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ORIGINAL ARTICLE

Molecular characterization of *Vibrio cholerae* O1 strains circulating in Assam: a north eastern state of India

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ABSTRACT

Background and Objectives: Information on the genetic epidemiology of cholera in Assam, a northeastern state of India is lacking despite cholera being a major public health problem. The study aimed to determine the virulence genes and genes encoding antibiotic resistance in *Vibrio cholerae* isolates and to determine the prevalent genotypes based on the presence or absence of the virulence genes and *ctxB* genotype.

Materials and Methods: Twenty-five *V. cholerae* strains were subjected to conventional biotyping and serotyping followed by multiplex PCR to detect *ctxA, ctxB, zot, ace, O1rfb, tcpA, ompU, ompW, rtxC, hly* and *toxR* and antibiotic resistance genes. Cholera toxin B *(ctxB)* gene was amplified followed by sequencing.

Results: All the *V. cholerae* O1 isolates were El Tor Ogawa and showed the presence of the core toxin region representing the genome of the filamentous bacteriophage CTXø. The complete cassette of virulence genes was seen in 48% of the isolates which was the predominant genotype. All the isolates possessed amino acid sequences identical to the El Tor *ctxB* subunit of genotype 3. *sulII* gene was detected in 68% of the isolates, *dfrA*1 in 88%, *strB* in 48% and *SXT* gene was detected in 36% of the isolates.

Conclusion: Toxigenic *V. cholerae* O1 El Tor Ogawa strains of *ctxB* genotype 3 carrying a large pool of virulence genes are prevailing in Assam. Presence of a transmissible genetic element *SXT* in 36% of the strains is of major concern as it indicates the emergence of multiple drug resistance among the *V. cholerae* isolates.

Keywords: *Vibrio cholerae* O1; Cholera toxin; Virulence; Genotype; Drug resistance

INTRODUCTION

Cholera still remains a global threat to public health in the developing countries where access to safe water and adequate sanitation cannot be assured for all. There are 2.9 million cases of cholera with 95,000 deaths occurring annually in endemic countries between 2008-2012. India is a country with more than 100,000 cases occurring annually (1). However, the actual global burden of cholera is not known as the vast majority of cases are not reported. WHO estimates that only 5-10% of the cases occurring annually are officially reported (2).

The causative agent of cholera, *Vibrio cholerae* is antigenically diverse organism and based on antigenic diversity of their outer membrane lipopolysaccharides, 206 serogroups (O1-O206) have been identified (3, 4). *V. cholerae* serogroup O1 is further classified into two biotypes, classical and El Tor, and two major serotypes, Ogawa and Inaba (5). Global

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replacement of *V. cholerae* classical biotype by El Tor biotype, emergence of O139 serogroup and rapid spread of antibiotic resistant strains indicate the continuous evolution in *V. cholerae*. The altered variant of El Tor strains containing the classical type of *ctxB* currently dominates globally.

Pathogenesis of *V. cholerae* is a complex process that involves coordinated expression of several virulence-associated genes to cause disease. The complete virulence profile of *V. cholerae*, including direct and indirect expression of genes involved in its survival and stress adaptation in the host are yet to be fully explored. The two major virulence factors that are associated with the generation of the symptoms of cholera are the cholera toxin (CT) responsible for the production of profuse rice-watery diarrhea and the toxin-coregulated pilus *(tcp)*, a type IV pilus that mediates adherence, microcolony formation and intestinal colonization (6). Interestingly, the production of these virulence factors (CT and tcp) is strongly influenced by environmental conditions. Cholera toxin (CT), and toxin co-regulated pilus (TCP) are part of the genome of two horizontally acquired Mobile Genetic Elements (MGEs), CTXΦ, and Vibrio pathogenicity island 1 (VPI-1), respectively (7). Apart from CT, the pathogenesis of cholera also relies on the synergistic action of a number of other genes like *tcp*, *ace* (accessory cholera enterotoxin), *zot* (zonula occludens toxin) and *toxR* the regulatory gene for CT production. Furthermore, the hemolysin of *V. cholerae (hly)*, is extracellular membrane damaging proteins plays important role in manifestation of cholera (8). *ompW* is the species-specific gene and *rfbO1* genes confirm O1 serogroup of the isolates (9).

