

How to Cite:

Ullah, H., Naeem, M., Ali, L., Alam, I., Masood, A., & Aslam, M. (2023). Stool culture & sensitivity for vibrio cholera in acute out break of diarrhea. *International Journal of Health Sciences*, 7(S1), 402–408. <https://doi.org/10.53730/ijhs.v7nS1.14214>

Stool culture & sensitivity for *vibrio cholera* in acute out break of diarrhea

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Abstract--Background: Acute watery diarrhea is a common clinical entity encountered daily in emergency department worldwide. In adults more than 90% of cases of acute diarrhea are caused by infectious agents spread by ingestion of feces contaminated food and water. Objective: To assess the stool Culture & Sensitivity for *Vibrio Cholera* in Acute Out Break of Diarrhea. Methodology: This study was prospective cohort study targeting all acute bacterial diarrhea cases in patients of either sex and all ages attending the emergency department of Qazi Hussain Ahmed Medical Complex, Nowshera Pakistan. The overall sample size was 86 on the basis of WHO sample size calculator. Culture and sensitivity was done for all the isolates of *Vibrio cholera*. All the data was documented in a special designed proforma and then analyzed by SPSS version 24. Results: In the current study, totally 86 patients were enrolled. There were 50 (58.14%) males and 36 (41.86%) females' patients in our study. Based on identification, 11 (12.79%) samples were positive for *vibrio cholera*

while 3 (3.49%) samples were positive for *Salmonella typhi*. Ampicillin was susceptible to 2 (18.18%) isolates while 3 (27.27%) isolates were resistant to ampicillin. The most susceptible antibiotic was Azithromycin, susceptible in 11 (100%) isolates and no isolate was resistant to it. The second most susceptible antibiotic was Ciprofloxacin in 10 (90.91%) cases while one isolate (9.09%) shows Intermediate resistance. Highest resistance was shown to Co-Trimoxazole in 8 (72.73%) isolates while only one isolate (9.09%) was susceptible to it. Conclusion: Our study concludes that the prevalence of *Vibrio cholera* is high amongst patients with acute outbreak of diarrhea. Our study also concludes that resistance has been developed by *Vibrio cholera* to many antibiotics like Ampicillin, Co-Trimoxazole and Doxycycline. Our study recommend to do culture and sensitivity before prescription of antibiotics to patients with acute diarrhea.

Keywords---stool culture, sensitivity, vibrio cholera, acute out break.

Introduction

Acute watery diarrhea is a common clinical entity encountered daily in emergency department worldwide. According to WHO 2017 report, diarrhea is the second leading cause of death in children under five years old. Moreover, it is responsible for killing around 525 000 children every year.¹ The 2017–2018 Pakistan Demographic and Health Survey (PDHS) reported the national prevalence of diarrhea among children <5 y of age is 19%.² Pakistan has the third-highest burden of diarrhea-related mortality globally. There are approximately 39 500 diarrhea related deaths in children below 5 years of age annually.³ In adults more than 90% of cases of acute diarrhea are caused by infectious agents spread by ingestion of feces contaminated food and water. *Rotavirus* and *Escherichia coli*, are the two most common etiological agents in low-income countries. Other less common pathogens include *cryptosporidium* and *shigella*. Millions of people have been affected by the acute form of diarrhea known as cholera over the years. Robert Koch discovered *Vibrio cholera*, the cholera-causing microbe, in 1883.⁴

