

Original Article

Spread of Haitian Variant *Vibrio cholerae* O1 Causing Cholera Outbreaks in Odisha, India

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SUMMARY: Cholera posed a significant threat causing outbreaks/epidemics with high morbidity and mortality in Odisha. This study envisages the characterisation of isolated pathogen from two cholera outbreaks reported in 2018 and 2019 from Bargarh and Rayagada districts of Odisha respectively. *Vibrio cholerae* O1 were isolated following standard techniques. The different virulent and drug resistant genes were detected by multiplex PCR assays; whereas the *ctxB* genotypes were characterised through double mismatch amplification mutation (DMAMA) PCR assay. The *ctxB* genes were further sequenced and pulse-field gel electrophoresis (PFGE) was done on some selected strains. The clinical and water isolates of Haitian variant (HCT) *V. cholerae* O1 Ogawa biotype El Tor with multi drug resistant strains were isolated from both the places. All the *V. cholerae* O1 strains were positive for virulence genes. The antibiotic resistant genes like *dfxA1* (100%), *strB* (76.9%), *intSXT* (61.5%) were detected. The PFGE results on *V. cholerae* O1 strains exhibited two different pulsotypes. These cholera outbreaks were due to multidrug resistant HCT variant *V. cholerae* O1 strains which were circulating and caused the cholera outbreaks in Odisha. So continuous surveillance on diarrheal disorders is highly essential to prevent the future diarrheal outbreaks in this region.

INTRODUCTION

Cholera is a life threatening acute secretory diarrhea caused by *Vibrio cholerae* that predominantly occurs in the developing or underdeveloped countries of South Asia, Latin America and Africa (1-3). Based on the somatic O-antigen, more than 200 serogroups of *V. cholerae* have been identified, out of which only the O1 and O139 serogroups are designated as pathogenic strains responsible for epidemics and outbreaks throughout the world (4). On the basis of phenotypic and genotypic differences, *V. cholerae* O1 is further divided into Classical and El Tor biotypes (1). Over time, the biotypes of *V. cholerae* O1 have undergone rapid genetic assortments in the *ctxB* gene (encoding the B subunit of cholera toxin), resulting in different variants which range from the *ctxB1* to the *ctxB9* (2,5,6). Following the cholera outbreak in Haiti in 2010, cholera outbreaks around the world in recent years have been attributed to the *ctxB7* (Haitian variant) genotypes of *V. cholerae* O1. Outbreaks caused by this variant were

reported in Nepal in 2012, Eastern Africa (Kenya, Tanzania and Uganda) during 2015 to 2016, and more recently in Yemen during 2016-2017 (7,8). Moreover, this Haitian variant of *V. cholerae* O1 was isolated in Kolkata, India in 2006 (9) as well as in Odisha in 1999 (10). The present study reports on the characterization of the causative pathogens of two diarrheal outbreaks in the Areigudi village of Bargarh district in August, 2018 as well as in the Kalyansinghpur block of Rayagada district in April, 2019 from Odisha, India.

MATERIALS AND METHODS

Study area: An assigned microbiologist from Regional Medical Research Centre, Bhubaneswar, along with medical officers of local Community Health Centre (CHC), visited the diarrhea affected the Areigudi village in the Bheden block of the Bargarh district in August, 2018 as well as the Podachuan village in the Kalyansinghpur block and the adjoining villages of Pedaguda and Mandalpiteshu in the Kolnara block of the Rayagada district in April, 2019. Information on the index cases, date-wise line listing of all diarrhea cases, deaths, as well as the clinical signs and symptoms of the diarrhea patients were collected. The index cases were traced by partaking in discussions with the villagers, along with examination of the hospital records. The cause of these outbreaks, sources of drinking water, chlorination of drinking water sources and prevailing sanitary conditions of the diarrhea affecting villagers in

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these blocks were also recorded.