Emergence of new strains in any species of bacterial population have always been challenging. *V. cholerae* being no different from other bacteria, persists around the globe and undergo evolution over time. Its dual characteristic feature of being able to be sustained in the environmental (less infectious) and infectious state, and changing or acquiring various virulence genes or mobile genetic elements makes it difficult to understand the pathogenesis of *V. cholerae*. Increased poverty, overall reduction in water quality, excessive misuse of antibiotics and climatic changes are encouraging further genetic exchange of virulence and antibiotic resistance (6).

Assam is a state which is fed by the very dynamic and unstable river Brahmaputra and its tributaries causing frequent floods during monsoon due to incessant rainfall. Increased rainfall is associated with increased risk of cholera because during the flood, sewage water contaminates surface and groundwater that individuals rely on for drinking, bathing, and washing clothes which is a natural fauna of cholera in Assam. Apart from that, earlier studies have reported that the spatiotemporal serotype shifts and genetic diversity of *V. cholerae* strains between epidemics and pandemics may be due to climatic variations influencing the switching of virulence factors (10-12). Globally, increased cases of dynamic *V. cholerae* strains, their serological switching and disease occurrence with respect to climate change (warm climate- more, cool climate- less) have attracted the attention of the public health sector (13).

Also, Assam is a state with highly diverse population in terms of ethnicity, religion and language. Of the 220 separate ethnic groups of North east India (NER), more than 30 of them are in Assam, which has over 70% of the North east region's population. There has been a consistent flow of migration in this region because of employment opportunities in tea gardens, availability of cultivable land and other related factors. Assam also has interlinked borders with other states of NER as well as other countries like Bhutan and Bangladesh and may have different genotypic divergence in their virulence profile and antibiotic resistance pattern, the study of which may help to formulate the prevention and control strategy of cholera in the state.

To the best of our knowledge, no published literature has been found describing the virulence and antibiotic resistance gene profile of *V. cholerae* in Assam. As per Integrated Disease Surveillance project (IDSP) data, 48 outbreaks of cholera have been reported from various districts of Assam during the period 2012 to 2015. No data related to the molecular characterization of the isolates from these outbreaks have been published so far. The emergence of antimicrobial resistance is also a global phenomenon. Multiple drug-resistant *V. cholerae* strains was reported from this region earlier also (14).

In this context, the present study was undertaken to characterize the *V. cholerae* strains based on distribution of virulence associated genes and antibiotic resistance genes among *V. cholerae* strains in Assam and to compare the virulence profile of the strains with that of the strains circulating in other regions of India.

MATERIALS AND METHODS

Study design. The study was carried out after obtaining approval from the Institutional Ethics Committee. Acute watery diarrhea (AWD) cases referred to the hospital from various districts of Assam were included in the study after taking written consent. Twenty-five *Vibrio cholerae* O1 strains El Tor isolated by standard bacteriological methods during a period of two and half years were included in the study (15, 16). For further confirmation, serotyping was done using antisera obtained from DENKA, SEIKEN CO LIMITED, Tokyo, Japan.

Multiplex PCR for detection of biotypes and virulence genes of *V. cholera*. *V. cholerae* strains were subcultured on Luria-Bertani agar, suspended in sterile deionized water to a turbidity of 2.0 McFarland standard and then boiled for 10 minutes. Two sets of multiplex PCR assay were performed to detect the presence of diverse gene traits. The first multiplex PCR detected *tcp A* (El Tor), *ctxA, O1rfb, rtxC, ompU, hlyA, zot,* and *ompW* genes. The second set of multiplex PCR detected *tcpA* (Classical), *ace, toxR* genes. The primers are listed in Table 1 (9, 17-21).

The reaction mixture consisted of dNTPs (200 µM), $MgCl_2$ (1.5 mM), Taq polymerase (1U), primers specific for target gene (2 µl each), template DNA (100 ng), and milli-Q water. The thermal cycling condition for both the multiplex PCR was pre-incubation at 94°C for 2 min followed by denaturation for 1 min at 94°C, annealing for 1 min at 59°C, extension for 2 min at 72°C and incubation at 72°C for 10 min for a final extension.

