

Short Communication

Antimicrobial susceptibility profile of *Vibrio cholerae* strains isolated at a tertiary care medical centre in New Delhi, India

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Received: 05 January 2017

Revised: 18 January 2017

Accepted: 02 February 2017

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ABSTRACT

Background: *Vibrio cholerae* is a common cause of acute diarrhea in India. Extensive and injudicious use of antimicrobials has led to the emergence of *V. cholerae* strains that are resistant to several antibiotics. The present study was conducted to document the antibiogram of *V. cholerae* strains isolated at an urban health centre in New Delhi. The present study was a retrospective, record-based analysis conducted from January to December 2015, at the Department of Microbiology of a tertiary care teaching institute.

Methods: Stool samples received in the laboratory were processed as per standard microbiological techniques and all the *V. cholerae* strains isolated were subjected to antimicrobial susceptibility testing as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines. Data was analyzed using the Epi info software, version 3.5.3, and results depicted in the form of descriptive statistics.

Results: *Vibrio cholerae* was isolated in 70 of the 2340 stool samples processed during the study period. Serogrouping revealed that 66 (94.3%) of the isolates were *V. cholerae* O1 serotype Ogawa, while non-O1 non-O139 serogroups constituted 4 (5.7%) isolates. The strains exhibited resistance to nalidixic acid (94.3%), ampicillin (92.9%), ciprofloxacin (55.7%), cefotaxime (41.4%), gentamicin (22.9%) and amikacin (7.1%).

Conclusions: Our study highlights the significance of regular surveillance of antimicrobial resistance profile of *V. cholerae* strains for proper management of cholera.

Keywords: Antibiogram, Cholera, Diarrhea

INTRODUCTION

Vibrio cholerae (*V. cholerae*) is a common enteric bacterial pathogen causing acute diarrheal disease, particularly in the developing world.¹ Cholera is endemic in several parts of the Indian subcontinent.^{2,3} In addition, one or the other part of the country continues to endure outbreaks of this illness every year, especially during monsoon seasons.⁴ Though, it is the fluid and electrolyte replacement that plays a critical role in management of cholera, antibiotic intervention may be warranted, particularly in severe cases. For the patient, it is

beneficial since it reduces the intensity and duration of diarrheal disease and prevents any lethal complications. Moreover, antibiotic therapy reduces the duration of fecal shedding of the bacillus, thereby reducing the chances of disease spread in the community.⁵

Vibrio cholerae was initially susceptible to several antimicrobial agents. However, extensive and injudicious use of antimicrobials has led to the emergence of drug resistant strains.⁶ Of particular concern is the emergence of multiple antibiotic-resistant (MAR) isolates of *V. cholerae*.^{3,5,7,8} Moreover, marked spatial and temporal

fluctuations have been reported in in-vitro susceptibility of *V. cholerae* isolates to antimicrobial agents.⁹

The present study was designed to determine the role of *V. cholerae* as a cause of acute diarrheal disease and to monitor the antibiogram of *V. cholerae* strains isolated during the year 2015 at a tertiary care health facility in New Delhi, India.

METHODS

The present retrospective, record-based study was conducted from January to December 2015, at the Department of Microbiology of a tertiary care teaching hospital in New Delhi. A total of 2340 consecutive stool samples collected from diarrheic/dysenteric patients of all age groups were processed during this period. Fresh stool samples were obtained from diarrheic patients and transported immediately to the laboratory for further processing. In the laboratory, stool samples were examined for macroscopic findings. In addition, a hanging drop preparation was made to look for darting motility and a stool routine microscopic examination was performed for pus cells, red blood cells and cysts and trophozoites of parasites. The study protocol was approved by the ethical committee of the institution.