Isolation and identification: Rectal swabs from diarrhea patients of affected villages and hospitals were collected prior to the administration of antibiotics and after obtaining due consent. They were transferred in Cary-Blair transport medium, enriched with alkaline peptone water and streaked on MacConkey agar, Hektoen enteric agar, as well as Thiosulfate-citrate-bile-salts-sucrose (TCBS) agar (Becton, Dickinson and Company [BD], NJ, USA) plates. Subsequently, sucrose fermenting colonies from TCBS agar plates were selected and assessed via selective biochemical tests. The suspected contaminated water samples were once more enriched with alkaline peptone water (APW), followed by 24 h incubation at 37°C and streaking on TCBS agar plates in order to selectively isolate the *V. cholerae* serogroups. Serological confirmation was performed using *V. cholerae* polyvalent O1 and monovalent O1 Ogawa and Inaba antisera. The isolation and identification of the pathogens were conducted as per our earlier practices adopted in the laboratory, in accordance with the WHO guidelines (11).

Antibiotic susceptibility assay: The resistance and sensitivity patterns of *V. cholerae* O1 strains were assessed using commercial antibiotic disks (BD), as per our earlier methodology (12, 13). The antibiotics used included 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), erythromycin (E, 15 µg), and polymyxin B (PB, 50U).

Polymerase chain reaction (PCR) assay: Two multiplex PCR (mPCR) assays were performed in order to detect the various traits of *V. cholerae* O1 strains. The mPCR1 was conducted for the confirmation of *V. cholerae*, along with its toxigenicity and serogroup, using primers for gene associated with O1 somatic antigen (*rfbO1*), toxin coregulated pilus (*tcpA*), zonula occludens protein (*zot*), outer membrane protein OmpW (*ompW*). The mPCR2 was carried out to confirm the presence of other toxigenic and virulence genes encoding accessory cholera enterotoxin (*ace*), hemolysin (*hlyA*), repeats-in-toxin protein (*rtx*), toxin regulator (*toxR*), as well as outer membrane protein OmpU (*ompU*). The PCR assays were performed as per the protocol standardized by Kumar et al. (Table 1) (14).

The *V. cholerae* O1 strains were further evaluated via mPCR assay for the detection of antibiotic resistant genes encoding sulfamethoxazole (*sulIII*), trimethoprim (*dfrA1*), streptomycin (*strB*), and the SXT genetic element (15).

Double mismatch amplification mutation assay (DMAMA) PCR for the *ctxB* genotype & detection of the *tcpA* gene: A DMAMA-PCR assay was employed to differentiate between the Classical *ctxB* genotype (*ctxB1*), El Tor *ctxB* genotype (*ctxB3*), and Haitian *ctxB* genotype (*ctxB7*) by observing the mutations at nucleotide position 58 and 203 of the *ctxB* gene (9). Standard strains of *V. cholerae* O1 for classical (O395), El Tor (N16961), and Haitian (2010EL-1786) were used as control strains. Biotype specific characterization was

confirmed via molecular characterization that included identification of variant of the *tcpA* gene (Classical, El Tor, and Haitian) using a PCR assay. Three specific primers were utilized in this PCR assay: one common reverse primer for Haitian and El Tor type *tcpA* as well as two specific forward primers for Haitian and El Tor type *tcpA*. (16).

Sequencing of the *ctxB* gene: The PCR results for the *ctxB* gene obtained via the DMAMA-PCR assay were validated on selected *V. cholerae* O1 strains by sequencing, using primers and conditions reported in previous studies (9). The sequences were compared and aligned with the sequences of standard strains of *V. cholerae* using CLUSTAL W in MEGA 7.0.

Pulse field gel electrophoresis (PFGE): The PFGE protocol standardized by pulsenet was followed for typing the selected *V. cholerae* O1 strains isolated from the above outbreaks (17,18).

RESULTS

A sudden increase in diarrhea patients with clinical symptoms such as abdominal pain, rice watery stool, severe dehydration, and muscular cramping was reported on 25.08.2018 in the Areigudi village, by the CHC of Bheden block, Bargarh district. The source of infection was suspected as pond water used for cooking purposes in a local festival. Individuals who did not participated in the festival were not infected, which substantiated the aforementioned hypothesis. The population of this amounted to 1387 in total, of which fifty-five diarrhea patients were admitted to the nearest health centers between 25.08.2018 and 28.08.2018. The infection rate was 3.96%, with no fatalities (Fig. 1a).