After the completion of 30 cycles of reactions, 10 µl of each of the PCR products were mixed individually with 2 µl of gel loading buffer. Electrophoresis was done on 2% agarose along with 100bp ladder as a molecular marker and stained with 0.5 Mg/ml of ethidium bromide and the bands were observed using a UV transilluminator (Fig. 1A and B). Genotyping was done based on the presence or absence of the virulence genes (Table 2).

Multiplex PCR procedure was standardized using reference strains of Gastrointestinal Tract Pathogen Repository (GTPR) work *V. cholerae* O1 Ogawa (GTPR ID 413), *V. cholerae* O1 Inaba (GTPR ID 842), *V. cholerae* O139 (GTPR ID 1144), *V. cholerae* classical (MTCC 3904) and *V. cholerae* O139 (MTCC 3906) obtained from National Institute of Cholera and Enteric Diseases (NICED), Kolkata and Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India (MTCC) respectively.

Multiplex PCR for detection of antibiotic resistance genes of *V. cholerae* O1. Another Multiplex PCR assay was performed to detect antibiotic resistance genes for *sulII* (encoding sulfamethoxazole resistance), *dfrA1* (O1-specific trimethoprim resistance), *strB* (Streptomycin B resistance), *SXT* (novel transmissible genetic element which contains the genes encoding resistance to these antibiotics), *dfr18* (O139-specific trimethoprim resistance) (Fig. 1B). The primers are listed in Table 1 (22-25).

Sequencing of *ctxB* gene. To determine the genotype of cholera toxin, Cholera toxin B *(ctxB)* gene was amplified from the isolates using the *ctxB* F GCCGGGTTGTGGGAATGCTCCAAG and *ctxB* R CATGCGATTGCCGCAATTAGTATGGC primers as described earlier (17). Purification of amplified product was performed by Exonuclease I-SAP (Shrimp Alkaline phosphatase) from Thermo Scientific, USA. Eluate was used as a purified gene product for sequencing which was performed for both the strands using the above-mentioned primers and BigDye® Terminator Cycle Sequencing Kit, Version 3.1 (Applied Biosystems, Foster City, USA). The sequencing reactions were analyzed on ABI 3500 Genetic Analyzer (Applied Bio systems® Foster City, USA). The consensus sequences were prepared using BioEdit sequence alignment editor (version 7.2.5) and searched against the GenBank database of the National Center for Biotechnology Information (NCBI) using the BLASTN algorithm (http://blast.ncbi.nlm.nih.gov/). The sequences obtained here were deposited in GenBank and the accession numbers are MK016537, MK016538, MK016539, MK029003, MK029004, MK29005.

RESULTS

All the strains tested were positive for the species-specific gene *ompW* and serogroup- specific *O1rfb*, thus confirming that all the isolates were *V. cholerae* O1. Detection of biotype-specific *tcpA* gene and *rtxC* gene revealed that, all the isolates belonged to biotype El Tor. Serotyping revealed that, all the isolates belonged to serotype Ogawa.

Table 1. List of primers used for detection of biotypes, virulence, and antibiotic resistance genes

