ORIGINAL ARTICLE

FREQUENCY AND ANTIMICROBIAL RESISTANCE PATTERN OF VIBRIO CHOLERAE IN STOOL SAMPLES

KhurramBaqai[1], Faisal Iqbal Afridi[2], Nasir Laique[3]
[1]Department of Gastroenterology, Dr. Ziauddin University Hospital, North Nazimabad Campus, Karachi
[2]Department of Microbiology, Dr. Ziauddin University Hospital, North Nazimabad Campus, Karachi

ABSTRACT

Background: Cholera, one of the most common cause of acute watery diarrhea in developing world, requires, along with correction of fluid and electrolyte imbalance, antibiotics in to shorten the duration of disease. Emergence of reistance with recommended antimicrobials is becoming an issue to treat *Vibrio Cholerae*. The purpose of this research was to determine the frequency and antimicrobial resistance pattern of Vibrio cholera isolated in stool samples at a tertiary care hospital.

Methods: Stool samples were received from both in-patients and out-patients in sterile leak proof containers. All *Vibrio Cholerae* isolated from stool samples were included in the study. The stool samples were inoculated on Thiosulphate-citrate-Bile salts-sucrose agar. Suspected *Vibrio cholerae* colonies were stained by Gram's stain Method. Oxidase positive colonies were isolated on Mueller Hinton agar (MHA). Slide agglunation tests were done to confirm presence of *Vibrio cholera* and its serotypes.

Results: A total of 352 (6.37%) Isolates of *Vibrio Cholerae* archieved from 5524 stool samples.*Vibrio Cholerae* serotype Ogawa isolated 188/352 (352%), while 164(46.6%) were *Vibrio cholerae* serotype Inaba. Predominant the isolates were from male patients 184/352 (52.3%), while from females were 168/352 (47.7%). Male to female ratio was 1.095:1. Mean age of patients with positive *Vibrio cholerae* isolates was 27.15 ± 25.61 years. The Frequency of resistance with Co-trimoxazole, Tetracyline, Ampicillin, Chloramphanicol and Ciprofloxacin was 66.2%, 40.9%, 8.8%, 2.6% and 1.4% respectively.

Conclusion: The multiple antimicrobials recommended to treat Cholera have been found to develop alarming resistance among *Vibrio Cholerae* isolates. This situation needs a regular surveillance of commonly used antibiotics and urgent discovery of alternative antibiotics.

KEYWORDS: Frequency.Antimicrobial resistance pattern.*Vibriocholerae*. Stool.

Corresponding Author
Dr. KhurramBaqai
Consulatant Gastroenterologist,
Ziauddin University Hospital.
North Nazimabad, Karachi.
E-mail: khbaqai@yahoo.com

INTRODUCTION

Cholera, a serious community health issue in developing nations, has characteristic features of profuse watery diarrhea, sometimes with vomiting, causing dehydration. When not treated urgently it can be severe enough to lead to death. Along with rehydration and hygiene as part of management, it is important to control cholera with antibiotic treatment especially with tetracycline.[1] An effective antibiotic helps in reduction of loss of fluids and period, intensity, transmission, morbidity and mortality of the disease.[2,3]

According to World health Organization (WHO), Cholera remains a leading community health issue in the world, particularly in Africa and Asia,[4] and if not diagnosed and treat properly it can be transmit-

ted to non-endemic regions.[5] Over 3.5 million population worldwide is affected annually, and between 100,000-130,000 deaths reported in last few years.[6] Notified cases of Cholera have been increased tremendously and most of the under developed nations are at risk of having outbreaks of Cholera epidemics.[4] Rehydration and replacement of electrolyte is the main treatment of Cholera. Antibiotic treatment is administered routinely to reduce severity and to shorten duration of illness and to decrease the chances of spread of infection. However, strains of *Vibrio cholera (V.cholerae)* showing resistance to antibiotics in different parts of world, as evidenced by studies.[7-12] Injudicious use of antibiotics is documented as a cause of development of resistance in cholera epidemics.[13] Prescribing inappropriate or wrong antibiotics can be dangerous and may have long term consequences.

Centers for disease control and prevention (CDC) has recommended antimicrobials from the group of tetracyclines, fluoroquinolones, macrolides and co-trimoxazole for the Cholera treatment in general,[14] yet local microbial susceptibility should be considered to choose the specific antibiotics.

In Pakistan, concerning susceptibility of antibiotic profile of *V.cholerae*, there is scanty information available. Consequently, there is no assurance of current antibiotic range in controlling and management of *V. cholerae*. In the current study, we reviewed and classified *V. cholerae* archived strains, determined frequently used antimicrobials resistance profile, and assessed patterns of resistance during last few years. This will help the physicians for selecting the appropriate antimicrobial management and their judicious use.

