Original Research Article

# Antibiotic Susceptibility Pattern of *Vibrio cholerae* Causing Diarrohea Outbreaks in Bidar, North Karnataka, India

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**A B S T R A C T**

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To evaluate the serogroups, antimicrobial susceptibility pattern, age and gender wise distribution of *Vibrio cholera* isolates from cholera epidemics in Bidar of north Karnataka, India during the years 2008 to 2015 till date. Stool samples from outbreak investigations were tested in the department of Microbiology, Bidar Institute of medical sciences BRIMS, Bidar, Karnataka. A total 500 stool samples of cholera outbreak from Year 2008 to 2015 till date were collected and processed at Department of Microbiology, Bidar Institute of Medical Sciences (BRIMS) Bidar as per the routine microbiological investigations. The isolates were identified as *Vibrio cholera*e and confirmed by serological tests with Polyvalent O1, O139 and mono specific Ogawa and Inaba antisera. Antibiotic sensitivity testing was done as per the CLSI guidelines. *Vibrio cholera* biotype ElTor, sero group O1 grown in 155 samples (31%). Among 155 isolates obtained during study period, of which, 136 (87.74%) were belonged to Ogawa, 13 (8.3%) belonged to Inaba and 06 (03.8%) to Hikozima. The isolates showed Multi drug resistance to Ampicillin (81.93%), Nalidixic acid (66.45%) and Ofloxacin (32.90%) throughout the study period. The infection was predominant in male and among patients age between 0-10 years (26.45%). This study reflects the importance of control and monitoring of *V. Cholera* by serogroup and antibiogram typing for policy makers and health professionals of this region as incidence of cholera increased year wise.

## Introduction

Acute diarrhoea is the second most prevalent communicable disease and a fourth leading cause of death in India with 10762500 cases and 32218 deaths reported in 2013 (National Health Profiles, Government of India). Cholera is an acute diarrhoeal illness caused by toxigenic strains of *Vibrio cholera*e serogroups O1 and O139 which contributes a major part to these figures as it has high epidemic potential. Presently, *V. cholerae* O1 belonging to the ElTor biotype is the most common serogroup causing Cholera in India.

Cholera is also changing epidemiologically. The development of resistance in vibrio strains creates a major problem in treating the severe cases of diarrhoea which needs

antibiotic intervention. Antibiotic resistance has become a major medical and public health problem as it has direct links with disease management as multiple antibiotic resistant strains of *V. cholerae* have emerged (Amit *et al.,* 2008; Garg *et al.,* 2000).

Reports of drug resistant *V. cholerae* strains are appearing with increasing frequency and these have been documented (Okoh and Igbinosa, 2010). Multidrug resistance in diarrhoeal illness like Cholera has increased the morbidity rate in the patients from 1% to 5.3% in cholera epidemic of Guinea – Bissau in the year 1996–1997 (Anders *et al.,* 2000). Studies reported that a genetic element named as SXT element plays a role in the acquisition of antibiotic resistance to sulfamethoxazole, trimethoprim in *Vibrio cholera*e O1 and O139 strains. However, the antibiotic susceptibility of the organisms fluctuate spatially and temporally (Tomoko *et al.,* 2004).

Till now no studies have been done in Bidar to monitor the antibiotic susceptibility pattern of *V. cholerae* and development of resistance of cholera isolates to doxycycline and tetracycline and prevalence of cholera incidence in Bidar year wise. We isolated Vibrio pathogens and in this study, we report the antibiotic susceptibility patterns of the vibrio isolates as well as the percentage of infection gender wise and age wise.

## Materials and Methods

**Study area:** The study area includes both rural parts and town of Bidar, North Karnataka. As in any other regions of India, the cholera outbreaks have been frequently reported from one or the other parts Bidar every year especially during end of the summer and beginning of the monsoon season in the months of May, June & July months.

**Case definition of cholera:** In an area where there is a cholera epidemic, a patient aged 5 years or more develops acute watery diarrhoea with or without vomiting was considered as suspected cholera case (WHO).

