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Molecular epidemiology of vibrio cholerae causing outbreaks and sporadic cholera in and around Hassan district and its antibiotic susceptibility pattern

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Abstract

Introduction: Aim of the study is isolation and identification of V cholerae followed by study of clonal relationship between isolates by molecular typing methods, molecular characterization by detection of genes coding for cholera toxin (CTX) & toxin- co-regulated pili (TCP) and study of antibiotic susceptibility pattern of V cholerae isolates

Materials and Methods: 74 V cholerae isolates from 620 stool samples were included in study. Isolation, identification and antibiogram done by using standard techniques. Clonal relationship between isolates studied by RAPD assays. Molecular characterization done by detection of CTX and TCP using multiplex PCR.

Results: 74 V. cholerae 01 biotype El Tor serotype Ogawa isolated from 620 stool samples with isolation rate of 11.94%. 38 of 46 isolates were positive for CTX – A and TCP – A El Tor by multiplex PCR. All 46 isolates were negative for TCP-A classical. 38 isolates subjected to RAPD typing assays revealed 11 different RAPD types. Antibiotic sensitivity tests for the isolates revealed the presence of diverse sensitivity patterns. 97.3% of isolates were multidrug resistant (MDR). 2.7% of isolates remained sensitive to all tested antibiotics.

Conclusion: V. cholerae 01 biotype El Tor serotype Ogawa is currently circulating type in our geographical region. All isolates tested positive for CTX and TCP indicating that circulating strains are pathogenic in nature. Presence of different RAPD types indicates that cholera is endemic in this region with periodic epidemics. Presence of large number of MDR isolates necessitates curbing of irrational use of antibiotics to prevent further spread of drug resistance.

Keywords: Vibrio cholerae, CTX, TCP, RAPD, Antibiogram.

Introduction

Vibrio cholerae has been recognised as one of the common causes of bacterial diarrhea throughout the developing world. Epidemics of cholera caused by toxigenic V. cholerae O1 and V. cholerae O139 are major public health problems.[1] Recent years have witnessed resurgence in global incidence of cholera cases as reported by WHO. Globally, cholera alone causes 120,000 deaths annually. Cholera is endemic in southern Asia and in several parts of Africa and Latin America with seasonal outbreaks.[2] Increase in the number of multidrug resistant pathogens have accompanied rise in case fatality rates & hampered the treatment of many infectious diseases including cholera.[2]

Cases of cholera have been on the rise in India and more than doubled from 1939 cases in 2006 to 5155 in 2010. While half a century ago cholera was more of a problem of the eastern parts of India,[3] it is increasingly being reported from southern India recently.[4,5,6,7]

Even though cholera is endemic with periodic epidemics in India, data regarding molecular epidemiology and antibiogram of V. cholerae isolates is scarce. Not much is known about the molecular epidemiology of the cholera in Hassan and surrounding areas. There is also scarcity of knowledge in the changing drug susceptibility pattern in V. cholerae isolated from this region over period of time. Because in depth knowledge on the epidemiology and current resistance profile is essential for implementing prevention strategies, the current research work is done in our institute.

Materials and Methods

It is an observational study done for 3 years from April 2012 to April 2015 in the Department of Microbiology, Hassan Institute of Medical Sciences, Hassan, Karnataka.

Inclusion criteria; the study group includes patients presenting with acute watery diarrhea (Three or more abnormally loose or fluid stools in the past 24 hours with or without dehydration).

Criteria used to diagnose suspected cholera epidemic includes.

1. Area where disease is present: Severe dehydration or death from acute watery diarrhea in patient aged ≥ 5 years.
2. Area where Cholera is endemic: Acute watery diarrhea, with or without vomiting in a patient aged ≥ 5 years.
3. In an area where there is a cholera epidemic: Acute watery diarrhea, with or without vomiting, in any patient.

Exclusion criteri

a; samples which are not properly transferred and cases with incomplete details.

Details of the cases such as age, sex and clinical features were collected by examining case sheet, contacting patient attenders & referring medical officers. Human excreta such as stool samples (preferably), vomitus or rectal swabs collected from all suspected cases before giving antibiotics & immediately transported to laboratory. Cary - Blair media used as transport media in case of delay in transportation.

