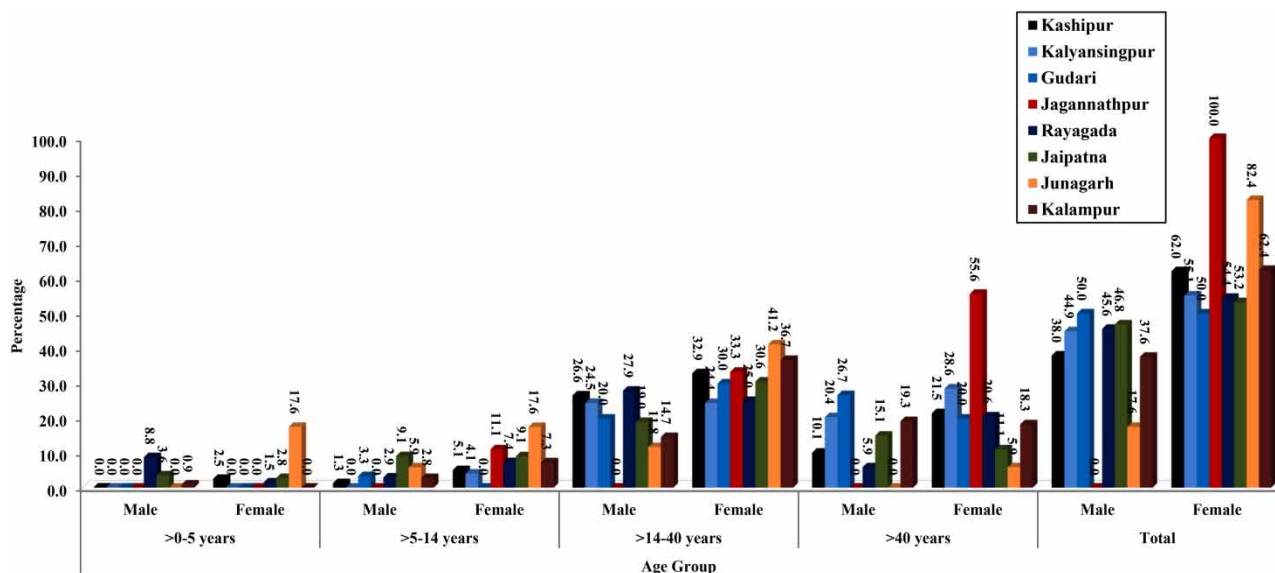


Figure 3 | Age/Sex-wise distribution of diarrhoea cases in both Rayagada and Kalahandi districts: July to October 2012.

Table 1 | Block-wise analysis of rectal swabs collected from diarrhoea patients from Rayagada and Kalahandi districts: July to October 2012

Districts	Blocks	July Cholera cases/ Total samples	August Cholera cases/ Total samples	September Cholera cases/ Total samples	October Cholera cases/ Total samples	Cholera cases /Total samples (%)	Antibiotics resistance profile
Rayagada	Kashipur	0	7/33	2/21	0	9/54 (16.6)	AM, NA, FR, E, C, COT
	Kalyansingpur	4/7	7/17	3/7	6/32	20/63 (30.3)	AM, NA, FR, E, S, COT
	Gudari	0	0	24/27	1/3	25/30 (83.3)	AM, NA, FR, E, S, COT
	Gunupur (Jagannathpur village)	0	8/8	0/1	0	8/9 (88.8)	AM, NA, FR, E, S, COT
	Rayagada	0	0	7/8	2/5	9/13 (69.2)	AM, NA, FR, C, S, COT
	Total		4/7 (57.1)	22/58 (37.9)	36/64 (51.7)	9/40 (22.5)	71/169 (42.0)
Kalahandi	Jaipatna	0/16	5/14	0	0	5/14 (35.7)	AM, NA, FR, C, S
	Junagarh	0	1/3	0	0	1/3 (33.3)	AM, NA, FR, C, S
	Kalampur	0/10	10/14	5/15	1/6	16/35 (45.7)	AM, NA, FR, C, S
	Total	0/26	16/31 (51.6)	5/15 (33.3)	1/6 (16.6)	22/52 (42.3)	

AM, ampicilin; NA, nalidixic acid; FR, furazolidone; E, erythromycin; COT, co-trimoxazole; S, streptomycin; C, chloramphenicol.