A total of 500 stool samples were collected from the outbreaks occurred from past 8 years from year 2008 to till July 2015 were received and processed in the Department of Microbiology, Bidar Institute Of Medical Sciences (BRIMS) Bidar. (The cases which were admitted in Private hospitals and other tertiary care hospitals of that region are not included in this study). The samples were collected in a sterile universal container and transported in Cary Blair transport medium to the laboratory. The routine microscopy was done for stool samples for detection of parasites, RBC's, & Pus cells. All stool samples were enriched in alkaline peptone water (APW) for 8 hours and hanging drop preparation was done from APW to see the darting type motility of the *Vibrio cholera*e which gives presumptive identification. It was followed by culture on blood agar, Mac Conkey agar, thiosulphate citrate bile salt sucrose agar (TCBS) at $37^{o}$C for 18 to 24 hours. The colonies with the characteristic appearance of *Vibrio cholera*e were identified by biochemical tests and conformed by serological tests with Polyvalent O1, O139 and mono specific Ogawa and Inaba antisera (Becton Dickinson Company, India).

**Antibiotic sensitivity tests:** Antibiotic sensitivity tests were carried on Muller Hinton agar (MHA) plates by Kirby Bauer disk diffusion method (Bauer *et al.,* 1966) using antibiotic discs (Himedia, Mumbai, India) Ampicillin (AMP 10mcg), Nalidixic acid (NAL 30mcg), Ofloxacin (OFX 5mcg), Tetracycline (TET 30mcg), Chloramphenicol (CHL 30mcg), Doxycycline (DO 10mcg) and Ciprofloxacin(CIP 5mcg). Characterization

of the strains as susceptible, intermediately resistant, or resistant was based on the size of inhibition zones around each disc as per the manufacturer's instructions, which matched the interpretive criteria as per CLSI guidelines (CLSI, 2007).

**Statistical analysis**

SPSS version 21 was used to generate graphs. Only descriptive statistics was used to generate results.

**Results and Discussion**

Among 500 stool specimens processed, 155 samples (31%) were found to be positive for *Vibrio cholera*e O1, Biotype ElTor. Among 155 isolates, 136(87.74%) were belonged to serogroup Ogawa, 13 belonged to Inaba (08.3%) and 6 to Hikozima (03.8%).

Highest infectivity of Cholera was found in age group between 0-10 with 41 cases (26.45%) and in Males with 84 cases (54.19%). And it is also found that the incidence of cholera also increased year wise from 2008 to July 2015.

The study focussed on the samples received both for outbreak investigation and routine diagnostic investigations in the laboratory.

**Antibiotic sensitivity:** The antibiogram profile revealed that, among 155 Vibrio isolates, all isolates showed Multi drug resistant to ampicillin, nalidixic acid and ofloxacin with resistance of 81.93%, 66.45% and 32.90% respectively. And even cholera isolates showed resistance to ciprofloxacin (22.58%), doxycycline (14.83%) and tetracycline (16.77%) indicating development of resistance of *V. cholerae* to the drugs which are commonly used to treat cholera infection.

Cholera continues to remain an important public health concern in India and other developing countries. Globally, the true number of cholera cases is known to be much higher than reported. Although rehydration therapy is the mainstay of therapy for cholera, antimicrobial treatment is also important to treat Cholera patients as their use results in a marked decrease in overall stool volume and a decreased length of illness. From past 30 years, doxycycline and tetracycline has been the drug of choice for treatment of cholera.