Another incidence occurred at a marriage ceremony organized on 22.04.2019 at Podachuan village of Kalyansinghpur block, Rayagada district. People from nearby villages i.e., Pedaguda and Mandalpiteshu of Kolnara block also attended the event. During the festivities, water was supplied from a stream flowing from a hill top and stored at the bottom of the hill. Onset of diarrhea began on 23.04.2019, and by 24.04.2019, 49 diarrhea cases had been registered. Most of the diarrhea cases presented symptoms of “rice watery stool”, along with severe dehydration and abdominal cramping (Fig. 1b). The total population of the three villages was 500 (Podachuan:125; Pedaguda:225; Mandalpiteshu:150). Seventy-three (Podachuan:46; Pedaguda:10; Mandalpiteshu:17) diarrhea patients (23.04.2019 to 27.04.2019) were admitted to the nearest health center, and only one death was reported. Overall, the attack rate was 14.6% and the case fatality rate was 1.36%.

Bacteriological analysis: Twenty-five rectal swabs out of 50 samples from Kalyansinghpur and 2 rectal swabs out of 15 samples from Bheden block tested positive for *V. cholerae* O1 Ogawa strains. Of the 14 water samples collected from Areigudi village, one sample (pond water) was positive for *V. cholerae* nonO1 and nonO139, while one out of 25 water samples collected from 3 villages in the Kalyansinghpur block was positive for *V. cholerae* O1 Ogawa (Podachuan-stream water) which was used during the marriage ceremony. The antibiotic susceptibility test was conducted on all the isolated *V. cholerae* O1 strains. All

Haitian Variant *V. cholerae* O1 in Odisha

Table 1. PCR primers used in this study

Target gene	Primer sequence (5'→3')	Amplicon Size (bp)	Reference	
mPCR1			14	
<i>ompW</i> -F	CACCAAGAAGGTGACTTTATTGTG	304		
<i>ompW</i> -R	GGTTTGTCTGAATTAGCTTCACC			
<i>rfbO1</i> -F	TCTATGTGCTGCGATTGGTG	638		
<i>rfbO1</i> -R	CCCCGAAAACCTAATGTGAG			
<i>tcpA</i> -F	CGTTGGCGGTCAGTCTTG	805		
<i>tcpA</i> -R	CGGGCTTTCTTCTTGTTTCG			
<i>zot</i> -F	TCGCTTAACGATGGCGCGTTTT	947		
<i>zot</i> -R	AACCCCGTTTCACTTCTACCCA			
mPCR2				14
<i>rtxC</i> -F	CGACGAAGATCATTGACGAC	265		
<i>rtxC</i> -R	CATCGTCGTTATGTGGTTGC			
<i>ace</i> -F	TAAGGATGTGCTTATGATGGACACCC	309		
<i>ace</i> -R	CGTGATGAATAAAGATACTCATAGG			
<i>hlyA</i> -F	GAGCCGGCATTCTCATCTGAAT	480		
<i>hlyA</i> -R	CTCAGCGGGCTAATACGGTTTA			
<i>ompU</i> -F	CCAAAGCGGTGACAAAGC	655		
<i>ompU</i> -R	TTCCATGCGGTAAGAAGC			
<i>toxR</i> -F	CCTTCGATCCCCTAAGCAATAC	779		
<i>toxR</i> -R	AGGGTTAGCAACGATGCGTAAG			
Antibiotic resistance genes			15	
<i>sulII</i> -F	TGTGCGGATGAAGTCAGCTCC	626		
<i>sulII</i> -R	AGGGGGCAGATGTGATCGAC			
<i>strB</i> -F	CCGCGATAGCTAGATCGCGTT	515		
<i>strB</i> -R	CGACTACCAGGCGACCGAAAT			
<i>SXT</i> -F	ATGGCGTTATCAGTTAGCTGGC	1035		
<i>SXT</i> -R	GCGAAGATCATGCATAGACC			
<i>dfrA1</i> -F	CAAGTTTACATCTGACAATGAGAACGTAT	278		
<i>dfrA1</i> -R	ACCCTTTTGCCAGATTTGGTA			
<i>ctxB</i> gene			9	
<i>Rv-cla</i>	CCTGGTACTTCTACTTGAAACG	191		
<i>ctxB</i> -F3 (Haitian)	GTTTTACTATCTTCAGCATATGCGA			
<i>ctxB</i> -F4 (Classical)	GTTTTACTATCTTCAGCATATGCGC			
<i>ctxB</i> -F	GGTTGCTTCTCATCATCGAACCAC	460		
<i>ctxB</i> -R	GATACACATAATAGAATTAAGGAT			
<i>tcpA</i> Gene			16	
<i>EL-Rev</i>	CCGACTGTAATTGCGAATGC	167		
<i>tcpA</i> -F1 (El Tor)	CCAGCTACCGCAAACGCAGA			
<i>tcpA</i> -F2 (Haitian)	CCAGCTACCGCAAACGCAGG			