METHODS

This observational study was conducted over a period of five years from January, 2011 to December, 2015 at the Department of Gastroenterology and the Department of Clinical Microbiology of Ziauddin University Hospital. Stool samples were received from both in-patients and out-patients in sterile leak proof containers. All *V. cholerae* isolated from stool samples were included in the study. All other stool bacterial pathogens were excluded from the study. Written approval from institiutional ethical committee was taken. Informed consent was taken from patient or any other patient's relative. The stool samples were inoculated on Thiosulphate-citrate-Bile salt-sucrose (TCBS) agar. Plates were incubated for 48 hours at 37°C aerobically. After 24 hour incubation plates were examined, yellow colonies suspected of *V. cholera* were stained by Gram's Method. Those colonies which stained as pink curved rods and were found to be oxidase positive were isolated on Mueller Hinton agar (MHA). The serological tests (slide agglutination test) were done to confirm the presence of V. cholera and its serotypes according to standard microbiological techniques.[15] The antisera used was from Denka Seiken co. Ltd containing specific antibodies against somatic antigen.

Antimicrobial susceptibility testing was performed on MHA medium (Oxoid Ltd., England) using modified Kirby Bauer's disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] *Escherichia coli* American Type Culture Collection (ATCC®) 25922 was used as control.

Data analysis was performed by using SPSS version-20. Frequency and percentages were computed for presentation of all categorical variables like micro-organisms, sex, and antimicrobial sensitivities. Mean and standard deviation was calculated for quantitative variables like age of patients.

RESULTS

Stool samples of 5524 of in and out patients were processed for culture and antimicrobial susceptibilities during the study period. From these stool samples a total of 352 clinical isolates of *V. cholerae* were archived. The frequency of *V.cholerae* isolates were 352/5524 (6.37%). In those 352 isolates 188 (53.4%) were *V.cholerae* serotype Ogawa and 164(46.6%) were *V.cholerae* serotype Inaba **(Table 1)**. Predominantly the isolates of *V.cholerae* were from male patients 184/352 (52.3%), while isolates from female patients were 168/352 (47.7%) **(Table 2)**. Male to female ratio was 1.095:1. Mean age of patients with positive *V.cholerae* isolates was 27.15±25.61 years. Five common antibiotics were used to screen those strains. The Antimircobial susceptibilities were shown in **Figure 1**.

Table:1 Vibrio Cholera Serotype

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Vibrio Cholerae Ogawa	188	53.4	53.4	53.4
	Vibrio Choleraenaba	164	46.6	46.6	100.0
	Total	352	100.0	100.0	

Table:2 Gender

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	184	52.3	52.3	52.3
	Female	168	47.7	47.7	100.0
	Total	352	100.0	100.0	

