Cholera is a diarrheal disease caused by *Vibrio cholerae*. It is of high epidemiological significance because of its potential to cause epidemics having high mortality. Though maintenance of fluids and electrolyte balance is enough to manage most cases, antibiotics do play an important role in severely dehydrated patients.\(^1,2\) Antibiotic resistance among *V. cholerae* has been frequently reported.\(^3,4\) Injudicious use of antibiotics has led to antibiotic resistance in *V. cholerae* also. Since antibiogram of isolates keeps changing depending on, change in biotype and serovar, careful epidemiological monitoring of circulating strains is recommended.\(^2,7,8\) Therefore, this study was conducted with the aim of finding the biotype, serotype and antibiotic susceptibility pattern of *V. cholerae* isolates encountered in our area.

**MATERIAL AND METHODS**

The study was conducted at the Department of Microbiology of a tertiary health care centre for 5 years between 2009 and 2013. A total of 1,975 consecutive stool samples from patients suffering from loose stools were processed. Specimens were inoculated on Blood agar, MacConkey’s agar, Xylose lysine deoxycholate (XLD) agar and Thiosulfate-citrate-bile salt-sucrose (TCBS) agar plates. The samples were also inoculated in alkaline peptone water and subcultures were made on MacConkey’s and TCBS plates after 8 hours of incubation. After
of stool passed, stopping diarrhea in 48 hours.\textsuperscript{1, 2} Hence antibiotics play a very important role in the treatment of cholera. However, emergence of drug resistance among \textit{V. cholerae} has narrowed the choices of antibiotics. There has been sudden surge of multidrug resistance (MDR) in Vibrio in last 20 years.\textsuperscript{3-5, 14-16} Besides, reports of biotype variation among \textit{V. cholerae} from Inaba to Ogawa and \textit{vice versa} are also known.\textsuperscript{13-15} Such variation is due to immune selection pressure in the local population. There have been reports of emergence of atypical variants of \textit{V. cholerae} presenting with severe illness and MDR.\textsuperscript{4,5,16} Hence it is important to monitor biotypes and resistance pattern of the isolates.

In our study 106 \textit{V. cholerae} O1 strains were isolated and all of them belonged to biotype El Tor, the predominant serotype was Ogawa. Phage typing revealed types T2 (Basu & Mukerjee) and T27 (new scheme) were most common. Classical and O139 serotypes were not encountered during our study. 89.62\% (95) and 83.01\% (88) of isolates showed resistance towards Ampicillin and Co-Trimoxazole respectively. Only 3 (2.83\%) isolates were resistant to Chloramphenicol. We observed that 9.43\% (10) of our isolates were resistant to Tetracycline (Figure 2). No major variation in antibiotic susceptibility pattern was seen between biotypes of \textit{V. cholerae} isolates.

\section*{DISCUSSION}

More than 4 million people are affected by cholera and more than 1 lakh deaths occur yearly.\textsuperscript{2} India reported about 37,000 cases from 68 outbreaks between 1997 to 2006.\textsuperscript{11} The number of cases in reality are predicted to be six times higher than reported.\textsuperscript{11, 12} Large, diverse population, suboptimal water sanitation and warm climate are conducive for epidemics in India.\textsuperscript{11}

Cholera is a self-limiting disease but can cause severe dehydration and death in a matter of hours.\textsuperscript{13} Rehydration is the mainstay of treatment. Antibiotics are indicated in cases of severe dehydration. Antibiotics reduce the total volume of stool passed, stopping diarrhea in 48 hours.\textsuperscript{1, 2}
Table 1. Year-wise distribution of *V. cholerae* between 2009 and 2013

| Year | Stool samples Screened | No. of isolates (%) |
|------|------------------------|---------------------|
| 2009 | 248                    | 17 (6.85)           |
| 2010 | 236                    | 7 (2.96)            |
| 2011 | 343                    | 10 (2.91)           |
| 2012 | 491                    | 34 (6.92)           |
| 2013 | 657                    | 38 (5.57)           |
| Total| 1975                   | 106 (5.36)          |