DMAMA-PCR assay

The DMAMA PCR assay on all the *V. cholerae* O1 isolates from both the districts revealed that out of 53 isolates from Rayagada district, 43 (81.1%) were positive for the classical *ctxB1* allele of *V. cholerae* O1 and 10 (18.9%) were *ctxB7* allele of Haitian variants. Interestingly, out of 20 *V. cholerae* O1 isolates from Kalahandi district, 18 (90%) were positive for *ctxB7*, possessing the Haitian variant cholera toxin (Table 2).

Table 2 | Distribution of *ctxB1* and *ctxB7* alleles of *V. cholerae* O1 isolated from stool and water samples from two districts

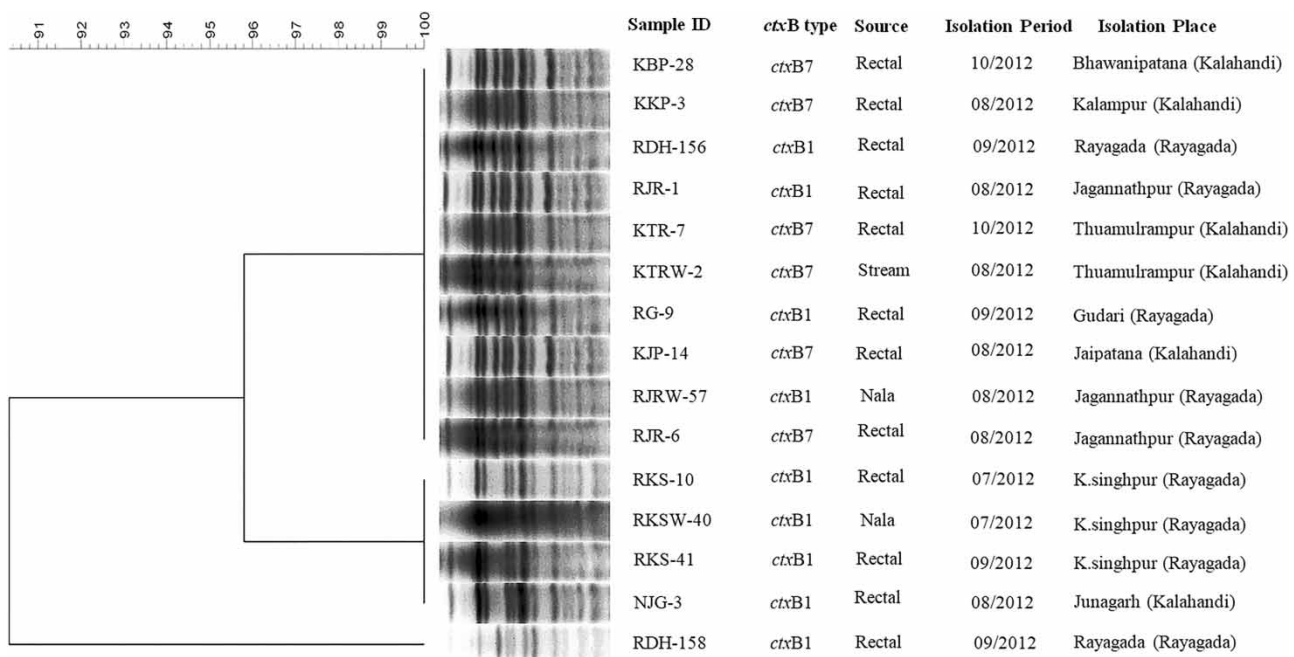
Rayagada			Kalahandi		
No. of <i>V. cholerae</i> O1 Ogawa	<i>ctxB1</i> n (%)	<i>ctxB7</i> n (%)	No. of <i>V. cholerae</i> O1 Ogawa	<i>ctxB1</i> n (%)	<i>ctxB7</i> n (%)
Stool (<i>n</i> = 47)	37 (78.7)	10 (21.3)	Stool (<i>n</i> = 17)	1 (5.8)	16 (94.1)
Water (<i>n</i> = 6)	6 (100)	0	Water (<i>n</i> = 3)	1 (33.3)	2 (66.6)
Total (<i>n</i> = 53)	43 (81.1)	10 (18.9)	Total (<i>n</i> = 20)	2 (10)	18 (90)

