



Prevalence of *Vibrio cholerae* O1 serogroup in Assam, India: A hospital-based study

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Background & objectives: Although cholera remains to be an important public health problem, studies on reliable population-based estimates of laboratory confirmed cholera in endemic areas are limited worldwide. The aim of this hospital-based study was to evaluate the prevalence of *Vibrio cholerae* serogroup in Assam, India, during 2003-2013.

Methods: Stool samples/rectal swabs were collected from acute watery diarrhoea (AWD) cases during 2003-2013 and processed by standard microbiological procedures. Antibiotic sensitivity test was done following the Clinical and Laboratory Standards Institute guidelines. Year-wise epidemiological trend of cholera was analyzed.

Results: Cholera contributed to 3.93 per cent of AWD cases. In Assam, cholera was found to be more prevalent in the rural areas (6.7%) followed by the tea gardens (5.06%), urban slum (1.9%) and urban areas (1.4%). Highest proportion of cholera (13.7%) was observed in 0-10 yr age group. Of them, 11.5 per cent belonged to 0-5 yr age group. *V. cholerae* O1 El Tor serotype Ogawa was the predominant isolate. Multiple drug-resistant isolates of *V. cholerae* O1 Ogawa were reported in the study.

Interpretation & conclusions: Emergence of resistance amongst *V. cholerae* towards many antibiotics is a matter of concern. Hence, continuous surveillance for diarrhoeal disorders is necessary to control the future outbreaks of cholera in this region.

Key words El Tor - Ogawa - serogroup - *Vibrio cholerae* O1

Cholera continues to be a major public health problem of growing concern in most developing countries¹. It is endemic in India and whole of the Ganges basin from where it has spread in the form of epidemics and pandemics to several other parts of the

world. Globally, cholera alone causes 120,000 deaths annually². In Asia, Indian subcontinent contributes about 78 per cent of cholera cases³. The incidence of cholera is estimated to be 1.6 cases/1000 population per year or 40/1000 cases of acute diarrhoea in India^{4,5}.

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Depending on somatic antigens, *Vibrio cholerae* have more than 200 serogroups, of which O1 and O139 are outbreak/epidemic strains⁶. *V. cholerae* O1 has two well-established biotypes, namely, classical and El Tor, which are differentiated primarily based on phenotypic characters. Observation during the last few years shows that many of the phenotypic and genotypic tests have been proven to be inadequate for classifying *V. cholerae* O1 strains into their biotype⁷. Strains having conventional phenotypic properties of both classical and El Tor are designated as 'hybrid biotype' and another which is similar to the El Tor biotype by conventional phenotypic traits, but produces classical type CT are designated as 'El Tor variant'⁷. This El Tor variant has caused several outbreaks/epidemics in Odisha, Chandigarh, Southern India, West Bengal, etc.⁸⁻¹³. This study was undertaken to evaluate the changing trend of epidemiological profile, prevalent biotypes and serotypes of *V. cholerae* and their antimicrobial resistance pattern during 2003-2013 in Assam, India.

Material & Methods

This hospital-based study was carried out at Gauhati Medical College, Guwahati, Assam, India, for a period of 11 years from 2003-2013. The study protocol was approved by the Institutional Ethics Committee. All acute watery diarrhoea (AWD) cases referred to Gauhati Medical College Hospital and Fakhruddin Ali Ahmed Medical College Hospital from various districts of Assam were included in the study after applying the inclusion and exclusion criteria¹⁴. Written informed consent was obtained from patients or parents in case of children.

All AWD patients with or without vomiting and/or dehydration of any age group were included in the study. Those presenting with acute bloody diarrhoea (acute diarrhoea with visible blood in stool with or without fever and/or pain in abdomen), and patients with AWD who were on antibiotics were excluded from the study.

During the study period, 1779 stool samples/rectal swabs were collected. The stool samples were collected in sterile container and the rectal swabs were collected in Cary-Blair Transport Medium. Enrichment was done in alkaline peptone water (APW, pH 8.0) for 6-8 h. Before the samples were subcultured, hanging drop preparations were made to detect the typical darting motility of *V. cholerae* if present, both directly from the sample and after enrichment with APW.