Apart from plasmid transfer of antibiotic resistance, there are other reasons for emergence of resistance among *V. cholerae*. Extensive antibiotic usage in veterinary and poultry industry, in order to increase the dairy and meat production, is one of the important reasons. Pressure exerted by the antibiotics lead to selection of resistant mutants. Studies have also shown that during an outbreak, if antibiotics are used for treatment, there is rapid development of resistance. Biotype switching is another cause of shift in drug resistance. Presence of a single biotype of *V. cholerae* in a geographical area leads to development of increased immunity levels in the community and
in such situations, *V. cholerae* are known to exhibit a revolutionary strategy by changing the biotype from Ogawa to Inaba and vice versa. Such biotype variation is linked to variation in antimicrobial resistance in some studies.\(^{14,17}\)

Since antibiotics play important role in limiting the disease severity, they should be wisely used. Clinical microbiology laboratories should adhere to the CLSI guidelines of testing only four antibiotics for *V. cholerae*.\(^{10}\) Testing and reporting sensitivity to antibiotics, not recommended by CLSI, would lead to their injudicious use adding to the menace of drug resistance. Many studies have also documented reversal of resistance with judicious use of antibiotics.\(^{7,24}\)

Clinical laboratories do not test antibiotic susceptibility of *V. cholerae* routinely. Therefore, unlike the other gram negative bacteria, only a little data on sensitivity pattern of this epidemiologically important pathogen exists.

The risk of cholera to community can be prevented by availability of good quality potable water and sanitation measures. Use of vaccines also is highly effective in halting the spread of cholera. Vaccination is an effective measure to prevent drug resistance in pathogens.\(^{25}\)

Our study had a few limitations. Only those cases presenting to our hospital were included in this study and the data does not include isolates from cases treated at other government or private health care centers in this area. And also we could not perform the minimum inhibitory concentration (MIC) of Tetracycline for resistant isolates. Inclusion of most isolates from a region would provide a comprehensive perspective of the prevalence of a pathogen.

**CONCLUSION**

*V. cholerae* El Tor biotype Ogawa is the most common serotype found in this area. Doxycycline remains the drug of choice for therapy. Resistance to Ampicillin and Co-Trimoxazole is common and this study also showed the presence of Tetracycline resistant isolates. Regular monitoring of antibiotic susceptibility profile of epidemiologically relevant bacterial pathogens must be carried out by tertiary health care centers.

**REFERENCES**

1. World Health Organization, Department of Child and Adolescent Health and Development. The treatment of diarrhoea: a manual for physicians and other senior health workers. Geneva: Dept. of Child and Adolescent Health and Development, World Health Organization; 2005.
2. WHO | Cholera [Internet]. WHO. [cited 2016 Jan 18]. Available from: http://www.who.int/mediacentre/factsheets/fs107/en/
3. Wang R, Lou J, Liu J, Zhang L, Li J, Kan B. Antibiotic resistance of Vibrio cholerae O1 El Tor strains from the seventh pandemic in China, 1961–2010. *Int J Antimicrob Agents*. 2012; 40(4):361–4.
4. Shrestha SD, Malla S, Adhikari BR, Shakya G, Basnyat SR, Sharma S. Antibiotic susceptibility patterns of Vibrio cholerae isolates. *JNMA J Nepal Med Assoc.* 2010; 49(179):232–6.
5. Miwanda B, Moore S, Muyembe J-J, Nguefack-Tsague G, Kabangwa IK, Ndjakani DY, et al. Antimicrobial Drug Resistance of *Vibrio cholerae*, Democratic Republic of the Congo. *Emerg Infect Dis*. 2015; 21(5):847–51.
6. Kar SK, Pal BB, Khuntia HK, Achary KG, Khuntia CP. 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.
7. Das S, Choudhry S, Saha R, Ramachandran VG, Kaur K, Sarkar BL. Emergence of multiple drug resistance Vibrio cholerae O1 in East Delhi. *J Infect Dev Ctries*. 2011; 5(04):294–298.
8. Saha A, Nair GB, Kong RYC. Evolution of new variants of Vibrio cholerae O1. *Trends Microbiol*. 2010; 18(1):46–54.
9. Tille PM. Bailey & Scott’s Diagnostic Microbiology. Thirteenth edition. St. Louis, Missouri: Elsevier; 2014, pp 1038.
10. CLSI (2007). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard M2-A10. Wayne, PA: Clinical and Laboratory Standards Institute.
11. Kanungo S, Sah B, Lopez A, Sung J, Paisley A, Sur D, et al. Cholera in India: an analysis of reports, 1997–2006. *Bull World Health Organ.* 2010; 88(3):185–91.
12. Sarkar BL, Kanungo S, Nair GB. How endemic is cholera in India? *Indian J Med Res*. 2012; 135(2):246–8.
13. Mahon CR, Lehman DC, Manuselis G Jr. Textbook of Diagnostic Microbiology. London: Elsevier Health Sciences; 2014.
14. Kitaoka M, Miyata ST, Unterweger D, Pukatzki S. Antibiotic resistance mechanisms of Vibrio cholerae. J Med Microbiol. 2011; 60(4):397–407.