PFGE analysis

Interesting results were obtained while comparing the PFGE analysis on *V. cholerae* O1 strains isolated from these districts, where three pulsotypes were noticed. The clinical and water isolates from Rayagada and Kalahandi districts were of one pulsotype (Bhawanipatna, Kalampur, Rayagada, Jagannathpur, Thuamulrampur, Gudari and Jaipatna) with different *ctxB* genotypes. The clinical and water isolates from Kalyansingpur block with the Junagarh isolates of *V. cholerae* O1 were of a different clone and belonged to *ctxB1* genotype only (Figure 4). This indicated that the water isolates of *V. cholerae* O1 had a similar clone with the clinical isolates showing its source of infection.

DISCUSSION

The earlier cholera outbreaks in published studies from Rayagada district during 2007 and 2010 indicated that Kashipur, Kalyansingpur and Bissam Cuttack blocks were involved (Pal *et al.* 2010; Kar *et al.* 2015). However, the present cholera epidemic was reported from other blocks like Rayagada, Gudari, Gunupur (Jagannathapur village) and also from Kashipur and Kalyansingpur blocks with 351,532 people at risk. Similarly, the cholera outbreak reported from Narla block of Kalahandi district during 2014 was different from the outbreak of 2012, where other blocks like Kalampur, Junagarh and Jaipatna were affected with 384,115 people at risk. The contamination of water sources is the main cause of transmission of cholera. During the rainy season, people go to the paddy field for farming, drink water in the field from nala, chua, stream, river, etc. and become infected this way. After returning home they suffer from loose motions, vomiting and fever. Unhygienic

**Figure 4** | PFGE analysis of different *ctxB* genotypes of *V. cholerae* O1 strains isolated from stool and water samples from Rayagada and Kalahandi districts.

conditions of the houses, person-to-person contact and migration of people from one village to another were noticed among the cholera-affected villages from both the districts. This might be the source of the spread of infection from house to house, village to village and different blocks in these areas. This was also published in our previous studies. The water samples collected from nala, stream and river were found positive for *V. cholerae* O1, which was also reported in 2007, 2010 and 2014 from the same area (Pal *et al.* 2010, 2017; Kar *et al.* 2015).

From these cholera outbreaks, it was noticed that the female population in the age group >15–60 years was more prone to cholera than males with a male:female ratio 1:1.3. Females were more in contact with contaminated water in their day-to-day life, from cooking in the home to farming in the field, where they depended on nala, chua and stream water for their daily work and livelihood. This may be the reason for more females being infected than males in these tribal areas. The current findings have coincided with earlier reports from very different settings such as Glasgow (1832), Indonesia (2005), Kenya (1990) and developed countries like the UK, USA and Canada in 2005, where adult women and school-aged girls had consistently higher rates of diarrhoeal disease and cholera than their male counterparts (Tornheim *et al.* 1990; Fauveau *et al.* 1991; Agtini *et al.* 2005; Scallan *et al.* 2005).