The stool samples were inoculated onto Mac Conkey agar, Thiosulphate citrate bile salts sucrose agar (TCBS) and Deoxycholate citrate agar (DCA). A part of the stool samples was inoculated in enrichment media such as alkaline peptone water and selenite F broth, from which subsequent subcultures were done after incubation at 37°C for 6 hours onto TCBS and DCA respectively. Any *V. cholerae* like colonies were subjected to conventional biochemical tests as per standard microbiological techniques.¹⁰ Organisms biochemically suspected to be *V. cholerae* were further confirmed serologically by slide agglutination employing serogroup specific O1 polyvalent and O139 antisera and monospecific antisera for Ogawa & Inaba strains (Denka Seiken Company Limited, Tokyo, Japan).

Antimicrobial susceptibility testing of *V. cholerae* isolates was performed on Mueller-Hinton agar employing the disc diffusion technique as per Clinical and Laboratory Standards Institute (CLSI) guidelines.¹¹ Each isolate of *V. cholerae* was tested against a panel comprising of the following antibiotics: amikacin (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), ampicillin (10 µg) and cefotaxime (30 µg). A strain of *Escherichia coli* ATCC 25922 was used as the control.

All the data was entered in a Microsoft excel sheet and the entries doubly checked for any possible keyboard errors. Data was analyzed using the Epi info software, version 3.5.3, Centers for Disease Control and Prevention, Atlanta, GA, USA and only descriptive statistics were employed to depict the results.

RESULTS

Of the total 2340 stool samples processed in the laboratory, *V. cholerae* was isolated in 70 samples. Thus, the prevalence of *V. cholerae*-associated diarrhea in the study population was 2.9%. Serogrouping revealed that 66 (94.3%) of the isolates belonged to serogroup O1, while four (5.7%) were non-O1 non-O139. On further typing with monospecific antisera, it was found that 100% of the *V. cholerae* serogroup O1 isolates were of Ogawa serotype. The cases were seen from May to December, with the peak in the number of cases coinciding with the monsoon season and fairly high numbers being encountered till October-November. The month-wise distribution of the cholera cases at our centre is depicted in Figure 1.

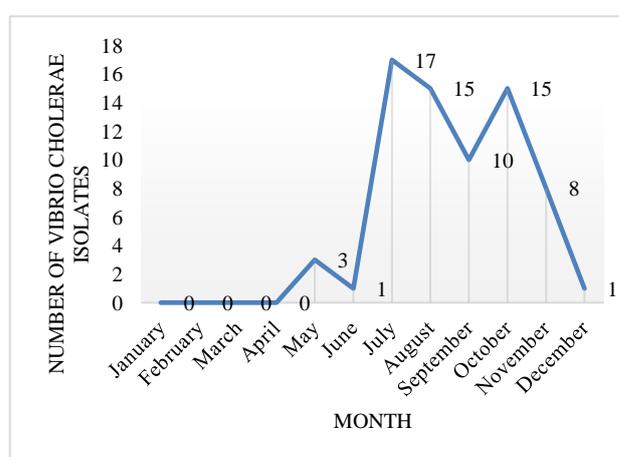


Figure 1: Month-wise distribution of *Vibrio cholerae* isolates (n=70).

The antibiogram of the *V. cholerae* strains isolated during the study period is summarized in Table 1. Strains were usually susceptible to amikacin and gentamicin. Conversely, resistance was high to nalidixic acid and ampicillin, with 94.3% (66 of 70) and 92.9% (65 of 70) of the strains showing resistance to each of the two antimicrobials respectively. Resistance rate of ciprofloxacin and cefotaxime was 55.7% (39 of 70) and 41.4% (29 of 70) respectively. All but two strains showed resistance to two or more antimicrobials while one strain was resistant to all the antimicrobials tested.

Table 1: Antimicrobial susceptibility pattern of *Vibrio cholerae* isolates (n=70).