the strains were found to be sensitive to tetracycline, chloramphenicol, azithromycin, erythromycin, gentamicin, norfloxacin, ciprofloxacin, ofloxacin, and doxycycline. However, they were resistant to ampicillin, nalidixic acid, furazolidone, streptomycin, neomycin, and co-trimoxazole.

Demographic analysis: A demographic analysis was performed on the patients, who were subdivided into 4 different age groups, i.e., > 0-5 years, > 5-14 years, > 14-40 years and > 40 years. The age group > 14-40 years in the Areigudi village was the worst affected, followed by the age groups > 40 years, > 5-14 years, and

> 0-5 years. Females were infected more than the males across all the age groups, except in the > 0-5 years age group. Contrastingly, in the Kalyansinghpur block, the age group > 5-14 years was the worst affected, followed by the age groups > 14-40 years, > 40 years, and > 0-5 years. In this outbreak, as well, females were infected more than the males, except for the > 14-40 years age group (Fig. 2a and 2b).

Molecular characterization: Two sets of mPCR were conducted for screening the various genes associated with toxigenicity and pathogenicity. From the mPCR1 results, it was revealed that all *V. cholerae*

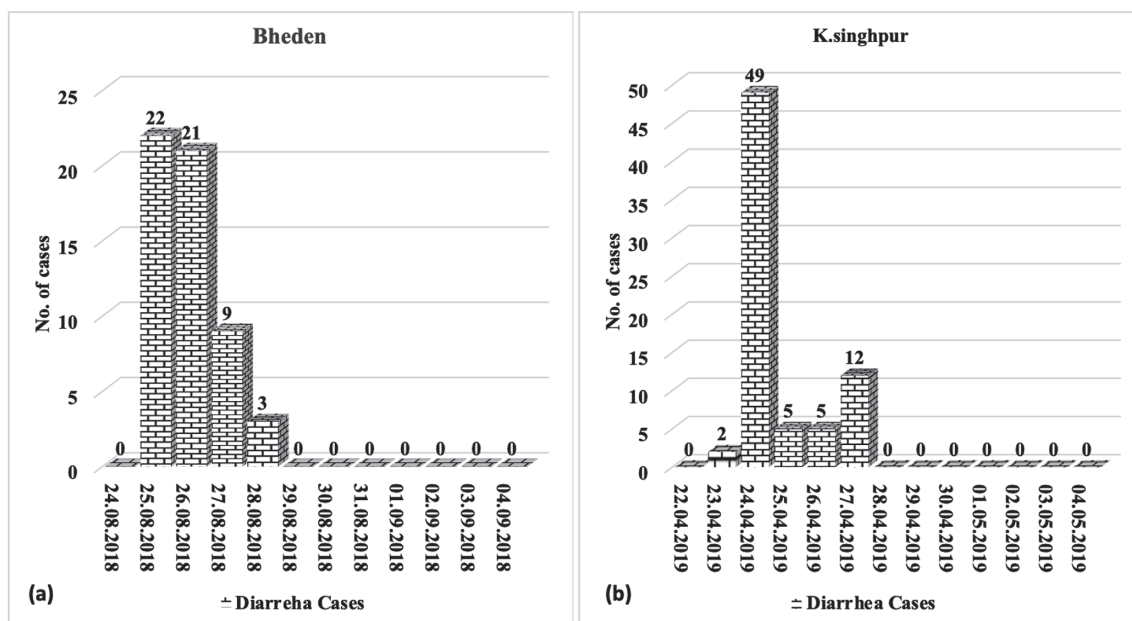


Fig. 1. Epicurve showing date wise severe diarrhea cases in two blocks of Odisha.

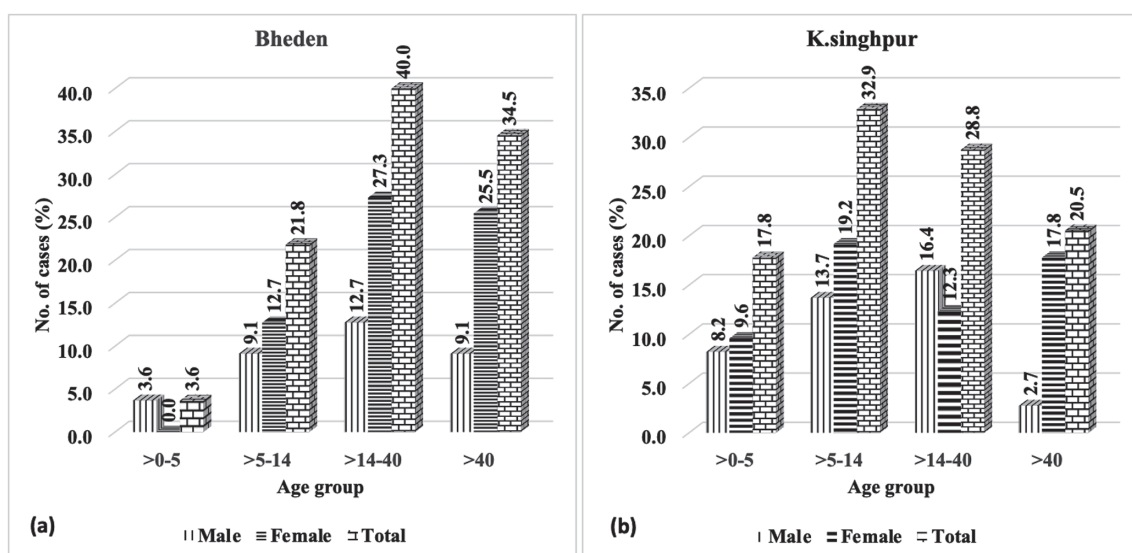