The subculture was done on MacConkey agar and thiosulphate-citrate-bile salt-sucrose agar medium. The isolates were identified as *V. cholerae* according to the standard laboratory methods using Gram stain, wet mount for motility, oxidase test, string test, triple sugar iron agar test and lysine iron agar test¹⁵. The culture media and reagents were obtained from HiMedia Laboratories, Mumbai.

For further confirmation, serotyping was done using *V. cholerae* O1 polyvalent antiserum followed by monovalent antiserum Ogawa and Inaba obtained from Denka Seiken Co., Ltd., Tokyo, Japan. Biotyping was done by standard conventional biotyping scheme using haemolysis of sheep blood, chick erythrocyte agglutination, Voges-Proskauer (VP) test and polymyxin B sensitivity with 50 µg disc¹⁵. Some of the representative strains were sent to National Institute of Cholera and Enteric Diseases (ICMR-NICED), Kolkata, for phage typing.

Antibiotic sensitivity was done as per the Clinical and Laboratory Standards Institute (CLSI) guidelines using antimicrobial agents (µg/disc) such as amoxicillin (10), co-trimoxazole (25), tetracycline (30), chloramphenicol (30), amikacin (30), ciprofloxacin (5), erythromycin (15) and furazolidone (50)¹⁶. *Escherichia coli* ATCC 25922 was used as quality control strain. Antibiotic sensitivity profile of each isolate was recorded as resistant, intermediate and sensitive based on the zone diameter according to CLSI guidelines. The antibiotic discs were obtained from HiMedia Laboratories Mumbai.

Data were collected and recorded using a predesigned questionnaire to study the trend of epidemiology of cholera during the study period. Year-wise proportions of cholera cases by age, gender, season and antibiotic resistance profile were analyzed to determine the trend. The descriptive statistical analysis was done manually and using statistical software Epi Info 7.1.2.0 (Centre for Disease Control and Prevention, USA).

Results

The AWD cases included in the study belonged to Dhubri, Barpeta, Nalbari, Kamrup (metro), Kamrup (rural), Nagaon, Dima Hasao, Cachar, Sonitpur, Karbi Anglong and Golaghat districts of Assam. The prevalence of cholera was 6.7 per cent (39 of 582 cases) in rural areas, 1.4 per cent (7 of 478 cases) in urban areas, 1.9 per cent (8 of 403 cases) in urban slums and 5.06 per cent (16 of 316 cases) in the tea gardens.

During 2003 to 2013, 1779 stool samples/rectal swabs were processed. *V. cholerae* was isolated from 70 (3.93%), *Shigella flexneri* from 30 (1.68%) and non-typhoidal *Salmonella* from seven (0.4%) cases. Other isolates included *Aeromonas* species in five (0.28%) and *Staphylococcus aureus* in pure culture in 10 (0.56%) cases. In 92.3 per cent of the AWD cases, normal enteric flora was found which predominantly included pure growth of *E. coli* in 1020 (57.3%) cases. However, serotyping of these *E. coli* isolates was not done to exclude enterotoxigenic and enteropathogenic *E. coli*. *Vibrio cholerae* isolation rate was highest in 2007 (8.7%) followed by 7.8 and 7.3 per cent in 2010 and 2011, respectively. In 2005 and 2006, no cholera case was found. *S. flexneri* was more prevalent in 2003 (10.6%) followed by 3.7 and 3.4 per cent in 2004 and 2011, respectively. Non-typhoidal *Salmonella* (3.9%) was isolated only in 2011. Table I depicts year-wise isolation of various enteropathogens.