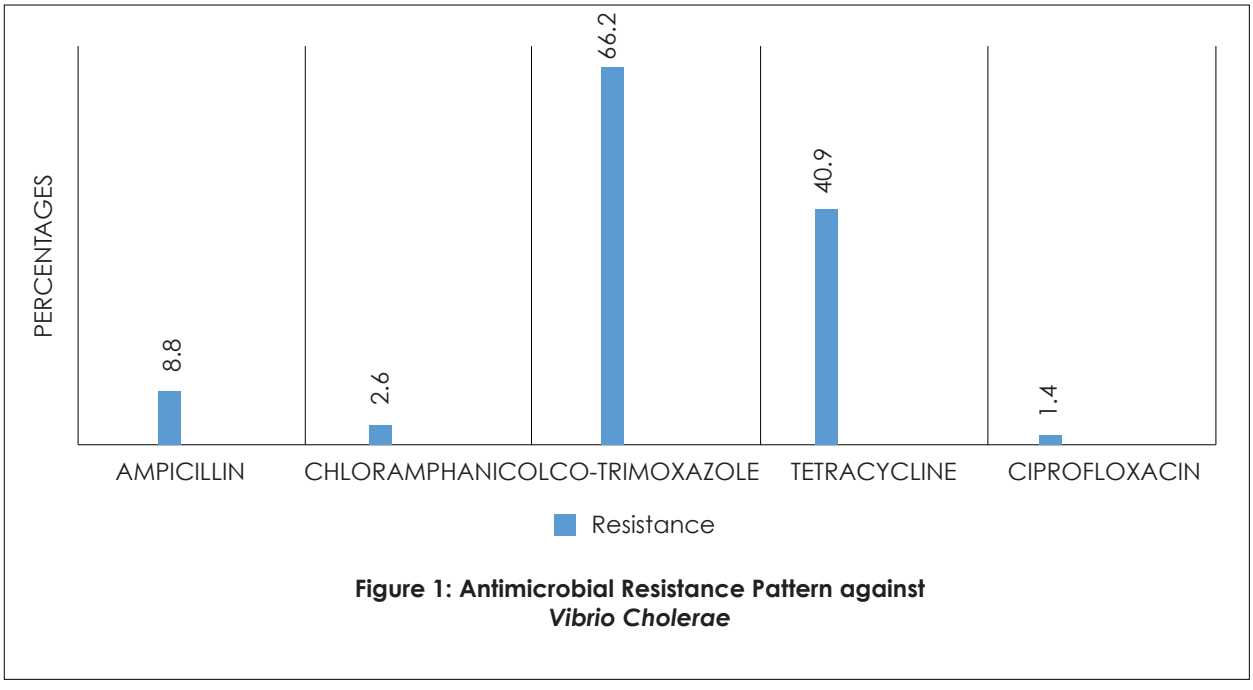

Figure 1: Antimicrobial Resistance Pattern against *Vibrio Cholerae*

DISCUSSION

Cholera is an endemic disease in Pakistan.[17] Contamination of water and food, unhealthy environment and poor sanitation are the causes of outbreaks of Cholera.[18] Incidence of Cholera in Pakistan is estimated as 1.64/1000 annually.[19]

V.cholerae is a curved gram negative bacilli that comprises both pathogenic and non-pathogenic stains. It has three serotypes (Ogawa, Inaba and rarely Hikojima). Clinical manifestations and sign and symptoms of Ogawa serotype are similar to Inaba serotype. Irrespective of serotype patients with concurrent infections and immunodeficiency are at high risk of having morbidity and mortality. Emergence of resistance with antimicrobials has remained a problem for years in Pakistan with changing pattern of sensitivity among Ampicillin, Chloramphanicol, Co-trimoxazole and Tetracycline.[20]

In studies from China the pattern of antimicrobial susceptibility was found 38.5% to Co-trimoxazole,

11% to tetracycline and 45.9% to nalidixic acid.[12] In India, strains during epidemics have shown an increasing pattern of resistance with nalidixic acid and Co-trimoxazole.[21] The antibiotic of choice does not only depend on the prevailing effectiveness of the antibiotic against *V.cholerae* isolate involved, but a number of factors such as age, sex, and physiological status.

In the current study of *V.cholerae* isolates, not only the pattern of resistance was observed but also the resistance with multiple antimicrobials among isolates of *V.cholerae* was studied. In this study the frequency of *V.cholerae* isolates was 6.37%. A recent study in Baghdad showed that 3.4% of stool samples were positive for *V.cholerae*.[22] A similar study in India showed isolation of *V.cholerae* in 24.8% of stool samples of pediatric patients presented with acute watery diarrhea.[23] In our sudy serotype Ogawa was isolated in 46.5% of stool samples while serotype Inaba was present in 53.5% of stool samples. It was in contrast to a study from India, Kranata, where the burden of V.cholerae serotype Ogawa was 87.7% and V.cholera serotype, Inaba, 8.3%.[24] Another study from India also showed frequency of V.cholera serotype Ogawa and V.cholerae serotype Inaba as 69.5% and 30.5% respectively.[25]

Our study showed that 66.2% isolates were resistant to Co-trimoxazole, while pattern of resistance of *V.cholerae* with Tetracycline, Ampicillin, Chloramphanicol and Ciprofloxacin was 40.9 %, 8.8%, 2.6% and 1.4% respectively. A study from Iran also showed similar pattern of resistance of antimircobials with *V.cholerae* strains in stool. This study exhibited the resistance with Co-trimoxazle, Tetracycline, Ampicillin and Ciprofloxacin as 43%, 29%, 27% and 0% respectively.[26] Cholramphanicol was not used in this study to treat *V.cholerae*. Similarly, a study from Pakistan, conducted in 2001-2, in Rawalpindi, showed a better trend of resistance of antimicrobials with *V.cholerae*. In this study *V.cholerae* showed resistance with Co-trimoxazole, Tetracycline, Ampicillin and Ciprofloxacin with a frequency of 95.8%, 0%, 4.17% and 0% respectively.[27] This situation is alarming for a developing country, as in the past, Tetracycline, Co-trimoxazole have been used as the drug of choice in patients with Cholera related diarrhea. This predicts that there should be a continuous surveillance of antibiotics used in the treatment of Cholera because of ever changing pattern of resistance of *V.cholerae*.

CONCLUSION

The current study concludes the emergence of resistance with commonly used antibiotics to treat *V. cholerae*, including some of the antibiotics recommended by WHO for treatment in Pakistan. The frequency of resistance with antimicrobials is increasing with Co-Trimoxazole, Tetracycline and ampicillin, which are the antibiotics of choice for Cholera. The situation is alarming, especially in a country where Cholera outbreaks are common especially after floods. However, Chloramphanicol and Ciprofloxacin are still highly effective for treatment of Cholera. To prevent further spread of resistance, these antibiotics should be use judiciously, and should be limited to those patients who are moderate to severely dehydrated due to Cholera[28]. The multi-durg resistance in *V. cholerae* demands the continuous surveillance of commonly used antimicrobials. Also, there is a need to search for alternative antimicrobials which can help in treating Cholera effectively.

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