Antimicrobial agent	Number (%) of drug-resistant strains of <i>Vibrio cholerae</i>
Amikacin	5 (7.1 %)
Gentamicin	16 (22.9 %)
Ciprofloxacin	39 (55.7%)
Nalidixic acid	66 (94.3 %)
Ampicillin	65 (92.9 %)
Cefotaxime	29 (41.4 %)

DISCUSSION

Cholera is a major public health concern in India. We found *V. cholerae* O1 serotype Ogawa to be the predominant circulating strain at our centre, a finding in concordance with other Indian studies.^{2,12} We also observed an increase in the number of cholera cases during the monsoon season, implying lack of proper sanitary systems. However, while other studies report a gradual decline following this peak, cholera cases in our study population continued to occur in similar numbers till October-November.¹²

Rehydration therapy is the mainstay of management of cholera, with requirement of antibiotic treatment arising only in severe cases. Several studies on antimicrobial resistance patterns in *V. cholerae* have been undertaken and great variations in the antimicrobial susceptibility profile of *V. cholerae* isolates have been reported from different parts of India and across the world.^{1-3,5,8,12,13} In the present study, the isolates exhibited greatest susceptibility to amikacin and gentamicin. High susceptibility of *V. cholerae* isolates to aminoglycosides has also been reported by several other Indian researchers.^{12,14} In contrast, Das *et al* found 41.3% of the *V. cholerae* O1 strains circulating in East Delhi during the year 2007 to be resistant to gentamicin.¹

Fluoroquinolone resistance was first detected in *V. cholerae* in 2002 and has increased since then.¹⁵ We report 55.7% of the *V. cholerae* isolates in our study to be resistant to ciprofloxacin. Other studies report this figure to be around 30-40%.² Circulation of ciprofloxacin resistant strains has also been confirmed by several other Indian workers.⁸ On the contrary, Kumar *et al* reported only 2.4% of the *V. cholerae* strains in their study to be fluoroquinolone resistant.¹²

Appearance of nalidixic acid resistance among *V. cholerae* O1 strains was reported in Calcutta by Mukhopadhyay *et al*.¹⁶ Since then resistance to this drug has drastically increased and some of the studies from Delhi and other parts of the country, now report nearly 100% resistance to nalidixic acid.^{1,8} Our study reinforces these findings, with very high resistance to nalidixic acid documented among the *V. cholerae* strains isolated at our centre.

While Kumar *et al* report a resistance rate of 26.8% for ampicillin, higher resistance rates, particularly among *V. cholerae* O1 strains, have been reported by Sharma *et al*.^{3,12} Our study also reveals high ampicillin resistance among *V. cholerae* strains circulating in and around our region.

For a long time, resistance to third generation cephalosporins was rarely reported in *V. cholerae*. Das *et al* in their analysis of drug resistance profile of *V. cholerae* O1 strains isolated during 2007-2009 also found more than 90% of their isolates to be susceptible to

cefotaxime.¹ In contrast, we report 41.4% of *V. cholerae* strains isolated at our centre to be cefotaxime resistant. This could have serious implications since third generation cephalosporins were till date being considered a potential therapeutic alternative in the management of multidrug resistant *V. cholerae*, and with emergence of strains resistant to this class of drugs, the treatment options are further restricted.

We found majority of *V. cholerae* strains isolated at our centre to be resistant to multiple antibiotics. Emergence of multiple antibiotic resistant *V. cholerae* has been reported by several Indian authors and is a matter of public health concern.^{3,5,7,8} The changing antibiogram of *V. cholerae* has limited the available therapeutic options.

CONCLUSION

Our study reveals the emergence of multidrug resistant strains of *V. cholerae* in and around Delhi region. The widespread and irrational chemoprophylactic use of some of the common enteric antimicrobials has led to the emergence of resistant profiles among these isolates. This rise in antibiotic resistance poses an imminent therapeutic challenge and cannot be ignored. Based on our findings, we strongly emphasize on the need to monitor the antibiotic resistance trends among clinical strains of *V. cholerae*. Continuous and regular vigilance of resistance patterns is imperative for proper treatment of cholera.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Uppal B, Mehra B, Panda PS, Kumar SK. Antimicrobial susceptibility profile of *Vibrio cholerae* strains isolated at a tertiary care medical centre in New Delhi, India. Int J Community Med Public Health 2017;4:868-71.