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Original Article

Epidemiology and Antibiogram Profile of *Vibrio cholerae* Isolates between 2004–2013 from Odisha, India

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SUMMARY: Cholera is an acute diarrheal disease caused by *Vibrio cholerae* serogroups O1 and O139, which are known to cause epidemics of cholera in Odisha. The present study was intended to document the antibiotic resistance pattern among clinical isolates of both serogroups of *V. cholerae* (O1 and O139) isolated during 2004–2013. Nine-hundred nine isolates of *V. cholerae* were included in this study and were identified by standard procedures. An antibiotic sensitivity test was performed by the disc diffusion method. The seasonality of cholera in this region indicated that there was one peak in the rainy season only. The number of cholera cases started increasing from July and declined starting from the month of October onward. The adult age group of patients was the worst affected among all age groups of patients. The 2 different serogroups of *V. cholerae* (O1 and O139) showed different prevalence rates (%) of resistance to all the antibiotics in each year. Serogroup O1 showed uniformly high resistance to co-trimoxazole, furazolidone, and nalidixic acid throughout the study. Chloramphenicol encountered resistance only during 2009, but the strains were sensitive in the other years. The emergence of multiple drug-resistant *V. cholerae* strains may significantly influence the control of future outbreaks and epidemics of cholera in this region.

INTRODUCTION

Cholera is a widespread endemic and neglected disease causing high morbidity and mortality throughout the world, especially in the developing nations including India. *Vibrio cholerae* strains belonging to serogroups O1 and O139 are the causative agents of cholera. Recently, a new variant El Tor, evolved, which was reported initially in Bangladesh, Asia. Until October 2015, 10,700 cholera cases and 170 deaths had been reported by the WHO in 5 countries in the East Mediterranean region and Africa (1). The current pandemic is the 7th pandemic that started in Indonesia during 1961 and still continues. Worldwide cholera cases were reported in the Middle East, Africa, South America, Democratic Republic of Congo, Zimbabwe, and Bangladesh (2).

As in a developing country, sanitation and public health system in India are poorly developed. Therefore, cholera continues to be a public health problem. In terms of the number of cholera cases, India was behind Afghanistan and Philippines in 2014 (3). Over the last decade, cholera outbreaks were reported in different parts of the country with high frequency (4, 5). Cholera outbreaks are some of the most frequent public health problems in Odisha. Several outbreaks and epidemics were reported during the supercyclone of 1999, flood of 2000, cholera epidemics of 2007 in tribal areas, cholera outbreaks in the Kendrapada district in 2009 and Rayagada and Kalahandi districts in 2012 (6–8), and in the Narla block of Kalahandi in 2014 (JJID, accepted for publication).

Prior to monsoon, *Vibrio* species undergo genetic assortment and reassortment for better adaptability, which account for antibiotic resistance (9). Prior to 1977, antibiotic-resistant strains had been rare, but indiscriminate use of antibiotics and frequent outbreaks of cholera gave rise to antibiotic resistance. Multidrug-resistant *V. cholerae* strains were reported in Rayagada in 2007 (7). Tetracycline-resistant *V. cholerae* strains were reported in the Rayagada district of Odisha in 2010, but *V. cholerae* strains at this location were sensitive earlier (8). Fluoroquinolone-resistant *V. cholerae* strains were reported in South India in 2002 (10).

Therefore, the present study was envisaged to determine the seasonal pattern of cholera and trends in multiple types of antibiotic resistance among clinical isolates of both *V. cholerae* serogroups O1 and O139 obtained during 2004–2013 in different parts of Odisha.

MATERIALS AND METHODS

Bacteriology and serogrouping: Rectal swabs from patients with diarrhea were collected into the Cary–Blair transport medium at various public health centers and in the houses of cholera-affected villages. The rectal swabs were inoculated into thiosulphate citrate bile salt sucrose (TCBS: BD, Franklin Lakes, NJ, USA) agar for the isolation of *V. cholerae*. Prior to inoculation, enrichment was carried out in alkaline peptone water (APW). The inoculated plates were incubated at 37°C for 24–48 h,

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and growth was examined for further analysis. *V. cholerae* was identified by standard biochemical tests. Serotypes of *V. cholerae* were confirmed using antisera procured from BD (7). Nine-hundred nine *V. cholerae* isolates collected from hospitalized diarrhea patients from outbreak areas of different parts of Odisha from 2004 to 2013 were included in the study. All the isolates were stored in soft agar supplemented with 1% NaCl at room temperature (20–30°C). Patients were subdivided into 4 age groups, i.e., <15 years, 15–30 years, 30–60 years, and > 60 years.