Serial No.	Target	Nucleotide sequence (5'→3')	Amplicon Size (bp)	Reference
First set of PCR primers				
1	*tcp A* (F), El Tor	GAAGAAGTTTGTAAAAGAAGAACAC	472	Keasler & Hall (1993) (18)
	tcp A (R), El Tor	GAAGGACCTTCTTTCACGTTG		
2	*ompU* (F)	CCAAAGCGGTGACAAAGC	655	Kumar et al. (2009) (19)
	ompU (R)	TTCCATGCGGTAAGAAGC		
3	*rfb O1* (F)	TCTATGTGCTGCGATTGGTG	638	Goel et al. (2007) (17)
	rfb O1 (R)	CCCCGAAAACCTAATGTGAG		
4	*rtxC* (F)	CGACGAAGATCATTGACGAC	265	Chow et al. (2001) (20)
	rtxC (R)	CATCGTCGTTATGTGGTTGC		
5	*ctx A* (F)	CTCAGACGGGATTTGTTAGGCACG	301	Keasler & Hall (1993) (18)
	ctx A (R)	TCTATCTCTGTAGCCCCTATTACG		
6	*zot* (F)	TCGCTTAACGATGGCGCGTTTT	947	Singh et al. (2001) (21)
	zot (R)	AACCCCGTTTCACTTCTACCCA		
7	*hlyA* (F)	GAGCCGGCATTCATCTGAAT	480	Kumar et al. (2009) (19)
	hlyA (R)	CTCAGCGGGCTAATACGGTTTA		
8	*ompW* (F)	CACCAAGAAGGTGACTTTATTGTG	304	Nandi et al. (2000) (9)
	ompW (R)	GGTTTGTCGAATTAGCTTCACC		
Second set of PCR primers				
9	*tcp A* (F), Classical	CACGATAAGAAAACCGGTCAAGAG	618	Keasler & Hall (1993) (18)
	tcp A (R), Classical	ACCAAATGCAACGCCGAATGGAGC		
10	*ctxB* (F)	GCCGGGTTGTGGGAATGCTCCAAG	536	Goel et al. (2007) (17)
	ctxB (R)	CATGCGATTGCCGCAATTAGTATGGC		
11	*ace* (F)	TAAGGATGTGCTTATGATGGACACCC	309	Kumar et al. (2009) (19)
	ace (R)	CGTGATGAATAAAGATACTCATAGG		
12	*toxR* (F)	CCTTCGATCCCCTAAGCAATAC	779	Singh et al. (2001) (21)
	toxR (R)	AGGGTTAGCAACGATGCGTAAG		
Primers used for detection of antibiotic resistance genes				
1	*SulII* (F)	TGTGCGGATGAAGTCAGCTCC	626	Hochhut et al. (2001) (22)
	SulII (R)	AGGGGGCAGATGTGATCGAC		
2	*dfrA1* (F)	CAAGTTTACATCTGACAATGAGAACGTAT	278	Falbo et al. (1999) (23)
	dfrA1 (R)	ACCCTTTTGCCAGATTTGGTA		
3	*strB* (F)	CCGCGATAGCTAGATCGCGTT	515	Ramachandran et al. (2007) (24)
	strB (R)	CGACTACCAGGCGACCGAAAT		
4	*SXT* (F)	ATGGCGTTATCAGTTAGCTGGC	1035	Bhanumathi et al. (2003) (25)
	SXT (R)	GCGAAGATCATGCATAGACC		
5	*dfr18 (F)*	ACTGCCGTTTTCGATAATGTGG	389	Hochhut et al. (2001) (22)
	Dfr 18 (R)	GGGTAAGACACTCGTCATGGG		

Among the virulence genes, *ctxA* gene was present in 88%, *ompU* in 64%, *zot* in 64%, *hlyA* in 84%, *ace* in 92% and *toxR* in 100% of the isolates. Based on the virulence genes analyzed, five genotypes of *V. cholerae* O1 were identified. Genotyping revealed, genotype *tcpA (El)*+ *ctxA*+ *ompU*+ *rtxC*+ *ompW*+ *zot*+ *O1rfb*+ *hlyA*+ *ctxB*+ *ace*+ *toxR*+ i.e. complete cassette of virulence genes was identified in 48% of the *V. cholerae* strains and was the most predominant genotype (Table 2).

Detection of antibiotic resistance genes. *sulII* was detected in 68% indicating resistance to sulphamethoxazole, *strB* in 48% indicating streptomycin B