The *V. cholerae* O1 isolates from the 2007 cholera epidemic in the Kashipur block of Rayagada district were due to *ctxB1* genotype which was highly resistant to ciprofloxacin, norfloxacin, nalidixic acid and furazolidone (Pal *et al.* 2010). Further tetracycline-resistant strains of *V. cholerae* O1 were reported from Kashipur, Kalyansingpur and Bissam Cuttack blocks of the same district in 2010 (Kar *et al.* 2015). From the present study the reversal of sensitivity to ciprofloxacin, norfloxacin and tetracycline was observed. The present findings from Kalahandi show similar patterns of sensitivity to tetracycline, azithromycin, norfloxacin, gentamicin, ofloxacin, ciprofloxacin as observed in the 2014 cholera outbreak from the same district (Pal *et al.* 2017). The common resistance profile of *V. cholerae* O1 strains from both the districts was ampicillin, nalidixic acid, furazolidone and co-trimoxazole with little variation. Similar resistance patterns were also published in a study from Kolkata and Southern India (Kumar *et al.* 2014).

The bacteriological and DMAMA-PCR analysis indicated that the outbreak in Rayagada district was due to the predominance of classical *ctxB1* allele (81.1%) of *V. cholerae* O1, whereas the epidemic in Kalahandi district was due to the HCT variant (90%) of *V. cholerae* O1 strains. The earlier reports from Rayagada district in 2007 and 2010 reported that *ctxB1* variant of *V. cholerae* O1 was the causative agent of the cholera outbreaks (Pal *et al.* 2010; Kar *et al.* 2015), whereas the epidemic in Narla block in 2014 was completely due to the HCT variant of *V. cholerae* O1 strain (Pal *et al.* 2017). These findings indicated that the classical *ctxB1* genotypes of *V. cholerae* O1 were spreading in Rayagada district causing the present outbreak in these areas. The cholera outbreak in Kalahandi district was predominantly due to HCT variant of *V. cholerae* O1, which might be the precursor of the 2014 epidemic in Narla block of the same district, demonstrating its correlation and spread to other blocks. Further PFGE analysis on *V. cholerae* O1 strains isolated in 2014 and the 2012 cholera outbreak from Kalahandi district will shed light on the spread of these variants in this district. Earlier studies also reported the spread of HCT-producing *V. cholerae* O1 to different parts of India during small cholera outbreaks from West Bengal, Bihar and Southern India (Naha *et al.* 2012; Kutar *et al.* 2013; Kumar *et al.* 2014). The cholera outbreaks in Baragarh district during 2018 and Kalyansingpur block of Rayagada district in 2019 were due to the HCT variant of *V. cholerae* O1 Ogawa (Nayak *et al.* 2021). The ongoing second wave of the cholera epidemic in Yemen which started in April 2017 was also caused by *V. cholerae* O1 serotype Ogawa harbouring the *ctxB7* allele (Weill *et al.* 2019). The hybrid biotype of *V. cholerae* O1 El Tor biotype (carrying cholera toxin of classical type) is known for its evolutionary fitness and heightened virulence that enhances the infection level with increased fluid loss and a higher fatality rate (Kumar *et al.* 2014). This was evident from the present investigation, in that one diarrhoea patient from Kalampur block was given 114 bottles of intravenous fluids within 4 days of treatment and recovered during this outbreak. This confirmed the severity and toxicity of *ctxB7* allele of *V. cholerae* O1 strains. Similar results were reported during the 2007 cholera epidemic in the tribal areas of Odisha (Pal *et al.* 2010).