Antibiotic sensitivity tests: The sensitivity and resistance patterns of *V. cholerae* O1 strains were tested with antibiotic-impregnated commercial disks (BD) containing ampicillin (A, 10 mg), chloramphenicol (C, 30 mg), co-trimoxazole (Co, 25 mg), ciprofloxacin (Cf, 5 mg), furazolidone (Fz, 100 mg), gentamicin (G, 10 mg), neomycin (N, 30 mg), nalidixic acid (Na, 30 mg), norfloxacin (Nx, 10 mg), streptomycin (S, 10 mg), or tetracycline (T, 30 mg). *V. cholerae* O1 and O139 strains were subcultured in Luria broth (BD) and plated on Mueller–Hinton agar (BD). The plates were incubated for 24 h at 37°C. Characterization of strains as susceptible or resistant was conducted based on the size of the inhibition zones around each antibiotic disk in accordance with the manufacturer's instructions following the Kirby–Bauer technique (11).

RESULTS

Nine-hundred nine *V. cholerae* isolates were obtained from 4,886 rectal swabs during the study period, and those isolates were confirmed by both biochemical and serological tests. It was observed that the *V. cholerae* O1 serogroup was completely dominant over the *V. cholerae* O139 serogroup. The *V. cholerae* O139 serogroup was the least frequently isolated in 2006, after which it was undetectable in this region. Similarly, the number of Ogawa serotype isolates was greater in comparison with the Inaba serotype throughout the study period, but Inaba serotype isolates were the most numerous in 2005 and gradually decreased in number; after 2008, no Inaba serotype isolates were detected in this region (Table 1). *V. cholerae* O1 Ogawa El Tor biotype isolates were reported in this region from 1995 to 2007. Nonetheless, the 1st epidemic of cholera due to the

Table 1. Yearwise distribution of *V.cholerae* strains from different areas of Odisha

Year	Total Samples	O1		O139	Place
		Ogawa	Inaba		
2004	148	35	12	0	Dhenkanal, Puri
2005	600	33	50	0	Kendrapara, Puri, Khurda, Dhenkanal, Cuttack
2006	652	30	17	8	Puri
2007	448	101	24	0	Ersama, Khurda, Kashipur, Dasmantpur, Mohana, Laxmipur, Kolnara, Th. Rampur
2008	618	125	2	0	Nayagard, Anugul, Kendrapada, Jajpur, Bargard Phulbani , Khurda, Puri, Ganjam, Boudh, Gajapati, Sambalpur & Sundergarh
2009	280	82	0	0	Mayurbhanj. Sundargarh, Ganjam, Rajnagar, Kandhamal, Kalahandi
2010	608	66	0	0	Rayagada, Kalahandi, Sundargarh, Nuapada, Ganjam, Malkangiri, Khurdha, Nabrangpur
2011	666	94	0	0	Puri, Gajpati
2012	500	144	0	0	Rayagada, Nuapada, Ganjam, Boudh ,Balangir, Keonjhar, Kalahandi
2013	366	86	0	0	Rayagada ,Koraput, Gajpati
Total	4886	796	105	8	