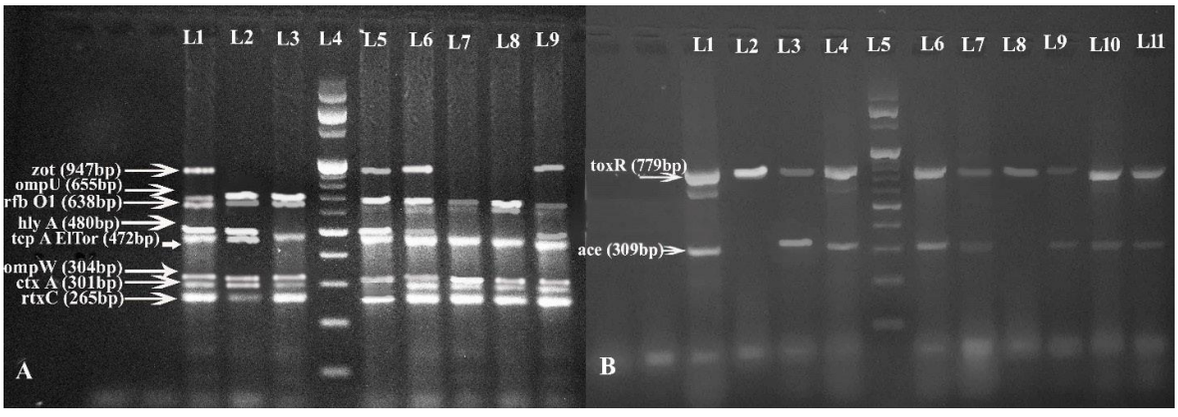

Fig. 1. A-Multiplex PCR 1- Lane 1, 6 & 9 (Genotype 1) showing complete cassette of virulence genes *tcpA* El Tor (472 bp), *rtxC* (265 bp), *ompU* (655 bp), *hly A* (480 bp), *zot* (947 bp); B- *ace* (309 bp), *toxR* (779 bp); Lane 2 (Genotype 2): Absence of *zot*; Lane 3, 7 & 8 (Genotype 3): Absence of *hlyA* and zot; Lane 4: 100 bp DNA ladder; Lane 5 (Genotype 4): Absence of *ctxA*; B-Multiplex PCR 2- Lane 1, 3, 4, 6, 7, 9-11 (Genotype 1): *ace* (309 bp), *tox R* (779 bp); Lane 2 & 8 (Genotype 5): Absence of *ace*; Lane 5: 100 bp DNA ladder.

Table 2. Representative genotypes of *Vibrio cholerae* O1 strains

Straina	District	Genotype
12	Kamrup Metro and Rural	1. *tcpA (El)b+ ctxA+ ompU+ rtxC+ ompW+ zot+ O1rfb+ hlyA+ ctxB+ ace+ toxR+*
5	Darrang	2. *tcpA (El)+ ctxA+ ompU+ rtxC+ ompW+ zot- O1rfb+ hlyA+ ctxB+ ace+ toxR+*
3	Barpeta	3. *tcpA (El)+ ctxA+ ompU+ rtxC+ zot- ompW+ O1rf+ hlyA- ctxB+ ace+ toxR+*
3	Barpeta & Nalbari	4. *tcpA (El)+ ctxA- ompU+ rtxC+ ompW+ zot+ O1rfb+ hlyA+ ctxB+ ace+ toxR+*
2	Morigaon and Golaghat	5. *tcpA (El)+ ctxA+ ompU+ rtxC+ ompW+ zot+ O1rfb+ hlyA+ ctxB+ ace- toxR+*

aData are numbers of strains showing the same genotype
bAbbreviations: El-El Tor

resistance, *dfrA1* in 88% indicating trimethoprim resistance. Detection of *SXT* element in 36% of the isolates indicates that a considerable proportion of the isolates possess the transmissible genetic element which contains the genes encoding resistance to multiple antibiotics.

Genotyping based on *ctxB* gene. The sequences of *ctxB* gene of reference strains *Vibrio cholerae* O1 El Tor N16961 (Accession No. NC_002505) and *Vibrio cholerae* O1 classical 569B (Accession No. U25679), El Tor, Australia (2463, 1988) were retrieved from GenBank. The deduced amino acid sequences of the *ctxB* gene from all strains were aligned using CLUSTAL W.

The deduced amino acid sequence alignment of partial *ctxB* subunit of *V. cholerae* O1 El Tor isolated in the present study against reference strains revealed that, the isolates of present study possessed amino acid sequences identical to the El Tor type of CT-B subunit, which was 100% identical to the amino acid sequence of El Tor reference strain N16961 of genotype 3 by having aspartate at position 28, tyrosine at position 39, phenylalanine at position 46 and isoleucine at position 68 (Table 3).