Age-wise proportion of cholera cases is shown in Table II. The highest proportion of cholera was observed in the age group 0-10 yr (13.7%) followed by 11-20 yr (4.3%) and 21-30 yr (3.2%). In 2004, 2007-2009 and 2011 and 2012, 0-10 yr age group was the most commonly affected age group. Analysis of year-wise trend showed that in 2008, 2009, 2011 and 2012, 25 per cent (1 of 4), 80 per cent (4 of 5), 15.4 per cent (2 of 13) and 14.3 per cent (1 of 7) cholera cases were found, respectively, in the age group 0-5 yr from the cholera endemic region. However, no such discernible pattern was observed in other years of the

study period. Of the 1779 cases, 993 (55.8%) were female and 786 (44.1%) were male. *V. cholerae* was isolated from 4.3 per cent (43 of 993) of the female cases and 3.4 per cent (27 of 786) of the male cases. The year-wise analysis revealed that proportion of cholera cases was more among the females in most of the years except in 2003 and 2007, where cholera was more prevalent among males than females (7.8 vs 3.7% and 10.6 vs 7.05%, respectively) (Figure).

Month-wise and year-wise AWD and cholera cases are presented in Table III. It was observed that during the period 2003-2009, most of the diarrhoea cases occurred in the monsoon season (July-October) which contributed 41.2 per cent (733 of 1779 cases) of the AWD cases as well as 71.4 per cent of the cholera cases (50 of 70 cases). The overall prevalence of cholera was highest 6.8 per cent (50 of 733 cases) during the monsoon. In 2013, only one cholera case was reported in monsoon season (Table III).

The predominant isolate in our study was *V. cholerae* O1 biotype El Tor serotype Ogawa (94.3%). In 2004, 2.9 per cent of the cases were found to be *V. cholerae* O1 biotype El Tor serotype Inaba. Two (2.9%) *V. cholerae* O1 isolates showed characteristics of El Tor biotype with some characteristics of classical variety (hybrid variety). These isolates were negative for VP reaction, sensitive to polymyxin B (50 µg), the traits typical for the classical biotype and were found to cause haemolysis of sheep erythrocytes, chick erythrocyte agglutination. These isolates were phage

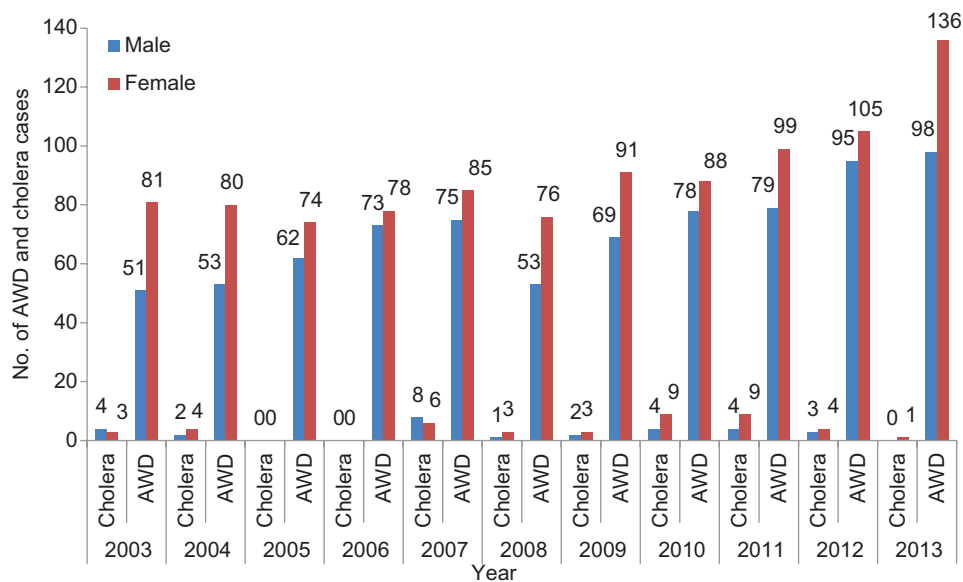


Figure. Year-wise and gender-wise distribution of acute watery diarrhoea (AWD) and cholera cases.