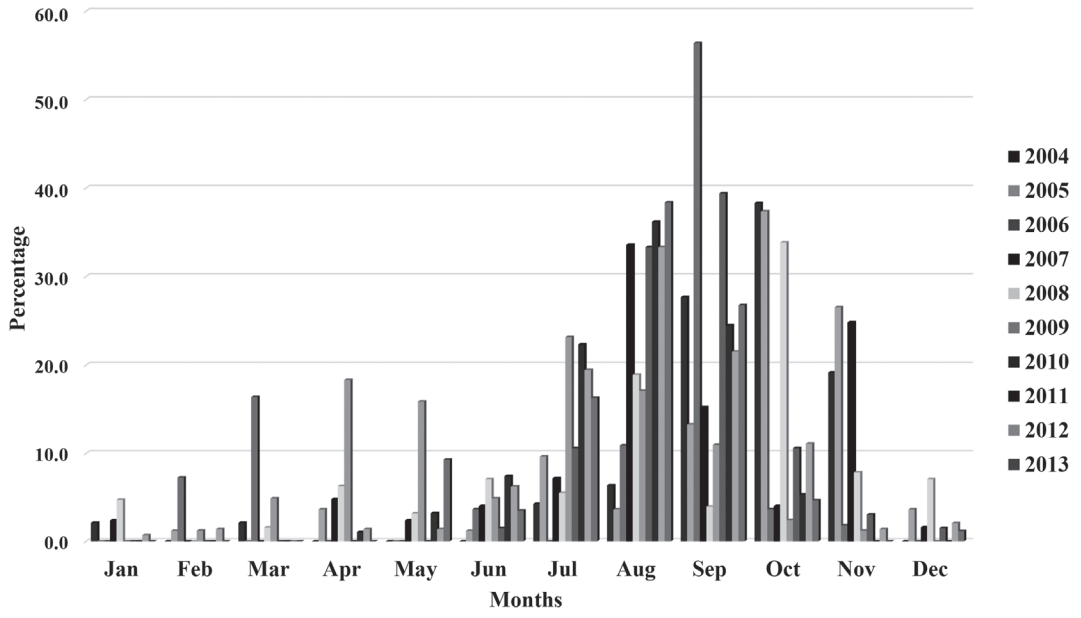

Fig. 1. Monthwise distribution of cholera patients (2004-2013).

El Tor variant carrying the *ctxB* gene of the classical strain was reported in 2007 in the tribal region and was spreading to different parts of the state. The monthwise distribution of cholera cases indicated that there was one peak, i.e., in the rainy season. The number of cases starts increasing from the month of July and declines from October onward (Fig. 1). The agewise distribution of the cholera cases indicated that people younger than 15 years old were the least affected, whereas people 15–60 years of age were more frequently infected. The distribution of cholera cases by sex indicated that both sexes were infected equally (Fig. 2).

Different prevalence rates (%) of resistance to different drugs were observed in different years (Table 2). Serogroup O1 showed uniformly high resistance to co-trimoxazole, furazolidone, and nalidixic acid throughout the study period. Increased resistance to ampicillin was observed after 2006. Chloramphenicol encountered resistance only in 2009, but the isolates were sensitive in the other years. Gentamicin,