DISCUSSION

The *V. cholerae* O1 El Tor isolates of the present study possessed different virulence genes like *ctxA, O1rfb, rtxC, ompU, hlyA, zot, ompW, ace, toxR* that indicates some toxigenic strain of *V. cholerae* are circulating in Assam. Cholera toxin (CT) is the most important epidemic marker among various toxins produced by *V. cholerae*, which is encoded by a mobile element, the genome of a filamentous CTX bacteriophage (26). Three strains of *V. cholerae* were negative for *ctxA* after repeated PCR. These findings may suggest that some *V. cholerae* strains can cause

Table 3. Genotypes of *Vibrio cholerae* O1 strains based on the DNA sequence of the *ctx* B subunit genes

Strain identification	Nucleotide at position					Amino acid at position					ctxB genotype
	83	101	115	138	203	28	34	39	46	68	
Classical 569B	A	A	C	T	C	D	H	H	F	T	Genotype 1
El Tor, Australia 2463	A	A	C	G	C	D	H	H	L	T	Genotype 2
El Tor N16961	A	A	T	T	T	D	H	Y	F	I	Genotype 3
MK016537	A	A	T	T	T	D	H	Y	F	I	Genotype 3
MK016538	A	A	T	T	T	D	H	Y	F	I	Genotype 3
MK016539	A	A	T	T	T	D	H	Y	F	I	Genotype 3
MK029003	A	A	T	T	T	D	H	Y	F	I	Genotype 3
MK029004	A	A	T	T	T	D	H	Y	F	I	Genotype 3
MK29005	A	A	T	T	T	D	H	Y	F	I	Genotype 3

illness in the absence of the cholera toxin. The absence of the *ctxA* gene may also be due to the ctxφ prophage genome being missing or disrupted by mutations (27). However, the study could not exclude the possibility of strain diversity which probably could not be detected by the primers used in the study. All the *ctxA* negative strains were positive for accessory cholera toxin *(ace),* zonula occludens toxin *(zot)* and haemolysin A *(hlyA)* genes. This finding is similar to Alishahi et al. (2013) and Abana et al. (2019) (27, 28). All isolates were positive for *tcpA* El Tor which is similar to the findings of Abana et al. (2019) and Zaw et al. (2019) (27, 29).

In the present study 8 (32%) isolates possessed the *ctx g*ene without the *zot* gene as well as 3 (8%) of the isolates possessed *zot* gene without the *ctx* gene, which is similar to a study conducted by Akoachere et al. (2014) (30). This may suggest that occurrence of *zot* gene may be independent of the *ctx* gene.

The screening for virulence genes revealed five different patterns or genotypes thus revealing the diversity among the strains even though all of them were clinical isolates pertaining to different districts of Assam. The predominant genotype included all the virulence genes investigated in the study. Determination of the genotypes based on the presence or absence of specific virulence genes only through *in vitro* test may not always indicate the severity of the strain as the expression of them in individuals may vary. However, the analysis of the virulence genes showed that the positive rates for the major virulence genes were mostly seen among the strains from Kamrup rural district which had large outbreak of cholera. A comparison of the virulence gene profile of *V. cholerae* isolates from different regions of India is presented in Table 4.

Sequencing of the partial *ctxB* gene revealed that the cholera outbreaks in certain districts of Assam were due to *V. cholerae* O1 El Tor carrying the *ctxB* gene of genotype 3. However, Borkakoty et al. (2012) reported circulation of *V. cholerae* O1 El Tor variant carrying the classical *ctxBC* gene in upper Assam (31). Chatterjee et al. (2009) and Goel et al. (2010) also reported the presence of *V. cholerae* O1 El Tor variant carrying the classical *ctxBC* gene in eastern and southern India respectively (32, 33).

Multiple antibiotic resistances among *V. cholerae* have emerged as a major problem worldwide. In India, there is a progressively increasing trend of antibiotic resistance towards common antibiotics like tetracycline and fluoroquinolones (14, 34-36). Resistance to these antibiotics could be due to the extensive use of these antibiotics for the treatment of other infectious diseases. The re-emergence of the cholera epidemic and the evolution of multidrug-resistant *V. cholerae* over the last decade, particularly in Asian countries, pose a great threat to the clinical diagnosis and treatment of cholera. The utmost concern is the acquisition of the *SXT* element by 36% of the isolates in the study. Similar to other bacteria, the spread of antibiotic resistance in *V. cholerae* is also facilitated by horizontal gene transfer via self-transmissible mobile genetic elements like *SXT.* Although identifying the serogroup and serotype of *V. cholerae* isolates is not necessary for treatment of cholera, this information may be of epidemiologic and public health importance. However, earlier studies have reported that, multiple drug resistance was more prevalent among the *V. cholerae* O1 El Tor Ogawa serotypes (31, 34). Hence, active surveillance is required for continuous monitoring of the serotype shift and emergence of antibiotic resistance.