But from time to time, however, antimicrobial resistance in *Vibrio cholera*e developed and many studies have been done and documented on it in India and other countries.
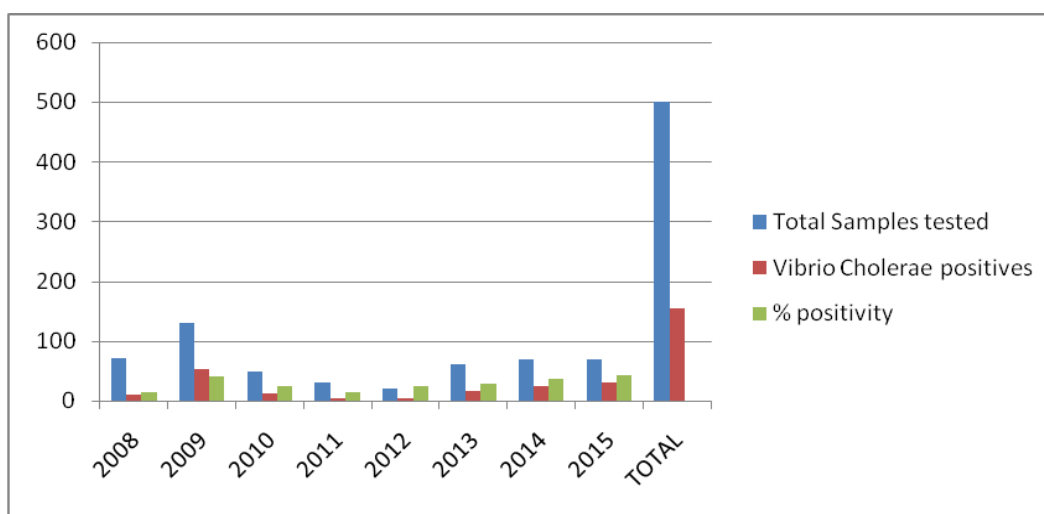
Plasmid coded high level resistance to ampicillin, sulphonamide, tetracycline, trimethoprim and gentamicin has been studied among strains of V.cholerae O1, isolated from Bangladesh and India (Tatsuo *et al.,* 1995). Mukhopadhyay *et al.* (1995) reported the ineffectiveness of Co trimoxazole and furazolidone to treat patients with *V. cholerae* O1 infection and appearance of nalidixic acid resistance among O1 strains from Calcutta patients. Amit *et al.* (2008) isolated and reported Tetracycline resistant strains in Kolkata in the year 2005.

Chandrasekhar *et al.* studied emergence of multidrug resistance among Vibrio strains in Hubli of Karnataka in the year 2008 and found that *V. cholerae*, which was earlier susceptible to the most enteric antimicrobials in 2000, was found to be multidrug resistant in subsequent years with the development of fluoroquinolone resistance since 2002. According to our study findings, *V. cholerae* O1 strains exhibited total resistance to Ampicillin and nalidixic acid and started developing resistance to doxycycline and tetracycline.

**Table.1** Percentage distribution of antibiotic sensitivity pattern of *Vibrio cholera*e strains

| Drug | Sensitivity | Resistant |
|------|-------------|-----------|
| Ampicillin | 18.06% | 81.93% |
| Chloramphericol | 86.45% | 13.54% |
| Ciprofloxacin | 77.41% | 22.58% |
| Ofloxacin | 67.09% | 32.90% |
| Nalidixic Acid | 33.54% | 66.45% |
| Doxycycline | 85.16% | 14.83% |
| Tetracycline | 83.22% | 16.77% |

**Graph.1** Total number of samples tested and positivity percentage of *Vibrio cholera*e strains year wise



**Table.2** Infectivity rate of cholera among age group

| Age group | Cholera cases | Percentage |
|-----------|---------------|------------|
| 0-10 | 41 | 26.45 |
| 11-20 | 22 | 14.19 |
| 21-30 | 22 | 14.19 |
| 31-40 | 22 | 14.19 |
| 41-50 | 20 | 12.90 |
| 51-60 | 15 | 9.68 |
| 61& above | 13 | 8.39 |
| **TOTAL** | **155** | |

All strains isolated were concurrently resistant to both ampicillin and nalidixic acid. The resistance to quinolones, ampicillin and Tetracycline is due to large

scale use of antibiotics for the treatment and prophylaxis of cholera. This study also highlighted the increased prevalence of cholera in Bidar district year wise showing the increased incidence of 14.08% in the year 2008 to 42.86% in 2015 with a slight fall in the year 2011 with occurrence of 13.33%.

In conclusion, this is the first study in the Bidar that describes the antibiotic susceptibility pattern of Vibrio isolates. Bidar experiences cholera epidemics every year. This study revealed the development of multidrug resistance among Vibrio strains in this endemic region. A highly cholera endemic area like Bidar need a continuous vigilance on the changing trend in the antibiotic susceptibility pattern of *V. cholerae* which is due to environmental factors and widespread use of antibiotics. As this region noticed high infectivity rate among children between age groups 0-10 years, it is necessary to control cholera by improving drinking water facilities and sanitation to control morbidity and mortality due to cholera.

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