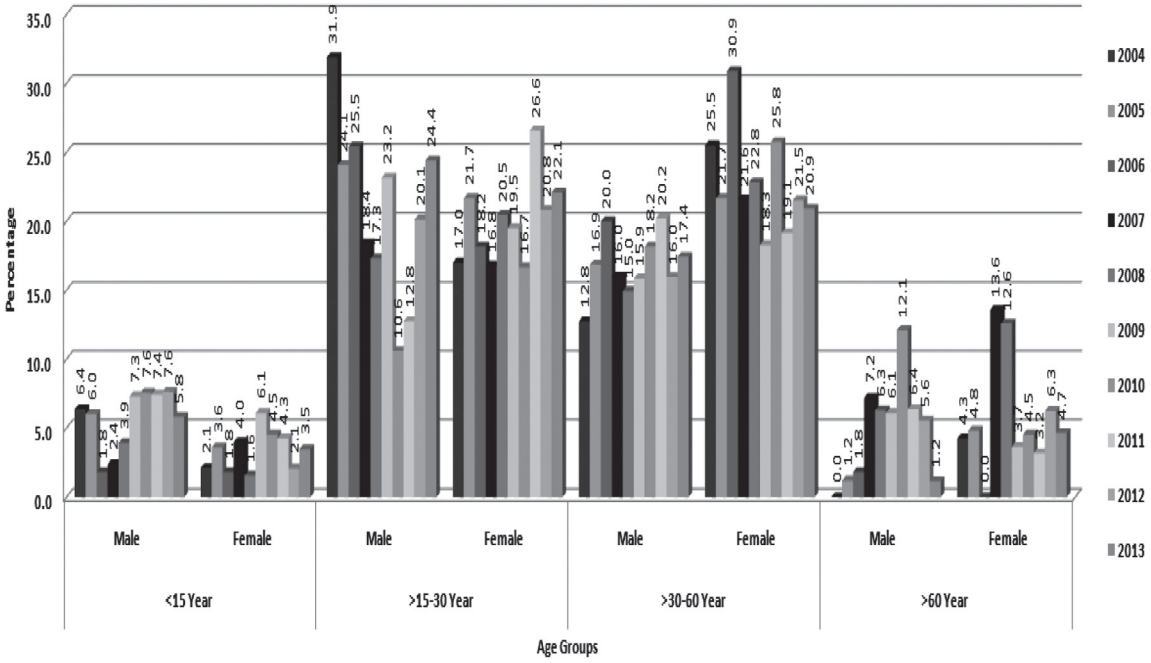

Fig. 2. Age and sexwise distribution of cholera patients (2004-2013).

Table 2. Percentage resistance of *V.cholerae* serogroups to different antibiotics

Year			2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
No. of isolates		O1	47	83	55	125	127	82	66	94	144	86
		O139	0	0	8	0	0	0	0	0	0	0
No. of resistant strains (%)	Ampicillin	O1	60	7.5	5.36	90.91	91.34	81.71	90.91	100	100	98.84
		O139			0							
	Chloramphenicol	O1	4	8.75	5.36	22.33	21.26	60.49	22.73	20.21	21.53	18.6
		O139			0							
	Tetracycline	O1	0	11.25	1.79	46.6	36.22	17.28	80.3	13.83	1.39	8.14
		O139			16.67							
	Streptomycin	O1	88	42.5	42.86	86.41	41.73	78.05	90.91	75.53	87.5	88.37
		O139			0							
	Gentamicin	O1	0	3.75	1.79	19.42	3.15	4.88	13.64	0	0	0
		O139			0							
	Nalidixic acid	O1	100	98.8	96.3	85.6	96.85	96.34	96.97	95.74	100	97.67
		O139			62.5							
	Co-trimoxazole	O1	96	48.75	92.86	89.47	93.7	87.5	98.48	74.47	93.75	89.53
		O139			83.33							
	Neomycin	O1	12	61.25	35.71	60.98	28.35	24.39	42.42	28.72	6.25	15.12
		O139			33.33							
	Ciprofloxacin	O1	25.5	3.6	14.5	64.8	14.17	22.22	31.82	2.13	0	1.16
		O139			0							
	Furazolidone	O1	100	90	91.07	95.95	99.21	97.56	96.97	98.94	97.22	100
		O139			33.33							
	Norfloxacin	O1	31.9	4.8	5.4	63.2	11.02	10.13	10.03	0	0.69	1.16
		O139			25							

Table 3. Antibiogram profile of *V.cholerae* serogroups from 2004 to 2013

YEAR	O1	O139
2004	A Fz Co S Na	-
2005	Fz Na N	-
2006	Fz Co Na	Co Na
2007	A Fz Co S Na Nx N Cf	-
2008	A Fz Co Na	-
2009	A C Fz Co S Na	-
2010	A Fz Co S Na T	-
2011	A Fz Co S Na	-
2012	A Fz Co S Na	-
2013	A Fz Co S Na	-

streptomycin, ciprofloxacin, norfloxacin, and neomycin tests showed varying ranges of sensitivity of the isolates. Tetracycline encountered high resistance during 2010, but the isolates were sensitive in the other years. According to the antibiotic sensitivity profiles over 10 years, multiple types of drug resistance were detected during the study period (Table 3). Sometimes, the strains showed different resistance patterns in different periods, for instance, multidrug-resistant strains of *V. cholerae* were observed during the supercyclone 1999, flood in 2000, and a cholera outbreak in 2007 and 2010 in tribal areas.

DISCUSSION

In this study, *V. cholerae* O1 Ogawa isolates were more numerous in comparison with Inaba serotype isolates, and the Inaba serotype was not detected from 2009 onward. In agreement with these findings, reports from Karnataka indicate that more Ogawa isolates were detected in comparison with Inaba strains (12). The *V. cholerae* O139 serogroup staged a reemergence in 2006 after its initial absence from 2004 to 2005. As per a WHO report, *V. cholerae* O139 serogroup isolates were detected in Philippines and China in 2014 in the smallest numbers (3).