Table I. Year-wise isolation of enteropathogens in the study

Enteropathogens	Year												
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013		
Total sample (n=1779)	132	133	136	151	160	129	160	166	178	200	234		
Culture positive, n (%)	131 (99.2)	131 (98.4)	136 (100)	149 (98.6)	158 (98.7)	127 (98.4)	159 (99.3)	166 (100)	176 (98.9)	200 (100)	231 (98.7)		
<i>Vibrio cholerae</i> , n (%)	7 (5.3)	6 (4.5)	ND	ND	14 (8.7)	4 (3.1)	5 (3.1)	13 (7.8)	13 (7.3)	7 (3.5)	1 (0.4)		
Non-typhoidal <i>Salmonella</i> , n (%)	ND	ND	ND	ND	ND	ND	ND	ND	7 (3.9)	ND	ND		
<i>Shigella flexneri</i> , n (%)	14 (10.6)	5 (3.7)	2 (1.5)	1 (0.7)	ND	1 (0.8)	ND	ND	6 (3.4)	1 (0.5)	ND		
Other isolates, n (%)	0	5 (3.7)	0	0	4 (2.5)	0	2 (1.2)	4 (2.4)	0	0	0		
Normal enteric flora, n (%)	110 (83.3)	115 (86.4)	134 (98.5)	148 (98.0)	140 (87.5)	122 (94.5)	152 (95.0)	149 (89.7)	150 (84.2)	192 (96.0)	230 (98.2)		
Culture negative, n (%)	1 (0.75)	2 (1.5)	0	2 (1.3)	2 (1.2)	2 (1.5)	1 (0.6)	0	2 (1.1)	0	3 (1.3)		

Normal enteric flora included *Escherichia coli*; *Klebsiella* spp.; *Citrobacter* spp.; *Enterobacter* spp.; *Proteus* spp.; *Pseudomonas* spp.; coagulase negative staphylococci and yeasts. Other isolates, *Aeromonas* species and *Staphylococcus aureus*.
 ND, not detected; n, number of cases

Table II. Year-wise proportion (%) of cholera cases in different age groups

Age group (yr)	Proportion of cholera cases by age	Year-wise proportion of cholera cases in different age groups (%)										
		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
0-10	13.7	0/14 (0)	3/12 (25)	0/7 (0)	0/12 (0)	4/13 (30.7)	1/9 (11.1)	4/19 (21)	0/6 (0)	5/19 (26.3)	4/20 (20)	0/22 (0)
11-20	4.3	1/16 (6.2)	2/20 (10)	0/19 (0)	0/25 (0)	3/25 (12)	0/17 (0)	0/24 (0)	2/18 (11.1)	2/27 (7.4)	0/26 (0)	1/37 (2.7)
21-30	3.2	5/34 (14.7)	1/41 (2.4)	0/33 (0)	0/35 (0)	4/50 (8)	1/50 (2)	1/45 (2.2)	1/39 (2.5)	2/45 (4.4)	0/45 (0)	0/46 (0)
31-40	2.7	0/30 (0)	0/33 (0)	0/41 (0)	0/35 (0)	2/28 (7.1)	1/18 (5.5)	0/24 (0)	4/32 (12.5)	2/35 (5.7)	1/42 (2.4)	0/49 (0)
41-50	2.4	0/18 (0)	0/16 (0)	0/24 (0)	0/21 (0)	0/20 (0)	0/17 (0)	0/18 (0)	3/36 (8.3)	2/24 (8.3)	1/24 (4.1)	0/33 (0)
51 and above	2.4	1/20 (5)	0/11 (0)	0/12 (0)	0/23 (0)	1/24 (4.1)	1/18 (7.7)	0/30 (0)	3/35 (7.4)	0/28 (0)	1/43 (3.2)	0/47 (0)

Table III. Month- and year-wise trend of acute watery diarrhoea (AWD) and cholera

Month	Year-wise trend of acute watery diarrhoea (AWD) and cholera											
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	
January	3	0	11	0	6	0	8	0	8	0	10	0
February	5	0	12	0	7	0	6	0	9	0	12	0
March	10	0	12	0	10	0	9	0	10	0	12	0
April	8	0	10	0	12	0	11	0	15	0	7	0
May	12	0	16	0	13	0	10	0	17	0	18	0
June	10	0	13	0	13	0	16	0	28	6	13	0
July	13	1	18	0	17	0	13	0	14	0	18	0
August	16	1	20	0	15	0	12	0	13	0	27	1
September	18	0	12	0	17	0	15	3	16	1	24	0
October	19	5	14	1	7	0	14	1	21	4	23	0
November	10	0	13	0	14	0	11	0	16	0	18	0
December	8	0	10	0	11	0	4	0	16	0	18	0

AWD, acute watery diarrhoea

typed in NICED, Kolkata, and were reported to be phage type T-4 (Basu & Mukherjee) and T-27 (New scheme).

