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### Original Article

# Characterization and Antibiogram of *Vibrio cholerae* isolates from a tertiary care hospital

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#### ABSTRACT

**Background:** Cholera, an acute diarrhoeal disease caused by *Vibrio cholerae* still ranks high in the etiology of diarrhoeal diseases in several parts of India. **Aim:** To study the characteristics and anti-microbial resistance pattern of the *Vibrio cholerae* strains isolated in our hospital. **Methods:** Over a 2 year period, stool samples from clinically suspected cholera cases were screened and the isolates were identified by standard laboratory techniques. *Vibrio cholerae* isolates were confirmed by slide agglutination using antisera and anti-microbial susceptibility was carried out by Kirby Bauer's disc diffusion method. **Results:** Of the 51 samples, 21 strains of *Vibrio cholerae* were isolated, all of which were identified as *Vibrio cholerae* bio type El Tor. Majority (85.71%) of the isolates were serotype Ogawa. Both sexes were equally affected with maximum number of cases seen in the age group 0 – 20 years. Resistance to ampicillin was seen in 61.9%, to tetracycline in 38.1% and to ciprofloxacin in 23.8% of the isolates. None of the isolates were resistant to gentamicin and cephotaxime. **Conclusion:** Our present situation regarding drug resistance in *Vibrio cholerae* is a matter of concern. Therefore, the anti-microbial profile of *Vibrio cholerae* isolates should be under constant surveillance.

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### 1. Introduction

*Vibrio cholerae* has been recognized as one of the most common causes of bacterial diarrhoea throughout the world [1]. More than half of the world's population lives in cholera endemic areas [2]. The Indian subcontinent alone accounts for almost 78% of all cholera cases in the world [3]. Cholera is endemic in most states of India & sporadic cases have also been reported throughout the year [4]. However, outbreaks are seen to occur from time to time and are usually associated with the monsoon season and have been reported from various parts of India [4,5,6].

Antibiotic resistance of *Vibrio cholerae* was unheard of before 1977. This however has changed rapidly and is primarily due to the indiscriminate use of antibiotics. Frequent outbreaks of cholera combined with rapidly emerging antibiotic resistance remain a major concern in our country as well as many other developing nations [7,8].

The present study was undertaken to identify the strains of *Vibrio cholerae* predominant in our area and to study the characteristics and anti-microbial resistance pattern of the strains isolated in our hospital.

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## 2. Material and Methods

All clinically suspected cases of cholera admitted to S. Nijalingappa Medical College & HSK Hospital & research Centre, Bagalkot between Aug 2010 and July 2012 were included in this study. Stool sample from these patients were collected in a wide mouth, capped sterile container & enrichment was done with alkaline peptone water at 37°C for 6 – 8 hours. Before plating, hanging drop preparation was made to confirm typical darting motility of *Vibrio cholerae* both directly from the sample and after enrichment in alkaline peptone water. The samples were then plated onto MacConkey agar, blood agar & Thiosulphate Citrate Bile salt Sucrose (TCBS) agar. The isolates obtained were identified according to recommended standard laboratory methods including polymyxin (50µg) [9] and serotypes were identified by slide agglutination with *Vibrio* non-differential O1, monospecific Ogawa & Inaba and *Vibrio cholerae* O139 antisera obtained from King Institute of Preventive Medicine, Guindy, Chennai. Antibiotic susceptibility was carried out by Kirby Bauer's disc diffusion method as per standard recommendations [10] using the following antimicrobial agents: Amoxycillin (10µg), tetracycline (30µg), ciprofloxacin (5µg), chloramphenicol (30µg), gentamicin (10µg), co-trimoxazole (25µg), cephalexin (30µg) and amikacin (30µg). Standard strain of *Escherichia coli* ATCC 25922 was used as control strain. The culture media and discs were obtained from Hi-media Ltd, Mumbai.

## 3. Results

Out of the 51 stool samples tested, 21 (41.18%) yielded isolates of *Vibrio cholerae*. 9 were isolated from Aug 2010-Jul 2011 and 12 from Aug 2011-Jul 2012. 14 (66.67%) of these patients were seen in the rainy season (June – Sept) (Fig 1). 9 (42.85%) were children, 4 of which were less than 5 years of age. 10 (47.62%) were seen in the less than 20 age group. Male to female ratio was 1:1.1. (Table 1)

All the isolates were *Vibrio cholerae* O1 bio type El Tor, 18 (85.71%) of which were serotype Ogawa and 3 (14.29%) were serotype Inaba (Table 2). The antibiotic resistance pattern of the isolates is shown in Table 3.