As per the report available from Chandigarh, more children younger than 5 years of age were affected by cholera in 2003 (13). In contrast, in the present study, the most affected age groups were 15–30 years followed by 30–60 years. The most likely reason may be direct exposure to contaminated water sources. Cholera incidence rates in the 2 sexes were similar in our study, as reported in other parts of the country (13).

Contaminated water sources are the major cause of cholera. In the population, the practice of defecation near a river bank or pond contaminates the water bodies and spreads the disease owing to heavy rainfall. During the rainy season, people usually work in paddy fields, drink water from traditional water sources like nala, chua, river, and stream in the tribal areas, and get infected, as already reported in different areas during the 2002 cholera outbreak and 2007 cholera epidemic in the tribal areas of Odisha (7) and a large outbreak in 2010 in tribal areas (8). In the present study, more cases were seen from July to November almost every year. The number of cases correlated well with the onset of monsoon in this region. Rains increase the water level and have been implicated in leakage of water pipes and mixing of stagnant water through broken pipelines (8). Such untreated water sources are used by people living in tribal areas and slum dwellings for bathing, cooking, and drinking purposes, and this practice increases the chances of infection (14).

Treatment of cholera is principally supportive; still, antimicrobial therapy helps to decrease the volume of stool and the duration of illness (15). Although tetracycline has been the mainstay empirical therapy, chloramphenicol, furazolidone, and co-trimoxazole are reported alternatives. According to the present study, it is evident that *V. cholerae* has continued to develop resistance to different antimicrobial agents used for the treatment of diarrhea in general and cholera in particular. In this study, overall, *V. cholerae* O1 isolates were resistant to ampicillin, streptomycin, nalidixic acid, co-trimoxazole, and furazolidone but sensitive to chloramphenicol, tetracycline, gentamicin, neomycin, ciprofloxacin, and norfloxacin. *V. cholerae* showed consistent resistance to ampicillin from 2007 to 2014. A report from Pune in 2015 showed 90% prevalence of ampicillin resistance and 88% prevalence of furazolidone resistance (16). In the present study, furazolidone encountered constant resistance at 90% to 100% prevalence among the isolates. Results similar to ours were reported in various regions of Kenya (17) and Cameroon (18). Because cholera is a noninvasive disease, drugs such as co-trimoxazole, which are not absorbed from gastrointestinal tract, were widely used for treatment. However, most of the *V. cholerae* isolates in the present study were resistant to co-trimoxazole. Several other investigators reported 100% resistance to co-trimoxazole in North Karnataka (19) and Cameroon (18).

Fluoroquinolones are commonly used for the treatment of enteric infections because of these drugs' good bioavailability and minimal adverse effects. Moreover, fluoroquinolones such as ciprofloxacin have higher rates of bacteriological and clinical use as compared to any other drugs used to treat *V. cholerae* infection though ciprofloxacin and norfloxacin are still the treatments of choice, toward which *V. cholerae* isolates have been highly sensitive. In this study, *V. cholerae* isolates were sensitive to these antibiotics. Kar *et al*. (2015) reported that *V. cholerae* O1 strains were sensitive to norfloxacin and ciprofloxacin but resistant to nalidixic acid during 2010 cholera outbreaks in Odisha (8). Dey *et al*. (2014) also reported sensitivity to ciprofloxacin in rural North Karnataka (19). Similar results were reported from Pune (16), Nepal (20), and Nigeria (21). The emergence of fluoroquinolone resistance among *V. cholerae* clinical strains needs attention because it complicates the therapeutic use of these drugs.