Stool samples from each patient were streaked on TCBS and MacConkey agar and enriched in alkaline

peptone water with subsequent plating. The isolates are identified by biochemical reactions & confirmed by serotyping using specific antisera.[8] The susceptibility of confirmed V.cholerae strains to antimicrobial agents performed on Mueller Hinton agar by Kirby Bauer disc diffusion technique with commercially available discs.[9] Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were used for internal quality control.

Further molecular studies conducted at Regional Medical Research Center, Belgaum. Biotyping of V. cholerae isolates and detection of genes coding for CTX & TCP done by multiplex PCR. Clonal relationship between isolates studied by Random Amplified Polymorphic DNA (RAPD) analysis.

Multiplex PCR was performed with ctxA-tcpA primers developed by exploiting biotype-specific variation of nucleotide sequences of tcpA, responsible for the expression of the major subunit protein (TcpA) of the toxin co-regulated pilus of V. cholerae.[10] Amplification of ctxA and tcpA El Tor produces bands of 301 and 472 bp, respectively. PCR was performed in a Bio-Rad iCycler in 25 ml reaction volumes. A quantity of 2.5 ml template DNA in the form of heat-treated rapid lysates from 18 hour cultures was used in PCR with 1 µM each primer, 250 µM each dNTP, 1.5 mM MgCl2 and 0.5 U Taq DNA polymerase in 10 mM Tris/HCl (pH 9.0) and 50 mM KCl. The temperature programme consisted of an initial denaturation of 5 min at 94 ^{0} C followed by 29 cycles of 1.5 min at 94 ^{0} C, 1.5 min at 60 ^{0} C and 1.5 min at 72 ^{0} C, and a final cycle of 1.5 min at 94 ^{0} C, 1.5 min at 60 ^{0} C and a final extension of 7 min at 72 ^{0} C.[11]

For Random amplified polymorphic DNA (RAPD) fingerprinting assay, purified genomic DNA was isolated from cultures of V. cholerae grown overnight in Luria broth (BD) following the cetyltrimethyl ammonium bromide method.[12] PB1 primer (59-GCG CTG GCT CAG-39) was employed in a RAPD fingerprinting assay with all isolates.[13] PCR was performed in a Bio-Rad iCycler in 50 ml reaction volumes. A quantity of 50 ng DNA extracted from 18 hr cultures was used in PCR with 2 mM primer, 250 mM each dNTP, 1.5 mM MgCl2 and 0.5 U Taq DNA polymerase in 10 mM Tris/HCl (pH 9.0) and 50 mM KCl. The temperature programme consisted of 1 cycle of 3 min at 97^{0}C, 1 min at 40^{0}C and 1 min at 72^{0}C; 4 cycles of 1 min at 97^{0}C, 1 min at 40^{0}C and 1 min at 72^{0}C; 24 cycles of 1 min at 95^{0}C, 1 min at 55^{0}C and 1 min at 72^{0}C; and 1 cycle of 1 min at 95^{0}C, 1 min at 55^{0}C and 7 min at 72^{0}C. PCR products were electrophoresed onto a 1% agarose gel, with gel red dye, viewed under UV light and documented in gel documentation system.[11]

Results

Out of total 620 stool samples received in the laboratory during the study period, Vibrio cholerae was isolated in 74 stool samples, giving a positivity rate of 11.94%. On serotyping and biotyping, all isolates were identified as V. cholerae 01 biotype El Tor serotype Ogawa. 46 out of 82 isolates studies for ctx- A, tcp–A El Tor and tcp–A Classical by PCR. 18 isolates subjected to PCR for O1 rfb gene. PCR results for O1rfb gene, ctx-A, tcp–A El Tor and tcp-A Classical shown in table 1. 8 isolates which were negative for ctx-A and tcp-A were considered as non agglutinable (NAG) vibrio and excluded from study.