In conclusion, this study showed circulation of multidrug resistant toxigenic *Vibrio cholerae* O1s-trains in Assam carrying a large pool of virulence genes. This study also revealed that the virulence gene profile of the *V. cholerae* isolates in Assam are diverse. The major limitation of this study was the small study population. Hence, further studies should focus on the analysis of the virulence profile of large numbers of epidemic, endemic and environmental strains of *V. cholerae* in Assam to find out the clonal origin of the *V. cholerae* strains of Assam and to monitor emergence of any new virulent strain.

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Table 4. Biotype and genotypic characteristics of *V. cholerae* isolates in different regions of India

Author (Year of publication)	Location of isolation	Year of isolation	Type of isolates	Strain type	*ctxB* genotype	Virulence gene profile
Bhowmick et al. (2009) (37)	Chennai (South India)	1970-2007	Clinical	O1 El Tor Ogawa & O1 classical	-	*ctxA+ zot+ ace+ hlyA+ ompU+ rtxA+ tcpI+ toxR+*
Kumar et al. (2009) (19)	Kolkata, West Bengal (East India)	Not known	Clinical	O1 El Tor Ogawa	El Tor carrying a ctxB sequence of the classical biotype	*ompW+ ctxAB+ zot+ tcpA+ rfbO1+ hlyA+ ompU+ rtx+ toxR+*
Goel et al. (2010) (33)	Chennai (South India)	2004	Clinical	O1 El Tor Ogawa	Hybrid ElTor with classical ctxØ	*ace+, ctxB+, hlyA+, ompU+, ompW+, rfbO1+, rtx+, tcpA+, tox R+ zot+*
Goel et al. (2010) (10)	Chennai (South India)	2005	Clinical	O1 El Tor Ogawa	Hybrid ElTor with classical ctxØ	*ompW+, rfbO1+, ctxB+, zot+, ace+, tcp+, hlyA+ toxR+*
Goel et al. (2010) (38)	Different regions of India	2004-2007	Clinical	O1 El Tor Ogawa	Hybrid ElTor with classical ctxØ	*ompW+, ctxB+, rfbO1+, tcp+, zot+, rtxC+, ace+, hlyA+, ompU+, toxR+*
Jain et al. (2013) (39)	Chennai, Tamil Nadu, Odisha, Maharashtra & Andhra Pradesh	2004-2010	Clinical	O1 El Tor Ogawa	El Tor variant carrying the classical CT	*ace+, ctxB+, hlyA+, ompU+, ompW+, rfbO1+, rtx+, tcpA+, toxR+, zot+*
Ratnam et al. (2015) (40)	Chennai (South India)	Not mentioned	Clinical	O1 El Tor Ogawa, Inaba, Hikojima, O139, non-epidemic strains	-	1. *ctxA+, toxR+, ace+, zot+, tcpA+, nanH+* 2. *ctxA+, toxR+, ace+, zot+, tcpA+, nanH-* 3. *ctxA+, toxR+, ace-, zot-, tcpA+, nanH+* 4. *ctxA-, toxR+, ace+, zot+, tcpA+, nanH+* 5. *ctxA+, toxR+, ace-, zot+, tcpA+, nanH+* 6. *ctxA+, toxR-, ace-, zot+, tcpA+, nanH+* 7. *ctxA+, toxR-, ace+, zot-, tcpA-, nanH+ ace+,*
Jain et al. (2016) (41)	Rayagada district, Odisha	2007 & 2010	Clinical	O1 El Tor	El Tor variant carrying classical CT	*ctxAB+, hlyA+, ompU+, ompW+, rfbO1+, rtxC+, tcpA+, toxR+, zot+*
Nayak et al. (2021) (42)	Odisha	1995-2019	Clinical	O1 El Tor and O1 classical	-	*toxR+, ompU+, ace+, rtxC+, ctxA+, tcpA+, rfbO1+, ompW+*

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