Tetracycline is the drug of choice for effective cholera treatment. A high prevalence rate of resistance to tetracycline during 2010 among *V. cholerae* O1 strains was observed in the present study (8). Emergence of tetracycline-resistant *V. cholerae* O1 in Assam was reported in 2012 (22). Similarly, tetracycline resistance to *V. cholerae* was reported in Bangladesh in 1979 (23). A study conducted in Kolkata indicated that a sudden upsurge of tetracycline-resistant cholera strains occurred in 2007 (24). Tetracycline-resistant strains were also detected during the cholera epidemics in Kolkata, Madagascar, Mozambique, and Tanzania (25–28). The

cause of the sudden rise of tetracycline resistance in 2010 is unclear. First, this phenomenon may be due to extensive irrational use of tetracycline during the 2007 cholera epidemic in the Kashipur area of Odisha. Second, new villages were affected by cholera in 2010 in the Kashipur block, in comparison with the villages affected in the same area during the 2007 cholera epidemic; this phenomenon may be due to acquired immunity. An increase in antibiotic pressure in the community may be the reason for the emergence for such resistant strains (11). Furthermore, *V. cholerae* O1 strains were sensitive to tetracycline from 2011 onward, in agreement with the results reported in Dhaka during a study from 2006 to 2011 (29).

Antimicrobials are generally used to shorten the duration of illness and reduce the volume of stool. The WHO recommends that cholera patients be treated with tetracycline, furazolidone, chloramphenicol, or ciprofloxacin as a treatment of choice. In contrast, in the present study, *V. cholerae* isolates were resistant to furazolidone. Thus, the other alternatives are chloramphenicol and ciprofloxacin along with norfloxacin and gentamicin. Similar results were reported by many researchers across the globe (12,16-18). During the study, the El Tor variants of *V. cholerae* were detected from 2007 onward, causing cholera outbreaks and epidemics in different parts of the state and have spread to different areas of Odisha (7,30). The present study suggests that the future outbreaks and epidemics may be due to the El Tor variant of *V. cholerae* O1 strains with multiple-drug resistance; continuous surveillance of such strains is necessary to track both the biotype, serogroup, serotype, and drug resistance patterns of *V. cholerae* for effective treatment and control of cholera outbreaks and epidemics in this region.

Our study clearly indicates that cholera cases were more numerous in rainy and postrainy seasons. Different drug resistance patterns were observed in different periods in different parts of the state. Again, because the El Tor variant of *V. cholerae* carrying the *ctxB* gene of the classical strain has spread to different parts of Odisha and caused epidemics from 2007 (30) onward, continuous surveillance is urgently needed to combat the future outbreaks and epidemics of cholera in this region.

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Conflict of interest None to declare.