Table 1: PCR test results for O1rfb gene, CTX–A, tcp–A El Tor and tcp–A Classical

	O1rfb gene	ctx–A	tcp–A El Tor	tcp–A Classical
No of isolates tested	18	46	46	46
Positive	18	38	38	Nil
Negative	Nil	°8 (NAG Vibrio)	°8 (NAG Vibrio)	46

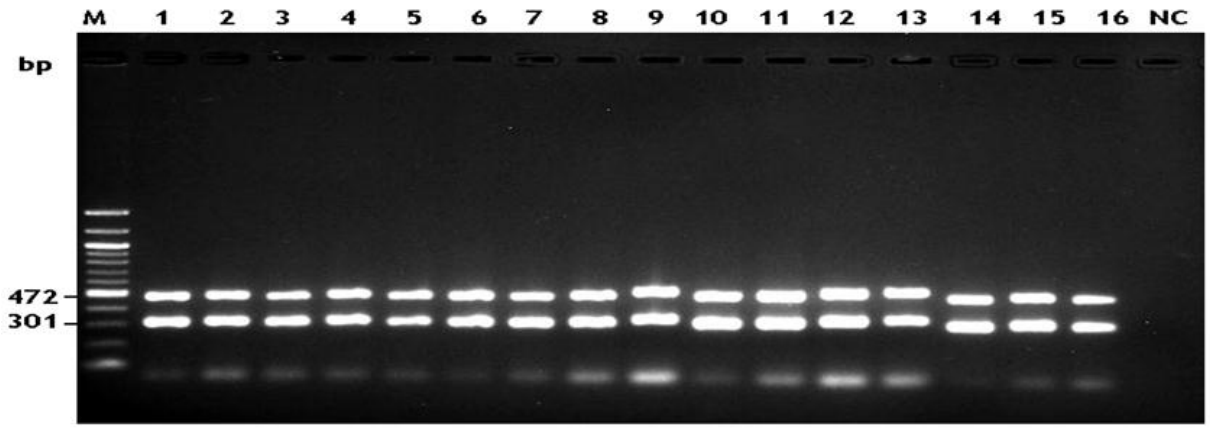

Fig. 1: ctxA-tcpA multiplex PCR results with 16 V.cholerae isolates. M denotes 1° °bp DNA ladder. NC denotes negative control. ctxA is of 3 °1 bp while tcpA is of 472 bp.

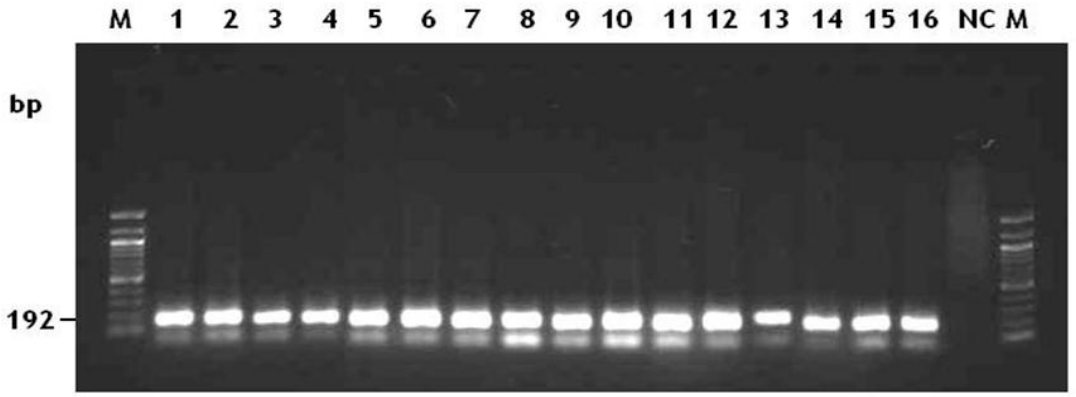

Fig. 2: O1rfb PCR results with 16 V.cholerae isolates. M denotes 1° °bp DNA ladder. NC denotes negative control. O1 rfb is of 192 bp.

RAPD done on 38 out of 74 isolates of V. cholerae isolated over period of 3 years. This revealed presence of 11 different patterns designated A–K among 38 isolates. Distribution of different RAPD types over the years is shown in table 2.