The PFGE analysis on *V. cholerae* O1 strains isolated from both clinical and water samples from these districts exhibited three pulsotypes. The rectal swab and water isolates of *V. cholerae* O1 from Kalyansingpur, Jagannathpur of Rayagada district and Thuamulrampur of Kalahandi district were similar to each other, and this confirmed the source of infection. Although *ctxB1* and *ctxB7* genotypes were reported from both the districts, they belonged to one clone which differed from the pulsotypes of *V. cholerae* O1 isolated from Kalyansingpur and Junagarh block having *ctxB1* genotypes. An interesting finding was observed from the PFGE analysis that there was 96% similarity between the clinical and water isolates of Kalyansingpur block of Rayagada district with the isolates of different places from Rayagada and Kalahandi districts (Figure 4). Similar reports have been published from Southern India during 2010 to 2014 from Belgaum, Karnataka (Bhattacharya *et al.*

2015). Different pulsotypes have been reported while comparing the normal biotype with hybrid biotypes having both El Tor and classical *ctxB* and El Tor variant strains of *V. cholerae* O1 of 1975 to 2017 isolates reported from Kenya (Bundi *et al.* 2019). Another PFGE analysis report from Kolkata showed a close relationship between classical and Haitian *ctxB* with >98% similarity (Naha *et al.* 2012).

CONCLUSIONS

In summary, we conclude that the outbreak strains of *V. cholerae* O1 shared a common resistance profile to antibiotics such as ampicillin, nalidixic acid, furazolidone and co-trimoxazole, whereas their sensitivity to tetracycline and ciprofloxacin again reappeared in both the districts. The water isolates of *V. cholerae* O1 were similar to the clinical isolates exhibited by PFGE analysis, which confirmed the source of infection. The *ctxB1* and *ctxB7* genotypes of *V. cholerae* O1 reported from these districts showed three pulsotypes with 96% similarity. Thus, continuous surveillance is highly essential to track the changing over of altered El Tor biotypes with antibiogram profiles of *V. cholerae* serogroups that caused cholera outbreaks/epidemics in the tribal areas of Odisha.

ACKNOWLEDGEMENTS

This work was supported by the Indian Council of Medical Research, New Delhi, India, Vide Project No. 5/8-1 (70)/2018-19-ECD-II that funded Dr B. B. Pal, and is highly acknowledged. The Chief District Medical Officer (CDMO) and medical staffs of different CHCs of Rayagada and Kalahandi districts are acknowledged for their help during field investigation and sample collection.

ETHICAL STATEMENT

No ethical approval was done as there were cholera outbreaks reported in Rayagada and Kalahandi districts of Odisha. However, this was approved by the scientific advisory committee of the institute.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