15. Nair GB, Qadri F, Holmgren J, Svennerholm A-M, Safa A, Bhuiyan NA, et al. Cholera Due to Altered El Tor Strains of Vibrio cholerae O1 in Bangladesh. J Clin Microbiol. 2006; 44(11):4211–3.

16. Marin MA, Thompson CC, Freitas FS, Fonseca EL, Aboderin AO, Zailani SB, et al. Cholera Outbreaks in Nigeria Are Associated with Multidrug Resistant Atypical El Tor and Non-O1/Non-O139 Vibrio cholerae. Vinetz JM, editor. PLoS Negl Trop Dis. 2013; 7(2):e2049.

17. Garg P, Nandy RK, Chaudhury P, Chowdhury NR, De K, Ramamurthy T, et al. Emergence of Vibrio cholerae O1 biotype El Tor serotype Inaba from the prevailing O1 Ogawa serotype strains in India. J Clin Microbiol. 2000; 38(11):4249–53.

18. Kulkarni S, Chillarge C. Antibiotic Susceptibility Pattern of Vibrio cholerae Causing Diarrhoea Outbreaks in Bidar, North Karnataka, India. Int J Curr Microbiol App Sci. 2015; 4(9):957–961.

19. Mandal J, Dinoop KP, Parija SC. Increasing Antimicrobial Resistance of Vibrio cholerae O1 Biotype El Tor Strains Isolated in a Tertiary-care Centre in India. J Health Popul Nutr. 2012; 30(1):12.

20. Chandrasekhar MR, Krishna BVS, Patil AB. Changing characteristics of Vibrio cholerae: emergence of multidrug resistance and non-O1, non-O139 serogroups. Southeast Asian J Trop Med Public Health. 2008; 39(6):1092–7.

21. Martinez JL. Antibiotics and Antibiotic Resistance Genes in Natural Environments. Science. 2008; 321(5887):365–7.

22. Teuber M. Veterinary use and antibiotic resistance. Curr Opin Microbiol. 2001; 4(5):493–9.

23. 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(8112):345–7.

24. Faruque AS, Alam K, Malek MA, Khan MG, Ahmed S, Saha D, et al. Emergence of multidrug-resistant strain of Vibrio cholerae O1 in Bangladesh and reversal of their susceptibility to tetracycline after two years. J Health Popul Nutr. 2007; 25(2):241.

25. Mishra RP, Oviedo-Orta E, Prachi P, Rappuoli R, Bagnoli F. Vaccines and antibiotic resistance. Curr Opin Microbiol. 2012; 15(5):596–602.