Table 2: RAPD types of V cholerae and their distribution over 3 years

Year		RAPD types
2 °12-13	17	A – 7, B - 6, C-2, D-2,
2 °13-14	12	A-1, B-1, E-3, F-1, G-2, H-1, I-2, J-1
2 °14-15	°9	J-7, K-2,
Total	38	A - 8, B - 7, C - 2, D - 2, E - 3, F - 1, G - 2, H - 1, I - 2, J - 8, K - 2

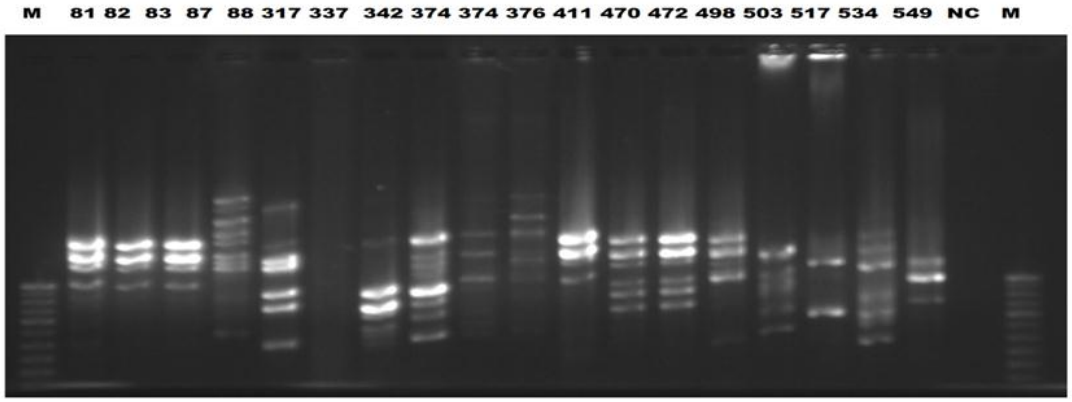

Fig. 3: RAPD fingerprints of the 18 isolates of V.cholerae with PB1 primer

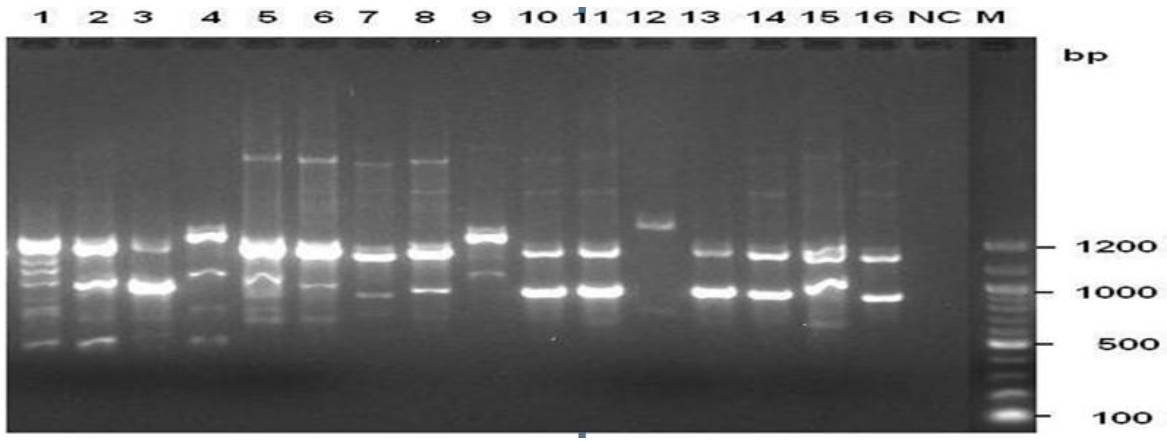

Fig. 4: RAPD fingerprints of the 16 isolates of V.cholerae with PB1 primer

72 out of 74 V cholerae isolates were resistant to more than one antibiotic with 6 distict patten for commonly used antibiotics as shown in table 3.