Fig. 2. Age and sex wise distribution of diarrhea patients in two blocks of Odisha.

O1 strains were positive for the *rfbO1*, *tcpA*, *zot*, and *ompW* genes. Contrastingly, the mPCR2 results indicated the presence of the *ace*, *hlyA*, *ompU*, *rtx*, and *toxR* genes in all the *V. cholerae* O1 strains (Table 2). The *ompW* gene is specific to *V. cholerae* at the species level, whereas *rfbO1* confirmed the O1 serogroup in all the isolated strains. The presence of the *rtx* gene further aids in biotyping the strains as the El Tor biotype, which *rtx* is specific to. Similarly, the *tcpA* gene confirms the presence of the pilus colonization factor—which acts as a receptor for CTXΦ bacteriophage—through which it infects a non-pathogenic *V. cholerae* and converts it into pathogenic *V. cholerae* strains via the lysogenic cycle. The remaining genes encode the core toxin of *V. cholerae*; they are closely located and get incorporated into its genome through the process of horizontal gene transfer.

Further, it was attempted to establish the resistance profile of the *V. cholerae* strains by detecting their

antibiotic resistance genes. The *dfra1* gene was detected in all the strains, whereas the *sulII* gene was absent. These genes provide resistance towards trimethoprim and sulfamethoxazole, respectively. The *strB* gene—which provides resistance for streptomycin—was 72.7% positive, while the *intSXT* was 54.5% positive.