The antibiotic resistance profile was analyzed during the study period and found none of the isolates were consistently sensitive to any of the antibiotics. The most important finding was that 52.8 per cent of the isolates were resistant to tetracycline. However, a decline in the rate of tetracycline resistance was observed since 2010. The low-level resistance of 7.1 and 14.3 per cent was observed against amikacin and ciprofloxacin, respectively (Table IV).

Discussion

The present study showed high prevalence of cholera in rural areas (6.7%) followed by the tea gardens (5.06%) of Assam. This finding corroborated with that of the earlier studies carried out in Assam^{17,18} which reported outbreaks of cholera in rural areas and tea gardens of Assam. Data from population-based diarrhoea surveillance in an endemic area of Kolkata revealed a cholera incidence of 2.2 cases per 1000 person-years¹⁹. During a 10-yr study period, the States having the highest number of reported cholera outbreaks were West Bengal, Odisha, Maharashtra and Kerala, which together accounted for 60 per cent of all reported outbreaks. Most of the cholera cases (91%) were identified in the States or union territories of Odisha, West Bengal, Andaman and Nicobar Islands, Assam and Chhattisgarh¹⁹. In India, there has been resurgence of cholera in many places such as Chandigarh and several north western States and outbreaks including major epidemics have occurred from time to time at various places^{8-10,17,20-22}. The attack and case fatality rate of cholera in Assam have been reported to be 11.6 and 0.8 per cent, respectively¹⁷.

Cholera contributed to 3.93 per cent of all AWD cases in the present study. Sur *et al*⁵ reported a similar prevalence rate (4%) of culture confirmed cholera cases among all the AWD cases in a community-based study in slums of Kolkata, India. Kaur and Lal²³ also reported similar isolation rate of *V. cholerae* 2, 2.6, 6.7, 7.08, 0.9 and 2.6 per cent during 1992-1997, respectively, in a hospital-based study in north India. In 2005 and 2006, no cholera case was reported and during 2003-2013, most of the stool/rectal swab cultures revealed neither *V. cholerae* nor any other enteropathogen which might be due to the prior usage of antibiotics by the patients. However, some studies reported higher prevalence rates 14.03 per cent²⁴, 20.8 per cent²⁵ and 16.2 per cent²⁶.

Table IV. Year-wise antibiotic resistance profile of the *Vibrio cholerae* isolates (resistance per cent)

Year (n)	Amoxicillin	Co-trimoxazole	Tetracycline	Chloramphenicol	Amikacin	Ciprofloxacin	Erythromycin	Furazolidone
2003 (7)	7 (100)	7 (100)	5 (71.4)	7 (100)	0 (0)	0 (0)	4 (57.1)	7 (100)
2004 (6)	6 (100)	4 (66.6)	4 (66.6)	3 (50)	0 (0)	0 (0)	3 (50)	4 (66.6)
2005 (0)	-	-	-	-	-	-	-	-
2006 (0)	-	-	-	-	-	-	-	-
2007 (14)	12 (85.7)	14 (100)	9 (64.2)	4 (28.5)	2 (14.2)	0 (0)	6 (42.8)	11 (78.5)
2008 (4)	3 (75)	4 (100)	4 (100)	2 (50)	1 (25)	1 (25)	3 (75)	4 (100)
2009 (5)	4 (80)	5 (100)	4 (80)	0 (0)	0 (0)	2 (40)	2 (40)	5 (100)
2010 (13)	7 (53.8)	13 (100)	6 (46.1)	2 (15.3)	1 (7.7)	2 (15.3)	4 (30.7)	13 (100)
2011 (13)	13 (100)	12 (92.3)	3 (23)	2 (15.3)	1 (7.7)	3 (23)	8 (61.5)	13 (100)
2012 (7)	7 (100)	7 (100)	2 (28.5)	3 (42.8)	0 (0)	2 (28.5)	3 (42.8)	7 (100)
2013 (1)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)
Total (70)	60 (85.7)	67 (95.7)	37 (52.8)	23 (32.8)	5 (7.1)	10 (14.2)	34 (48.5)	65 (92.8)