Table 3: Antibiotic resistance pattern of isolates for key antibiotics

S. No	Resistance pattern	Number of isolates	Percentage
1	A,Na,Co,T,Nor,Ch,Cef,G	8	1 °.81%
2	A,Na,Co,T,Nor,Ch	4	5.41%
3	A,Na,Co,T,Nor,	4	5.41%
4	A,Na,Co,T,	5	6.76%
5	A,Na,Co,	49	66.23%
6	A,Na,	2	2.7 °%

Note: A-Ampicillin, Na-Nalidixic acid, Co-otrimoxazole, T-Tetracycline, Nor- Norfloxacin, Ch-Chloramphenicol, Cef- Ceftriaxone, G- Gentamycin.

The v cholerae isolates were tested for susceptibility to 18 different antibiotics. Antibiotic sensitivity pattern for all 18 antibiotics given in table 4.

Table 4: The prevalence of resistance pattern of the isolated V. cholerae °1 strains during the study period

S. No	Antibiotics tested	No of isolates tested	Sensitive (%)	Intermediate sensitivity (%)	Resistant (%)
1.	Ampicillin	74	2 (2.7)	Nil	72 (97.3°)
2.	Amikacin	74	72 (97.3 °)	Nil	2 (2.7 °)
3.	Gentamicin	74	69 (93.24)	°1 (1.35)	4 (5.41)
4.	Azithromycin	3 °	28 (93.33)	Nil	2 (6.67)
5.	Ceftriaxone	74	64 (86.49)	2 (2.7 °)	8 (1°.81)
6.	Cephotaxime	74	64(86.49)	2 (2.7)	8 (1°.81)
7.	Ceftazidime	74	65 (87.84)	2 (2.7)	7 (9.5)
8.	Cefixime	5 °	44 (88)	1 (2)	5 (1 °)
9.	Chloramphenicol	74	6 ° (81. °8)	2(2.7)	12 (16.23)
10.	Ciprofloxacin	74	58(78.38)	2(2.7°)	14(18.92)
11.	Ofloxacin	58	47 (81. °3)	2 (3.45)	9 (15.52)
12.	Norfloxacin	74	57 (77. °3)	°1 (1.35)	16 (21.62)
13.	Nalidixic acid	74	2 (2.7)	Nil	72 (97.3°)
14.	Co-trimoxazole	74	4(5.41)	Nil	7 ° (94.59)
15.	Tetracycline	74	53 (71.62)	2(2.7 °)	19 (25.68)
16.	Doxycycline	41	3°(73.17)	1 (2.43)	1 °(24.39)
17.	Furazolidone	43	4 (9.3°)	Nil	39 (9°.7°)
18.	Imipenem	54	54 (1 ° °)	Nil	Nil

Discussion

Out of total 620 stool samples received in the laboratory during the study period, V. cholerae was isolated in 74 stool samples, giving an isolation rate of 11.94%. Many studies throughout India reported V cholera ogawa as commonest serotype with isolation rate varying from 5.79% to 56.6%.[1,3,7,14,15] Mandal J et al reported 5.79% isolation rate with 97.4% V. cholerae ogawa and remaining Inaba serotypes.[14] Chatterjee S et al reported 10.12% isolation rate and all are El tor V. cholerae ogawa serotypes.[3] Misra M reported 34.95% isolation rate of El tor V. cholerae Ogawa (76.8%), Inaba (1.52%) and Hikojima (2.02%).[15] Das S et al reported 56.6% isolation rate of El tor V. cholerae Ogawa (85%) followed by Inaba serotype.[1]The lower isolation rate in our study may be because; most of the cases included were inpatients in tertiary care hospital and might have received antibiotics before sample collection. All the V cholera isolates were identified as V. cholerae biotype El tor serotype Ogawa suggesting that, it is currently the predominant circulating serotype in our region.

PCR done for detection of O1rfb gene on 18 out of 74 biochemically confirmed isolates (Table; 1). All 18 isolates showed the presence of a 192 bp amplicon marker for the O1rfb gene indicating that isolates belong to V cholerae subgroup O1. Multiplex PCR done for detection of genes coding for ctx-A and tcp- A for 38 V. cholerae isolates and 8 isolates of non agglutinable vibrio (Table 1). All 38 isolates confirmed as V. cholerae serotype ogawa were positive for ctx-A (301 bp amplicon marker) and toxin co-regulated pilus gene tcp-A el tor (472 bp amplicon marker) and negative for tcp–A classical. This confirms that all the V. cholerae strains isolated during the outbreak were highly pathogenic with virulence markers ctx and tcp.

