



## CURRENT SCENARIO OF VIBRIO CHOLERAE

## Microbiology

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## ABSTRACT

Cholera is an acute diarrheal disease caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*. It is a Notifiable Disease known to cause serious epidemics and pandemics in developing countries like India. **Aim & objective:** To evaluate the proportion of *Vibrio cholerae* positive stool samples along with Find out Biotypes, Serotypes and antimicrobial susceptibility patterns of *V. Cholerae* isolates. **Material and Method:** Total 873 stool samples were received and processed in the Bacteriology laboratory, Department of Microbiology, Medical College Baroda, during the period January 2022 to December 2022. Stool specimens were examined microscopically. Further processing, identification, Biotyping & Serotyping of the isolates was done by standard Biochemical tests. Antibiotic susceptibility testing was done & interpreted according to CLSI guidelines. **Result:** Out of 873 stool samples 42(04.81%) samples were positive for *Vibrio cholerae*. Where is Classical(71.42%) Biotype & Serotype Ogawa was predominant. Maximum isolation was in rainy season (57.14%). The isolates were sensitive to Ceftriaxone (88.09%), Ciprofloxacin (88.09%), Ampicillin(35.71%), Cotrimoxazol (35.71%), Doxycycline (28.57%). **Conclusions:** Classical Biotype and Serotype Ogawa was predominant. Antibiotic susceptibility pattern of *V.cholera* shows high sensitivity to Ceftriaxone & Ciprofloxacin which should be the preferred agents for chemotherapy.

## KEYWORDS

*Vibrio cholerae*, antimicrobial susceptibility

## INTRODUCTION

Cholera is a diarrhoeal illness caused by *Vibrio cholera*, transmitted by ingestion of contaminated food or water [1]. It is one of the Notifiable Diseases and known to cause serious epidemics and pandemics in developing countries like India[1]

The causative agent, *V. cholerae*, has two biotypes, classical and El Tor. Classical biotype was responsible for the first six pandemics of cholera worldwide. El Tor biotype replaced the classical biotype by 1961 and caused the seventh pandemic of cholera [2]. Each biotype has three serotypes: Ogawa, Inaba and Hikojima [3]. Prevalent biotypes and their antibiotic resistance changes over time [4,5]. Presently, O1 serogroup which is belonged to El Tor biotype is most common in India [6]. The use of phage typing as a method of classifying *V. cholerae* has contributed greatly to the understanding of the epidemiology of cholera. Phage typing at the National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India includes the Basu S and Mukherjee S typing and new phage typing schemes. These two phage-typing schemes are specific for *V. cholerae* O1 and O139 and are being routinely used for the classification of strains at this institute [7,8]. Cholera can occur throughout the year, but maximum transmission is associated with high temperature, heavy rainfall, and flooding [9]. A change in seasonal pattern was observed in the present study, since the maximum number of cases in the study was seen during the month of November. Hence, this study was done with an objective to evaluate the proportion of *Vibrio cholerae* positive stool samples. along with Find out Biotypes, Serotypes and antimicrobial susceptibility patterns of *V. Cholerae* isolates.

## MATERIALS AND METHODS

Total 873 stool samples were received and processed in the Bacteriology laboratory, Department of Microbiology, Medical College Baroda, during the period January 2022 to December 2022. Stool specimens were collected on admission in a clean, wide mouthed, leak proof container preferably before starting antibiotics and transported immediately to the laboratory for processing. Specimens were cultured directly on Nutrient agar, Blood agar, MacConkey's agar, TCBS sucrose agar [Fig-1]. Nutrient agar was used to perform biochemical tests on the isolates [Fig-2]. Blood agar was used for biotyping. Further, the specimens were sub cultured on MacConkey's agar, TCBS agar after enrichment in Alkaline peptone water and were examined after overnight incubation at 37°C [10]. Antibiotic susceptibility testing was done on MHA (Muller hinton agar) by using Kirby Bauer disc diffusion method & interpreted according to CLSI guidelines.



[fig-1] Growth On Tcbs Medium



[fig-2] Growth On Nutrient Agar

Colonies suggestive of *V. cholerae* were identified by standard biochemical tests. Serotyping of isolates was done for agglutination using *V. cholera* polyvalent, *V. cholera* Ogawa and *V. cholera* Inaba antisera (Denka Seiken Co., Ltd., Japan). Biotyping was done by conventional Methods such as Polymyxin-B sensitivity and Sheep RBC haemolysis [10].