Detection of the *ctxB* and *tcpA* genes: Randomly selected *V. cholerae* O1 strains were analyzed via a DMAMA-PCR assay for the detection of the *ctxB* genotype. It was observed that all the El Tor biotypes harbored the *ctxB7* (191bp) genotype, irrespective of the location or source of isolation. Furthermore, PCR assay results for the detection of the *tcpA* gene (167bp) confirmed the presence of the Haitian type *tcpA* allele.

Sequencing of the *ctxB* gene: PCR-amplified *ctxB* genes (460bp) were additionally sequenced and the amino acid sequences were obtained using bioinformatics tools. Upon further analysis, it was observed that all the strains were identical to the

Table 2. Antibiotic resistant and virulent genes of *V. cholerae* O1 isolated from cholera outbreaks

Sl. No	Code (Source:S/W) ¹⁾	Resistance Profile	Antibiotic resistant genes					Virulent genes								
			sxt	strB	sulII	dfrA1	hly	ompW	r/bO1	tcpA	rtxC	ompU	toxR	ace	zot	
1	RDS-1558 (S)	AMP, FR, NA, S, COT, N	+	+	-	+	+	+	+	+	+	+	+	+	+	+
2	KYS-116 (S)	AMP, FR, NA, S, COT, N, GEN	+	+	-	+	+	+	+	+	+	+	+	+	+	+
3	KYS-117 (S)	AMP, FR, NA, S, COT, N, GEN	+	+	-	+	+	+	+	+	+	+	+	+	+	+
4	KYS-120 (S)	AMP, FR, NA, S, COT, N	-	+	-	+	+	+	+	+	+	+	+	+	+	+
5	KYS-122 (S)	AMP, FR, NA, S, COT, N	-	+	-	+	+	+	+	+	+	+	+	+	+	+
6	RDS-1560 (S)	AMP, FR, NA, S, COT, N	+	+	-	+	+	+	+	+	+	+	+	+	+	+
7	RDS-1568 (S)	AMP, FR, NA, S, COT, N	-	-	-	+	+	+	+	+	+	+	+	+	+	+
8	RDS-1569 (S)	AMP, FR, NA, S, COT, N	-	-	-	+	+	+	+	+	+	+	+	+	+	+
9	KYW-699 (W)	AMP, FR, NA, S, COT, N	-	-	-	+	+	+	+	+	+	+	+	+	+	+
10	RDS-1576 (S)	AMP, FR, NA, S, COT, N	+	+	-	+	+	+	+	+	+	+	+	+	+	+
11	RDS-1577 (S)	AMP, FR, NA, S, COT, N	+	+	-	+	+	+	+	+	+	+	+	+	+	+
12	BRD-5 (S)	AMP, FR, NA, S, COT, N	+	+	-	+	+	+	+	+	+	+	+	+	+	+
13	BRD-6 (S)	AMP, FR, NA, S, COT, N	+	+	-	+	+	+	+	+	+	+	+	+	+	+

¹⁾S, Stool; W, Water.

ctxB7 genotype of *V. cholerae* O1, with a mutation at position 20 of its amino acid sequence in comparison to *ctxB1* (GenBank accession numbers: MN396783, MN3967834; MN401681- MN401688).

Pulse field gel electrophoresis (PFGE): The PFGE analysis of above selected *V. cholerae* O1 strains exhibited 2 different pulsotypes. The *V. cholerae* O1 strains isolated from Bargarh in 2018 and from Rayagada district in 2019 were of a single clone. Contrastingly, the strain (RDS-1577) isolated from Kalyansinghpur block in the Rayagada district had a different pulsotype, sharing 97% similarity (Fig. 3).

DISCUSSION

Unhygienic living conditions, including open defecation with poor access to safe drinking water, were the major causes of diarrhea in the tribal areas of the state. Diarrhea being a waterborne disease, the use of contaminated water from traditional water sources like nala, chua, streams, and rivers made the population more susceptible to infection, especially during the monsoon, as previously reported in the Rayagada district of Odisha in 2007 and 2010 (13,19).