RAPD done on 38 isolates out of 74 V. cholerae isolated over period of 3 years. This revealed presence of 11 different patterns among 38 isolates. Reports shows that strains found in outbreaks as well as sporadic cases in present study belong to diverse genotypes. Presence of multiple different genotypes in single outbreak also noted in present study. This indicates possibility of origin of

outbreak from different sources. High prevalence of cholera in particular regions (Belur and Sakaleshpur talukas), prevalence of multiple and different RAPD types in an outbreak and higher incidence in children indicates that, cholera is endemic in these regions leading to frequent outbreaks. Most of the outbreaks associated with mass gatherings with evidence of breakdown of water supply and sanitation systems with an unhygienic environment.

The RAPD fingerprinting assay of 18 V. cholerae isolates from an epidemic in Belgaum done by Roy S et al. The study revealed presence of eight different patterns among the 18 isolates.[11]A RAPD fingerprinting study of V. cholerae isolates from Talikoti and Harnal outbreaks was done by Dey S et al in the year 2014. They identified that RAPD fingerprints of these isolates were found to be identical with one particular clone out of eight different clones of V. cholerae O1 obtained from an outbreak that occurred in Belgaum in 2010.[16] Prabhu DIG et al analysed genetic similarities between environmental and clinical isolates of V. cholerae using RAPD finger printing using A5 & A18 primers. They observed that environmental isolates showed very close genetic relationship (ranging from 80.81% to 90.91%) with clinical isolates.[17]These studies indicate that RAPD finger printing is simple, rapid and reliable method for typing V. cholerae isolates, phylogenetic studies and to find out possible source of infection.

Most of the cases are reported from 3 taluks; Belur, Arasikere and Sakaleshpura. All RAPD types found to be distributed in all three regions. This could be because; they are neighboring talukas with frequent travelling of people for functions and festivals.

In cholera cases fluid and electrolytes replacement is main stay of management and antibiotics are not required in all. Use of antimicrobial agents can only reduce the duration and severity of illness. Antibiotic sensitivity tests for the isolates revealed the presence of diverse patterns, and 97.3% of isolates were multidrug resistant (MDR) with resistance to more than one antibiotic. Only 2.7% of isolates remained sensitive to all antibiotics. Among all the antibiotics tested, only Imipenem found to be effective against all the isolates. MDR cholera is being reported from India by many investigators.[1,15,18,19] This wide spectrum of resistance found in V. cholerae in this study is not very common and a cause for serious concern. Large-scale, irrational antibiotic usage among humans and animals, inadequate therapy and lack of awareness regarding antibiotic policy and antibiotic stewardship may be responsible for development of high degree of drug resistance in our country. Antibiogram pattern also found to be fluctuating over the period of study. This could be because of changes in antibiotic choice of treatment which is not determined based on scientific data.

97.29% of isolates resistant to combination of antibiotics A, Na and Co with or without resistance to other antibiotics. (Table; 3) Most common resistant pattern is A, Na, Co (66.23%) followed by A, Na, Co, T, Nor, Ch, Cef, G (10.81%) and A, Na, Co, T (6.76%).These kind of resistance patterns with resistance to more than 8 antibiotics indicates that it could be plasmid mediated drug resistance. This is a matter of grave concern as plasmid mediated resistance can spread in environment very easily.