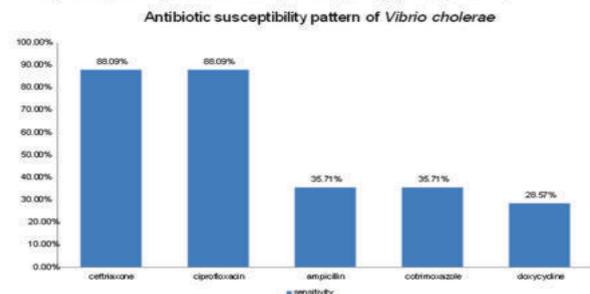
Phage typing was done by Basu S and Mukherjee S method and New Phage Typing method and susceptibility to phage IV and V for biotyping was done at NICED, Kolkata, West Bengal India [7,8].

Antibiotic susceptibility testing was performed by Kirby Bauer's Method as per CLSI guidelines [11]. The control used for

antimicrobial sensitivity testing was ATCC strain *Escherichia coli* 25922. The following antibiotics were used-Ceftriaxone (30 µg), Doxycycline (30 µg),

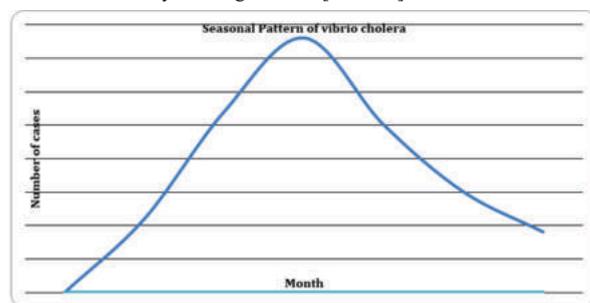
Ciprofloxacin, Ampicillin (30 µg) and Cotrimoxazole (25 µg). The results were seen after 16-18 hours of incubation at 37°C. The zone of inhibition for each antibiotic was interpreted as per CLSI guidelines [11].

- The isolates were sensitive to Ceftriaxone (88.09%), Ciprofloxacin (88.09%), Ampicillin (35.71%), Cotrimoxazole (35.71%), Doxycycline (28.57%).



## RESULTS

Out of 873 stool samples 42(04.81%) samples were positive for *Vibrio cholerae*, where is Classical 30 (71.42%) and EL tor 12 (28.5%) Biotype & Serotype Ogawa was predominant. Among them 38 were from urban and 4 from rural areas. The infection was more in females than males with maximum isolation in rainy season (57.14%) in the month of July and August 2022. [Table- 2]



**Table-2 Seasonal Pattern of vibrio cholera cases**

Phage typing was done at Vibrio Phage Reference Lab, NICED, Kolkata by Basu S and Mukherjee S method and New Phage Typing method. According to Basu S and Mukherjee S method. Out of 42 isolates 7 were sent for phage typing to NICED, Kolkata. 3 isolates were typed as Ogawa O1 T27 phagetype.

Antibiotic susceptibility was performed by Kirby Bauer disc diffusion method.

Antibiotic susceptibility pattern of *V. cholera* shows high sensitivity to Ceftriaxone & Ciprofloxacin which should be the preferred agents for chemotherapy.

## DISCUSSION

In this present study 42 samples positive for *V. cholera* where is Classical 71.42% and EL tor 28.5% Biotype & Serotype Ogawa was predominant where Kanungo S et in their study has reported that *V. cholerae* O1 belonging to the El Tor was the most common biotype in India and frequency of O139 biotype has been reduced [15]. Bibhuti Bhusan Pal et al. study report *V. cholerae* O1 Ogawa isolates were more numerous in comparison with Inaba serotype isolates(19) Devnikar AV et al. study has reported that more Ogawa isolates were detected in comparison with Inaba strains (20).

Usually, a seasonal trend in the isolation of *V. cholerae* is seen. Cases peak in July, taper after September and are negligible after October which closely mimics the seasonal trends in monsoon [12]. However, in present study though the onset of cases coincided with monsoon, they peaked in August and continued till December.

Ciprofloxacin susceptibility (88%) seen in the present study was similar to other studies by Mandal et al.[16] (97%) and Shrestha et al.[17] (100%) carried out in Puducherry and Nepal

respectively. An antibiotic susceptibility result reported by Chander et al.[18] was quite similar to the present study. No association between the phage type and antimicrobial susceptibility pattern was observed in the present study.

## CONCLUSION(S)

*V. cholerae* O1 Classical Biotype and Serotype Ogawa was predominant. Antibiotic susceptibility pattern of *V. cholera* shows high sensitivity to Ceftriaxone & Ciprofloxacin which should be the preferred agents for chemotherapy.

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