- Agtini, M. D., Soeharno, R., Lesmana, M., Punjabi, N. H., Simanjuntak, C., Wangsasaputra, F., Nurdin, D., Pulungsih, S. P., Rofiq, A., Santoso, H., Pujarwoto, H., Sjahrurachman, A., Sudarmono, P., von Seidlein, L., Deen, J. L., Ali, M., Lee, H., Kim, D. R., Han, O., Park, J. K., Suwandono, A., Ingerani, Oyofu, B. A., Campbell, J. R., Beecham, H. J., Corwin, A. L. & Clemens, J. D. 2005 *The burden of diarrhoea, shigellosis and cholera in North Jakarta, Indonesia: findings from 24 months surveillance*. *BMC Infect. Dis.* **5**, 89. <https://doi.org/10.1186/1471-2334-5-89>.
- Bauer, A. W., Kirby, W. M., Sherris, J. C. & Turck, M. 1966 *Antibiotic susceptibility testing by a standardized single disc method*. *Am. J. Clin. Pathol.* **45**, 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493.
- Bhattacharya, D., Dey, S., Pazhani, G. P., Ramamurthy, T., Parande, M. V., Kholkute, S. D. & Roy, S. 2015 *Vibrio cholerae* O1 El Tor variant and emergence of Haitian *ctxB* variant in the strains isolated from South India. *Med. Microbiol. Immunol.* **205**, 195–200. <https://doi.org/10.1007/s00430-015-0433-y>.
- Bundi, M., Shah, M. M., Odoyo, E., Kathiiko, C., Wandera, E., Miring'u, G., Guyo, S., Langat, D., Morita, K. & Ichinose, Y. 2019 *Characterization of Vibrio cholerae O1 isolates responsible for cholera outbreaks in Kenya between 1975 and 2017*. *Microbiol. Immunol.* **63**, 350–358. <https://doi.org/10.1111/1348-0421.12731>.
- Cooper, K. L., Luey, C. K., Bird, M., Terajima, J., Nair, G. B., Kam, K. M., Arakawa, E., Safa, A., Cheung, D. T., Law, C. P., Watanabe, H., Kubota, K., Swaminathan, B. & Ribot, E. M. 2006 *Development and validation of a PulseNet standardized pulsed-field gel electrophoresis protocol for subtyping of Vibrio cholerae*. *Food Borne Pathogen. Dis.* **3**, 51–58. <https://doi.org/10.1089/fpd.2006.3.51>.
- Dixit, S. M., Johura, F. T., Manandhar, S., Sadique, A., Rajbhandari, R. M., Mannan, S. B., Rashid, M., Islam, S., Karmacharya, D., Watanabe, H., Bradley Sack, R., Cravioto, A. & Alam, M. 2014 *Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant Vibrio cholerae O1*. *BMC Infect. Dis.* **14** (1), 392. <https://doi.org/10.1186/1471-2334-14-392>.
- Faruque, S. M., Albert, M. J. & Mekalanos, J. J. 1998 *Epidemiology, genetics, and ecology of toxigenic Vibrio cholerae*. *Microbiol. Mol. Biol. Rev.* **62** (4), 1301–1314. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC98947>.
- Fauveau, V., Koenig, M. A. & Wojtyniak, B. 1991 *Excess female deaths among rural Bangladeshi children: an examination of cause specific mortality and morbidity*. *Int. J. Epidemiol.* **20** (3), 729–735. <https://doi.org/10.1093/ije/20.3.729>.