No discernible pattern was noticed in year-wise prevalence of cholera in different age groups. However, finding of 11.5 per cent cholera cases in the age group 0-5 yr from cholera endemic region was an important finding of the study. The prevalence rate of cholera in 0-5 yr age group found in this study was lower than that reported by others (32.7²² and 31.4²⁷ % of cholera cases). Cholera was seen more among females in most of the years in our study except the year 2003 and 2007, during which male predominated the female cases. Increased prevalence among females has been reported by Pal *et al*²⁵, however, many studies have reported higher attack rate among males than females or no variation was reported in the attack rate between genders^{26,27}.

V. cholerae O1 biotype El Tor serotype Ogawa (94.3%) was the predominant isolate in the present study which was comparable to many studies done in India^{17,18,24,25,27}. Another important finding of the study was that 2.9 per cent of the *V. cholerae* isolates were hybrid biotype showing common characteristics of both classical and El Tor biotypes. However, VP reaction was variable and was not a recommended marker for the differentiation of classical and El Tor vibrios. The existing biotyping scheme has limitations and causes confusion as many of the hybrid biotype and El Tor variant strains have phenotypic and genetic changes.

The analysis of year-wise antibiotic resistance profile revealed that amoxicillin resistance decreased during 2003-2004, followed by an increase in resistance to 100 per cent during 2011-2013. A similar finding was also reported by Chander *et al*²⁷. A declining trend of tetracycline resistance was observed during 2003-2007 ranging from 71.4 to 64.2 per cent followed by a high-level resistance (100%) in the year 2008, after which the resistance again declined to 28.5 per cent in 2012. Overall, 52.8 per cent *V. cholerae* isolates were resistant to tetracycline, which was an important finding. There are reports of tetracycline-resistant *V. cholerae* strains responsible for major epidemics of cholera in Latin America, Tanzania, Bangladesh and Zaire²⁸. Hence, any level of resistance to tetracycline is significant and requires monitoring. In the study carried out in Odisha by Pal *et al*⁹, all clinical *V. cholerae* isolates were sensitive to gentamicin, azithromycin, chloramphenicol and tetracycline and resistant to ciprofloxacin, norfloxacin, ampicillin, streptomycin, neomycin, nalidixic acid, furazolidone and co-trimoxazole. A low-level resistance to tetracycline 4.34-15.38 per cent²⁷ and one per cent⁵ has also been

reported. Bhattacharya *et al*²⁹ reported a sudden upsurge in tetracycline resistance among *V. cholerae* isolates, from one per cent in 2004 to 76 per cent in 2007 before decreasing to 50 per cent in 2009. Kar *et al*³⁰ reported 100 per cent tetracycline-resistant *V. cholerae* O1 El Tor variant strains causing the cholera epidemic in Odisha.

In the present study, 34 per cent isolates were sensitive to co-trimoxazole and furazolidone in 2004; however, these were found to be the least effective antibiotics *in vitro* for the rest of the study period. Bhattacharya *et al*²⁹ also reported an increase in resistance to furazolidone and trimethoprim/sulphamethoxazole during the study period. A declining trend of chloramphenicol resistance was also observed during the study period ranging from 100 per cent resistance in 2003 to 0 per cent in 2013. A low-level resistance to chloramphenicol has already been reported^{21,22}. Emergence of resistance to ciprofloxacin was observed in our study during 2008-2012, which ranged from 15.3 to 40 per cent. This correlated with the findings reported by earlier workers^{27,30}.

In conclusion, multiple drug-resistant *V. cholerae* O1 Ogawa strains were reported during 2003-2013 from Assam. Development of resistance to commonly used antibiotics indicates a serious public health concern because it complicates treatment by extending the duration of hospital stay for patients. Hence, a continuous surveillance for diarrhoeal disorders is necessary to control the future outbreaks of cholera in this region.

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Conflicts of Interest: None.

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