REFERENCES

1. World Health Organization(WHO). Cholera. Available at <http://www.who.int/cholera/news/22october2015/en>. Accessed November 5, 2015.
2. Bharati K, Bhattacharya SK. Cholera outbreaks in South-East Asia. Curr Top Microbiol Immunol. 2014; 379:87-116.
3. World Health Organisation(WHO). Cholera, 2014. Weekly Epidemiol Rec. 2015; 90: 517-44.
4. Sharma NC, Mandal PK, Dhillon R, et al. Changing profile of *Vibrio cholerae* O1, O139 in Delhi & its periphery (2003-2005). Indian J Med Res. 2007; 125: 633-40.
5. Roy S, Parande MV, Mantur BG, et al. Multidrug-resistant *Vibrio cholerae* O1 in Belgaum, south India. J Med Microbiol. 2012; 61: 1574-9.
6. Chhotray GP, Pal BB, Khuntia HK, et al. Incidence and molecular analysis of *Vibrio cholerae* associated with cholera outbreak subsequent to the super cyclone in Orissa, India. Epidemiol Infect. 2002; 128: 131-8.
7. Pal BB, Khuntia HK, Samal SK, et al. Epidemics of severe cholera caused by E1 Tor *Vibrio cholerae* O1 Ogawa possessing the ctxB gene of the classical biotype in Orissa, India. Int J Infect Dis. 2010;14:e384-9.
8. Kar SK, Pal BB, Khuntia HK, et al. 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. 2015; 33:45-9.
9. Sack DA, Sack RB, Nair GB, et al. Cholera.Lancet. 2004; 363:223-33.
10. Krishna BVS, Patil AB, Chandrasekhar MR. Fluoroquinolone-resistant Vibrio cholerae isolated during a cholera outbreak in India. Trans R Soc Trop Med Hyg.2006;100:224-6.
11. Bauer AW, Kirby WM, Sherris JC, et al. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45:493-6.
12. Devnikar AV, Sonth SB, Baragundi MC, et al. Characterization and antibiogram of *Vibrio cholerae* isolates from a tertiary care hospital. Int J Biol Med Res. 2012;3: 2352-4.
13. Taneja N, Kaur J, Sharma K, et al. A recent outbreak of cholera due to *Vibrio cholerae* O1 Ogawa in and around Chandigarh, north India. Indian J Med Res. 2003;117:243-6.
14. Chander J, Kaistha N, Gupta V, et al. Epidemiology & antibiograms of *Vibrio cholerae* isolates from a tertiary care hospital in Chandigarh, north India. Indian J Med Res. 2009;129:613-7.
15. Greenough WB III, Gordon RS Jr, Rosenberg IS, et al. Tetracycline in the treatment of cholera. Lancet. 1964; 1: 355-7.
16. Palewar MS, Choure AC, Mudshingkar S, et al. Typing and antibiogram of *Vibrio cholerae* isolates from a tertiary care hospital in Pune: A 3 year study. J Glob Infect Dis. 2015; 7:35-6.
17. Mercy N, Mohamed AA, Zipporah N, et al. Phenotypic and genetic characterization of *Vibrio cholerae* O1 isolated from various regions of Kenya between 2007 and 2010. Pan Afr Med J. 2014;19:8.
18. Akoachere JF, Masalla TN, Njom HA. Multi-drug resistant toxigenic *Vibrio cholerae* O1 is persistent in water sources in New Bell-Douala, Cameroon. BMC Infect Dis. 2013;13:366.
19. Dey S, Parande MV, Parande AM, et al. Twin outbreak of cholera in rural North Karnataka, India. Indian J Med Res. 2014;140: 420-6.
20. Dixit SM, Johura F, Manandhar S, et al. Cholera outbreaks (2012) in three districts of Nepal reveal clonal transmission of multi-drug resistant *Vibrio cholerae* O1. BMC Infect Dis. 2014;14:392.
21. Marin MA, Thompson CC, Freitas FS, et al. Cholera Outbreaks in Nigeria Are Associated with Multidrug Resistant Atypical El Tor and Non-O1/Non-O139 *Vibrio cholerae*. PLoS Negl Trop Dis. 2013;7:e2049.
22. Borkakoty B, Biswas D, Devi U, et al. Emergence of classical ctxB genotype 1 and tetracycline resistant strains of *Vibrio cholerae* O1 El Tor in Assam, India. Trans R Soc Trop Med Hyg. 2012;106:382-6.
23. Glass RI, Huq I, Alim AR, et al. Emergence of multiply antibiotic-resistant *Vibrio cholerae* in Bangladesh. J Infect Dis.1980;142:939-42.
24. Bhattacharya K, Kanungo S, Sur D, et al. Tetracycline-Resistant *Vibrio cholerae* O1, Kolkata, India. Emerg Infect Dis.2011;17:568-9.
25. Roychowdhury A, Pan A, Dutta D, et al. Emergence of tetracycline-resistant *Vibrio cholerae* O1 serotype Inaba, in Kolkata, India. Jpn J Infect Dis.2008;61:128-9.
26. Dromigny JA, Rakoto-Alson O, Rajaonatahina D, et al. Emergence and rapid spread of tetracycline-resistant *Vibrio cholerae* strains,Madagascar. Emerg Infect Dis. 2002;8:336-8.
27. Mandomando I, Espasa M, Vallès X, et al. Antimicrobial resistance of *Vibrio cholerae* O1 serotype Ogawa isolated in Manhica District Hospital, southern Mozambique. J Antimicrob Chemother.2007;60:662-4.
28. Mhalu FS, Mmari PW, Ijumba J. Rapid emergence of El Tor *Vibrio cholerae* resistant to antimicrobial agents during first six months of fourth cholera epidemic in Tanzania. Lancet.1979;1:345-7.
29. Rashed SM, Mannan SB, Johura FT, et al. Genetic characteristics of drug-resistant *Vibrio cholerae* O1 causing endemic cholera in Dhaka, 2006-2011. J Med Microbiol. 2012;61:1736-45.
30. Khuntia HK, Pal BB, Samal SK, et al. Rapid spread of *Vibrio cholerae* O1 El Tor Variant in Odisha, eastern India 2008-09. J Clinic Microbiol.2013;51:1909-12.