**Table 1: Age wise distribution**

Age (Years)	MALE	FEMALE	Total (%)
0-5	1	3	4
6-10	-	2	2
11-20	3	1	4
21-30	2	-	2
31-40	1	1	2
41-50	1	1	2
51-60	2	2	4
>61	-	1	1
Total	10 (47.62%)	11 (52.38%)	21

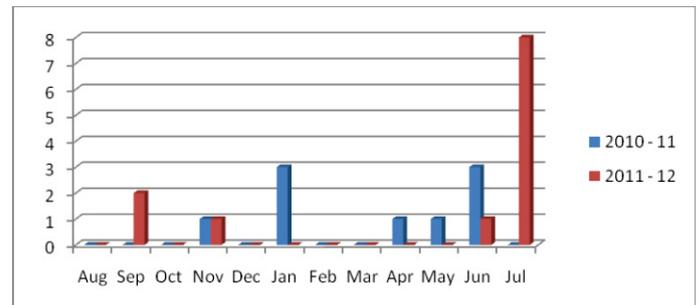
**Table 2: Year wise distribution of *Vibrio cholerae* isolates**

<i>Vibrio cholerae</i>	2010-11	2011-12	Total (%)
Ogawa	8	10	18 (85.71)
Inaba	1	2	3 (14.29)
Hikojima	-	-	-
NAG	-	-	-

**Table 3: Resistance pattern of isolates**

Drug	Resistant isolates (%)	Drug	Resistant isolates (%)
Tetracycline	8 (38.1)	Cephalexin	0 (0)
Gentamicin	0 (0)	Co-trimoxazole	9 (42.86)
Amikacin	1 (4.76)	Ciprofloxacin	5 (23.8)
Ampicillin	13 (61.9)	Chloramphenicol	6 (28.57)

**Fig 1: Seasonal variation of *Vibrio cholerae***



## 4. Discussion

By the end of 1965, *Vibrio cholerae* biotype El Tor replaced the age old classical biotype and no isolation of classical *Vibrio cholerae* has been reported after 1980 [11]. In the present study, all isolates were *Vibrio cholerae* biotype El Tor, majority of which were serotype Ogawa. In an 18 year study at NICED by Sarkar BL et al [12], it was found that 96.5% of the *Vibrio cholerae* isolates from all over India were serotype Ogawa. Other studies from India have similar findings [2,3,4,6]. There have been reports of emergence of *Vibrio cholerae* O139 from all over India in the previous decade [1,13,14,15,16], after which the incidence of O139 has declined [16]. In this study, not a single strain of *Vibrio cholerae* O139 was isolated. Mathur M et al [2], Mishra M et al [6] and Kulkarni RD et al [17] did not isolate strains of O139 in their areas either.

The isolation rate was 41.17% which is similar to a 3 year study by Sharma NC et al [13] where the overall isolation rate was 43.1%. Taneja Net al [18] gave an account of 58.5% patients being children of which 19.5% were less than 5 years of age. In the present study, 42.85% of the cases were children and 19.04% were less than 5 years of age. The youngest patient was 11 months old. Sharma NC et al [13] reported a higher incidence in the less than 5 age group (32.7%). They have also reported that men are affected more than

women but in our study we found that cholera incidence in both sexes is same, which is similar to findings by Mishra M et al [6] and Taneja Net al [18].

Although, oral rehydration is the mainstay of treatment, antimicrobial therapy is a useful adjuvant as it substantially reduces the duration and volume of diarrhoea, thereby lessening fluid requirements and shortening the duration of hospital stay [19]. WHO recommends either the use of tetracycline or ciprofloxacin as the treatment of choice. However in recent studies in India and in the present study, resistance to these antimicrobials has been noted. The next line of action is third generation cephalosporins [20]. Although emerging resistance to this drug has been reported [13], in our study all the isolates were sensitive to cephalexin. Antimicrobial resistance has attained importance as a global public health problem. The increase in magnitude of bacterial species resistant to multiple antimicrobial agents relies on various factors which the organism faces over the years. The availability of over the counter drugs and wide spread use of irrational chemoprophylaxis in developing countries play an important role in emergence of resistance resulting into the drug becoming ineffective. In the present study, 38.1% resistance to tetracycline and 23.8% resistance to ciprofloxacin is worrisome and noteworthy.

## 5. Conclusion

The most important responsibility of the laboratory is to define the beginning of the epidemic, monitor the changes in antimicrobial resistance during the epidemic and also to confirm that an epidemic is over. The current scenario regarding drug resistance in *Vibrio cholerae* is a matter of concern. The emergence of such resistance may significantly influence the control strategies in future outbreaks. Therefore, continued monitoring and surveillance of all cholera outbreaks and the antimicrobial profile becomes a necessity.

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