The *V. cholerae* O1 strains were resistant towards ampicillin, furazolidone, nalidixic acid, streptomycin, co-trimoxazole, neomycin, and occasionally to gentamicin. The current antibiogram profile differs from the cholera outbreak reported in 2014 in the Kalahandi district of Odisha (10). Another report suggested the prevalence of multi drug resistant (MDR) *V. cholerae* O1 strains from Odisha between 2004 and 2013 (20), as well as from other parts of the country, like Pune and rural areas of North Karnataka, and globally in Kenya, Cameroon, and Mozambique (21,22,23,24,25). Resistance of *V. cholerae* O1 strains towards several antibiotics like co-trimoxazole, trimethoprim, and streptomycin indicate the lateral acquisition of *intSXT* into the chromosome (26). Indiscriminate use of different antibiotics at various levels, including agriculture, is giving rise to MDR strains that pose numerous challenges to antibiotic therapy worldwide. The multiplex PCR assay in this study further confirmed the presence of antibiotic genes. Although *intSXT* is normally associated with the SXT element, a few strains were negative for *intSXT* and *strB*. Moreover, it was observed that although all the *V. cholerae* O1 strains were negative for *sulII*, they nonetheless showed resistance to corresponding antibiotics, potentially indicating the presence of alternative mechanisms involved in antibiotic resistance (14,15).

In the present study, the most affected age group was > 14-40 years, followed by > 40 years in the Bheden block. Similarly, in the Kalyansinghpur block, the age group > 5-14 years was the worst affected, followed by the age group > 14-40 years. Both these reports proved contradictory to a previous report from Chandigarh, where the age group < 5 years were the most affected (27). The study in Chandigarh reported that the age groups > 5-14 years and > 14-40 years were the most affected by cholera cases, which is similar to this study (28). Additionally, they reported that the male to female ratio among the diarrhea patients was 120:71, over a period of 16 years (2000 to 2015). An investigation

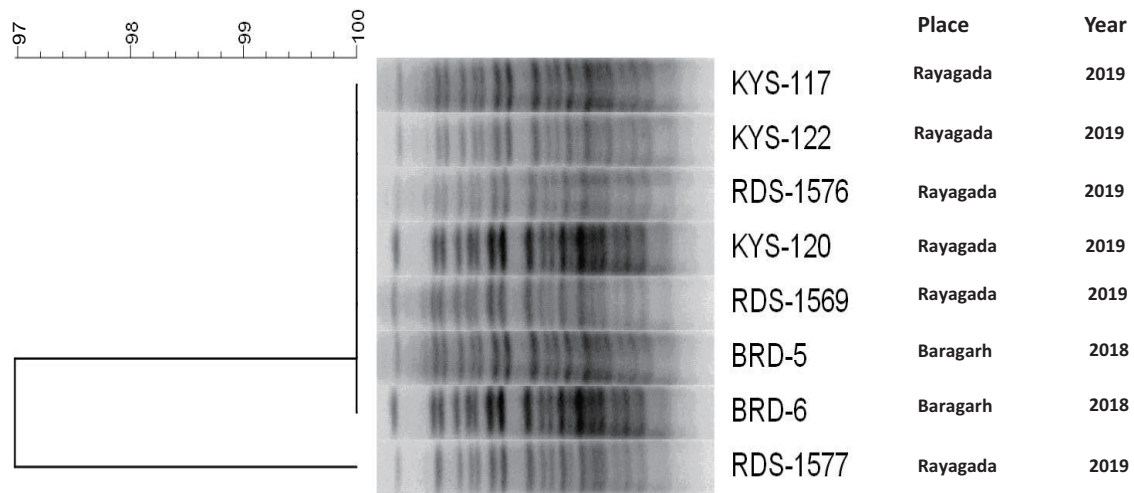


Fig.3. PFGE patterns of NotI digested *V. cholerae* O1 strains with dendrogram analysis using BioNumeric 7.2 (Applied Maths, Belgium) having two different pulsotypes with 97% similarity.

undertake in Kolkata during 1992 reported the male to female ratio as 1.4:1, while a report from Delhi and its peripheral regions indicated the male:female ratio to be 1.5:1 (28,29,30). Both the reports were in contrast with the current findings, wherein females were more affected than males. During the field visits, discussions about the outbreak were held with villagers from both the villages of these districts. As previously mentioned, both these outbreaks were associated with a marriage ceremony in Podachuan village, Kalyansinghpur block of Rayagada district and an annual religious festival in the Areigudi village, Bheden block of Bargarh district. It should be noted that both these events featured greater female participants in comparison to males.