- Kar, S. K., Pal, B. B., Khuntia, H. K., Achary, K. G. & Khuntia, C. P. 2015 Emergence and spread of tetracycline resistant *Vibrio cholerae* O1 El Tor variant during 2010 cholera epidemic in the tribal areas of Odisha, India. *Int. J. Infect. Dis.* **33**, 45–49. <https://doi.org/10.1016/j.ijid.2014.12.025>.
- Keymer, D. P. & Boehm, A. B. 2011 Recombination shapes the structure of an environmental *Vibrio cholerae* population. *Appl. Environ. Microbiol.* **77** (2), 537–544. <https://doi.org/10.1128/AEM.02062-10>.
- Kumar, P., Mishra, D. K., Deshmukh, D. G., Jain, M., Zade, A. M., Ingole, K. V. & Yadava, P. K. 2014 Haitian variant *ctxB* producing *Vibrio cholerae* O1 with reduced susceptibility to ciprofloxacin is persistent in Yavatmal, Maharashtra, India, after causing a cholera outbreak. *Clin. Microbiol. Infect.* **20**, O292–O293. <https://doi.org/10.1111/1469-0691.12393>.
- Kutar, B. M. R. N. S., Rajpara, N., Upadhyay, H., Ramamurthy, T. & Bhardwaj, A. K. 2013 Clinical isolates of *Vibrio cholerae* O1 El Tor Ogawa of 2009 from Kolkata, India: preponderance of SXT element and presence of Haitian *ctxB* variant. *PLoS. One* **8** (2), e56477. <https://doi.org/10.1371/journal.pone.0056477>.
- Lacey, S. W. 1995 Cholera: calamitous past, ominous future. *Clin. Infect. Dis.* **20**, 1409–1419. <https://doi.org/10.1093/clinids/20.5.1409>.
- Naha, A., Pazhani, G. P., Ganguly, M., Ghosh, S., Ramamurthy, T., Nandy, R. K., Balakrishna Nair, G., Takeda, Y. & Mukhopadhyay, A. K. 2012 Development and evaluation of a PCR assay for tracking the emergence and dissemination of Haitian variant *ctxB* in *Vibrio cholerae* O1 strains isolated from Kolkata, India. *J. Clin. Microbiol.* **50**, 1733–1736. <https://doi.org/10.1128/JCM.00387-12>.
- Nair, G. B., Qadri, F., Holmgren, J., Svennerholm, A. M., Safa, A., Bhuiyan, N. A., Shafi Ahmad, Q., Faruque, S. M., Faruque, A. S. G., Takeda, Y. & Sack, D. A. 2006 Cholera due to altered El Tor strains of *Vibrio cholerae* O1 in Bangladesh. *J. Clin. Microbiol.* **44** (11), 4211–4213. <https://doi.org/10.1128/JCM.01304-06>.
- Nayak, S. R., Nayak, A. K., Biswal, B. L., Pati, S. & Pal, B. B. 2021 Spread of Haitian variant *Vibrio cholerae* O1 causing cholera outbreaks in Odisha, India. *Jpn. J. Infect. Dis.* **74** (2), 137–143. <https://doi.org/10.7883/yoken.JJID.2020.364>.
- Pal, B. B., Khuntia, H. K., Samal, S. K., Kar, S. K. & Patnaik, B. 2010 Epidemics of severe cholera caused by El Tor *Vibrio cholerae* O1 Ogawa possessing the *ctxB* gene of the classical biotype in Orissa, India. *Int. J. Infect. Dis.* **14**, e384–e389. <https://doi.org/10.1016/j.ijid.2009.06.020>.
- Pal, B. B., Khuntia, H. K., Nayak, S. R., Mohanty, A. & Biswal, B. 2017 *Vibrio cholerae* O1 Ogawa strains carrying *ctxb7* allele caused a large cholera outbreak during 2014 in the tribal areas of Odisha, India. *Jpn. J. Infect. Dis.* **70**, 549–553. <https://doi.org/10.7883/yoken.JJID.2016.585>.
- Piarroux, R., Barraix, R., Faucher, B., Haus, R., Piarroux, M., Gaudart, J., Magloire, R. & Raoult, D. 2011 Understanding the cholera epidemic, Haiti. *Emerg. Infect. Dis.* **17**, 1161–1168. <https://doi.org/10.3201/eid1707.110059>.
- Safa, A., Nair, G. B. & Kong, R. Y. 2010 Evolution of new variants of *Vibrio cholerae* O1. *Trends. Microbiol.* **18** (1), 46–54. <https://doi.org/10.1016/j.tim.2009.10.003>.
- Scallan, E., Majowicz, S. E., Hall, G., Banerjee, A., Bowman, C. L., Daly, L., Jones, T., Kirk, M. D., Fitzgerald, M. & Angulo, J. M. 2005 Prevalence of diarrhoea in the community in Australia, Canada, Ireland and the United States. *Int. J. Epidemiol.* **34** (2), 454–460. <https://doi.org/10.1093/ije/dyh413>.
- Tornheim, J. A., Many, A. S., Oyando, N., Kabaka, S., O Reilly, C. E., Breiman, R. F. & Feikin, D. R. 1990 The epidemiology of hospitalization with diarrhea in rural Kenya: the utility of existing health facility data in developing countries. *Int. J. Infect. Dis.* **14** (6), e499–e505. <https://doi.org/10.1016/j.ijid.2009.07.021>.
- Weill, F. X., Domman, D., Njamkepo, E., Almesbahi, A. A., Naji, M. & Nasher, S. S. 2019 Genomic insights into the 2016–2017 cholera epidemic in Yemen. *Nature* **565** (7738), 230–233. <https://doi.org/10.1038/s41586-018-0818-3>.
- World Health Organization 1983 *Manual of Laboratory Infections of Acute Enteric Infections*. CDD/83.3. World Health Organization, Geneva, Switzerland.

First received 28 May 2021; accepted in revised form 4 October 2021. Available online 5 November 2021