Nalidixic acid resistance was 100% with a number of studies,[1,5,11] but our study reports lesser percentage of resistance (97.30%) similar to a study by Garg P et al who reported 94.3%.[20] 100% resistance with Furazolidone was noted with study by Das S et al[1] & Garg P et al.[20] But resistance to Furazolidone in our study was 90.70% and varied degree of resistance was noted with a number of studies like Palewar MS et al,88%[21] and Chander J et al, 45.4%[22] In the present study, 25.68% of isolates were resistant to Tetracycline and 24.39% resistant to Doxycycline. Resistance to tetracycline in other studies were as low as Mandal J et al, 18.6%[14] and Chander J et al, 15.4%.[22] Few other studies had higher percentage of resistance like Misra M et al, 31.1%[15] and Devnikar AV et al, 38.1%.[23] Resistance with Co-trimaxazole (94.59%) and Ampicillin (97.30%) were in higher range with majority of the studies including ours.100% resistance to Ampicillin, reported by Das S et al[1] and Garg P et al.[20] But few other studies reported very low degree of resistance like Roy S et al, 26.8% [11] and Misra M et al, 15.2%.[15] Similarly a study by Devnikar AV et al reported a lower resistance with Co-trimoxazole (42.86%).[23]

Ciprofloxacin resistance in this study was 18.92%. Similar degree of resistance was noted with Garg P et al (18.9%) [20]. Das S et al[1] (30.4%) and Misra M et al[15] (37.9%) reported higher percentage resistance compared to number of other studies which reported Kutar BMRNS et al, 12.6%[5], Mandal J et al, 4.2%[14] and Chander J et al, 8.33%[22] resistance. Among representative Cephalosporins of 2nd generation, Ceftriaxone had 10.81% resistance. But a number of studies reported no resistance with cephalosporins.[1,21,22,23] But study by Misra M et al reports resistance to Ceftriaxone as high as 34.9%.[15]

Conclusion

V. cholerae 01 biotype El Tor serotype Ogawa is currently circulating type in our geographical region. All isolates tested positive for CTX and TCP indicating that circulating strains are pathogenic in nature. Presence of different RAPD types indicates that cholera is endemic in this region with periodic epidemics. Presence of large number of MDR isolates necessitates curbing of irrational use of antibiotics to prevent further spread of drug resistance. Resistance to commonly used antimicrobial agents which were considered as drug of choice is a major public health concern. Drug resistance itself may result in longer hospital stays for patients thereby increasing mortality, morbidity and adds economical burden on community.

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identity and antibiogram and in molecular biology tests like PCR for ctx, tcp, O1rfb and RAPD.

Conflicts of Interest: None.