Two sets of mPCRs conducted in this study confirmed the presence of various core toxin proteins, along with associated proteins, indicating the pathogenicity and toxicity of the *V. cholerae* O1 strains isolated from these outbreaks. Furthermore, the presence of the *rtx* gene confirmed the El Tor biotype amongst these strains (31). The RTX toxin is widely prevalent among Gram-negative bacteria and possesses important virulence factors (32). The pathogenicity of cholera is a complex process associated with several genes which are responsible for the toxicity, including the cholera toxin (CT) gene (*ctxAB*), along with other marker genes like *tcp*, *zot*, *hly*, *ace*, and *toxR* etc. This CT gene gets incorporated into the *V. cholerae* genome by CTX bacteriophage via the TCP receptor. Any change in the CTX element may result in the emergence of a novel *V. cholerae* strain (27). The above toxicity genes were present in all the *V. cholerae* O1 strains, indicating the presence of a core toxic region in the CTXΦ genome. Similar findings were reported by other investigators from different places (14,33,34).

The *ctxB* gene in *V. cholerae* O1 has many variants as a result of point mutations in the nucleotide sequences and corresponding amino acids. It has eleven distinct genotypes associated with various serogroups of *V. cholerae*. Genotype *ctxB1*, 2, 3, 7, 10, and 11 were identified in the O1 serogroups of *V. cholerae*. Contrastingly, *ctxB3*, 4, 5, and 6 were observed in the *V. cholerae* O139 serogroups, while *ctxB8* and 9 were present only in the O27 and O37 serogroups (35). In

comparison to the *ctxB1* genotype, genotype *ctxB7* has a point mutation at nucleotide position 58 (C to A) corresponding to the 20th amino acid (His to Asp). In the present study, *ctxB7* genotypes were confirmed in all the isolated strains that were also isolated in Odisha in 1999, 2007, and 2014 (10,14). Similar reports were published by other investigators from West Bengal, Bihar, and Southern India as well (9,36). These *V. cholerae* O1 strains having *ctxB7* genotypes were also reported in other parts of India—like Chennai, Hyderabad, Solapur, and Assam (37,38,39). The same Haitian variant *V. cholerae* O1 strain is responsible for causing a devastating cholera epidemic in Haiti during 2010. It was assumed to have migrated through Nepal, causing outbreaks there since 2007 (7), and subsequently spreading in Africa and Yemen during 2015 to 2017 (8).

The PFGE analysis clearly indicated two distinct pulsotypes of *V. cholerae* O1 strains. It is assumed that the *V. cholerae* O1 strains, which caused the 2018 cholera outbreak, might also have been responsible for the 2019 cholera outbreak and a variation occurring towards the end of the outbreak resulted in the new pulsotype with 97% similarity. The strains isolated in these outbreaks proved to be highly clonal, suggestive of high genetic homogeneity in the *V. cholerae* O1 population.

The present study provides a comprehensive view on the cholera outbreaks and spread of multi drug resistant *ctxB7* variants of *V. cholerae* O1 with two distinct PFGE patterns, in two different districts of Odisha. The cholera outbreak that occurred in Rayagada during April, 2019 was particularly unusual for this region. Henceforth, public hygiene and distribution of safe drinking water should be of foremost importance for the prevention and control of future diarrheal outbreaks in the tribal areas. It is indeed essential to have an active and continuous surveillance of diarrheal disorders in order to prevent or minimize the future diarrheal outbreak in this region of India.

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Conflict of interest None to declare.

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