References

1. 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(4):294-298.
2. 2.Taneja N, Sangar G, Chowdhury G, Ramamurthy T, Mishra A, Singh M, Sharma M. Molecular epidemiology of Vibrio cholerae causing outbreaks and sporadic cholera in northern India. *Indian J Med Res* 2012;136:656-663.
3. Chatterjee S, Ghosh K, Raychoudhuri A, Pan A, Bhattacharya MK, Mukhopadhyay AK, Ramamurthy T, Bhattacharya SK, Nandy RK. Phenotypic and genotypic traits and epidemiological implication of Vibrio cholerae O1 and O139 strains in India during 2003. *J Med Microbiol* 2007;56:824–832
4. Pal BB, Khuntia HK, Samal SK, Kar SK, Patnaik B. Epidemics of severe cholera caused by El Tor *Vibrio cholerae* O1 Ogawa possessing the ctxB gene of the classical biotype in Orissa, India. *Int J Infect Dis* 2010;14(5):384-389.
5. Kutar BMRNS, Rajpara N, Upadhyay H, Ramamurthy T, Bharadwaj AK. Clinical Isolates of Vibrio cholerae O1El Tor Ogawa of 2009 from Kolkata, India: Preponderance of SXT Element and Presence of Haitian *ctxB* Variant. *PLoS One.* 2013;8(2):e56477.
6. Kannan B, Peter AO, Ramalingam T, Shunmugiah KP. Virulence and Multidrug Resistance Patterns of *Vibriocholerae* O1 Isolates from Diarrheal Outbreaks of South India during 2006–2009. *Microbial Drug Resis* 2012;54:354-361
7. Saha DR, Rajendra N K, Ramamurthy T, Nandy RK, Bhattacharya SK. Intestinal parasitism and *Vibrio cholerae* infection among diarrhoeal patients in Kolkata, India. *Epidemiol Infect*. 2008;136:661–664.
8. Collee JG, Miles RS. Vibrio, Aeromonas, Campylobacter, Arcobacter, Helicobacter, Wolinella. In Mackie and McCartney's Practical medical microbiology. 14th edn. Churchill livingstone. Edited by Collee JG, Duguid JP, Fraser AG, Marmion BP. 1996.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disc susceptibility tests. Approved standard M2-A10. Wayne, PA, USA: CLSI; 2007.
10. Keasler SP, Hall RH. Detecting and biotyping Vibrio cholerae O1 with multiplex polymerase chain reaction. *Lancet* 1993;341(8861):1661.
11. Roy S, Parande MV, Mantur BG, Bhat S, Shinde R, Parande AM, Meti RS, Chandrashekhar MR, Kholkute SD, Saini A, Joshi M, Gaonkar A. Multidrug-resistant Vibrio cholerae O1 in Belgaum, South India. *J Med Microbiol* 2012; 61:1574-79.
12. Ausubel FM. Short Protocols in Molecular Biology. 4th edn. New York: John Wiley& sons. Edited by Ausubel FM, Brent R, Kingston, RE, Moore DD, Seidman JG, Smith JA, Struhi K. 2003.
13. Roy S, Dutta B, Ghosh AR, Sugunan AP,Nandy RK, Bhattacharya SK, Sehgal SC. Molecular tracking of the lineage of strains of *Vibrio cholerae* O1 biotype El Tor associated with a cholera outbreak in Andaman and Nicobar Islands, India. *Tropical medicine and international Health* June 2005;10(6):604–611.
14. 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):12-16
15. Mishra M, Mohammed F, Akulwar SL, KatkarVJ, Tankhiwale NS, Powar RM. Re-emergence of El Tor *vibrio* in outbreak of cholera in & around Nagpur. *Indian J Med Res*, 2004;120:478-480.
16. Dey S, Parande MV, Parande AM, Lakkannavar SL, Rathore PK, Mantur BG, Kholkute SG, Roy S. Twin outbreak of cholera in rural North Karnataka, India. *Indian J Med Res,* 2014;140:420-426.
17. Prabhu DIG, Pandian RS, Vasan PT. Pathogenicity, antibiotic susceptibility and genetic similarity of environmental and clinical isolates of Vibrio cholerae. *Indian J of exp biol* 2007;45:817-823.
18. Ghosh A, Ramamurthy T. Antimicrobials & cholera: are we stranded? *Indian J Med Res* 2011;133:225-231.
19. Bhattacharya D, Sayi DS, Thamizhmani R, Bhattacharjee H, Bharadwaj AP, Roy A, Sugunan AP. Emergence of multidrug-resistant *Vibrio cholerae* O1 Biotype El Tor in Port Blair, India. *Am J Trop Med Hyg* 2012;86:1015–1017.
20. Garg P, Chakraborty S, Basu I, Datta S, Rajendran K, Bhattacharya T, Yamasaki S, Bhattacharya SK, Takeda Y, Nair GB, Ramamurthy T. Expanding multiple antibiotic resistance among clinical strains of Vibrio cholerae isolated from 1992±7 in Calcutta, India. *Epidemiol Infect* 2000;124;393-399.
21. Palewar MS, Choure AC, Mudshingkar S, Dohe V, Kagal A, Bhardwaj R, Jaiswal A**,** Sarkar B. Typing and antibiogram of Vibrio cholerae isolates from a tertiary care hospital in Pune: A 3 year study. *J Global Infect Dis* 2015;7(7):35-36.
22. Chander J, Kaistha N, Gupta V, Mehta M, Singla N, Deep A, Sarkar BL. Epidemiology & antibiograms of Vibrio cholerae isolates from a tertiary care hospital in Chandigarh, north India. *Indian J Med Res* 2009;129:613-617.
23. Devnikar AV, Sonth SB, Baragundi MC, Solabannavar SS, Kulkarni RB. Characterization and Antibiogram of *Vibrio cholerae* isolates from a tertiary care hospital. *Int J Biol Med Res* 2012;3(4):2352-2354.

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