More recently, severe cases of cholera have also been linked to *V. cholerae* 0139.⁵ *Vibrio cholerae* O1, the bacterium that causes epidemic cholera, is still a serious health concern in the majority of poor countries in the globe.⁶ Cholera epidemics have been often recorded from various regions of this nation, particularly during the monsoon season. Nonetheless, rare instances have sometimes been documented, mostly as a result of poor sanitation and contaminated water supplies.^{7,8} A clean environment, a reliable supply of clean water, and effective sewage treatment should all be part of a public health policy. In Pakistan in 2022, cholera outbreak was declared by the National IHR Focal Point of Pakistan according to WHO⁹. Most cases of acute diarrhea are mild and self-limiting and no investigation or treatment is necessary. However, keeping in view the frequent upsurges in acute diarrhea cases, regular stool cultures can prevent epidemics and identification of the bacterial pattern of sensitivity to antibiotics. Earlier, *Vibrio* sp. was responsive to a variety of antibiotics, but with time, they became

less effective against them. There is a limited body of research on the antibiotic susceptibility of cholera pathogens from the majority of underdeveloped nations. *V. cholerae* O1 strains that are resistant to "tetracycline, trimethoprim-sulfamethoxazole, and ampicillin" are widespread across the world.¹⁰⁻¹⁴ In several of these investigations, excessive antibiotic prophylaxis, unlawful administration, and usage of these medications in animal husbandry have been cited as the primary causes of the fast growth in antimicrobial resistance.^{15,16} Argentina¹⁷ and most recently Delhi, India, have both documented *V. cholerae* resistance to ceftriaxone.¹⁸ Moreover, there are no comprehensive data on the organisms' patterns of antibiotic sensitivity. Objective of our study was to identify common pathogenic organism of acute watery diarrhea in our population, and their sensitivity to the commonly used antibiotics.

Material and Methods

This study was prospective cohort study targeting all acute bacterial diarrhea cases in patients of either sex and all ages attending the emergency department of Qazi Hussain Ahmed Medical Complex, Nowshera Pakistan. The study duration was six months from July 2022 to December 2022. The study approval was taken from the ethical committee of the hospital. The overall sample size was 86 on the basis of WHO sample size calculator.

Inclusion criteria

- Cases of only acute watery are included in the study
- Cases of moderate – severe degree of severity were included
- Samples were taken from the patients who have not used antibiotics for the illness till sampling

Exclusion criteria

- Cases of chronic and bloody diarrhea
- Patients who have used antibiotics before sampling
- Formed stools
- Samples contaminated with urine or toilet paper

Each patient had a thorough history that included biographical data, the duration of the diarrhea, and a report of their stools. For every patient, stool cultures were performed. The preferred specimen was feces, which were obtained during a diarrheal disease's acute stage. Feces were gathered in a dry, clean, tight-lidded container that wasn't contaminated with toilet paper or urine. Demographic features (for example, age, sex, date of presentation, and history of recent travel); presenting symptoms (body temperature, passage of bloody stools, number of stools per day, and the duration of illness, abdominal pain, and vomiting) were documented for all the enrolled patients. All the data was collected by using pre-designed proforma. The collected data was analyzed by using SPSS version 24. Frequency and percentages were calculated for variables like gender; sensitivity and resistance pattern of antibiotics and culture positivity whereas for variables like age, means and standard deviations were computed.

Results

In the current study, totally 86 patients were enrolled. There were 50 (58.14%) males and 36 (41.86%) females' patients in our study. (Figure 1) The mean age was 28 (4.22) years. Out of 86 samples, 14 (16.28%) samples were culture positive. Based on identification, 11 (12.79%) samples were positive for *vibrio cholera* while 3 (3.49%) samples were positive for *Salmonella typhi*. (Figure 2) Antibigram assay was done for all the *Vibrio cholera* isolates. Ampicillin was susceptible to 2 (18.18%) isolates while 3 (27.27%) isolates were resistant to ampicillin. The most susceptible antibiotic was Azithromycin, susceptible in 11 (100%) isolates and no isolate was resistant to it. The second most susceptible antibiotic was Ciprofloxacin in 10 (90.91%) cases while one isolate (9.09%) shows Intermediate resistance. Highest resistance was shown to Co-Trimoxazole in 8 (72.73%) isolates while only one isolate (9.09%) was susceptible to it. (Table 1)

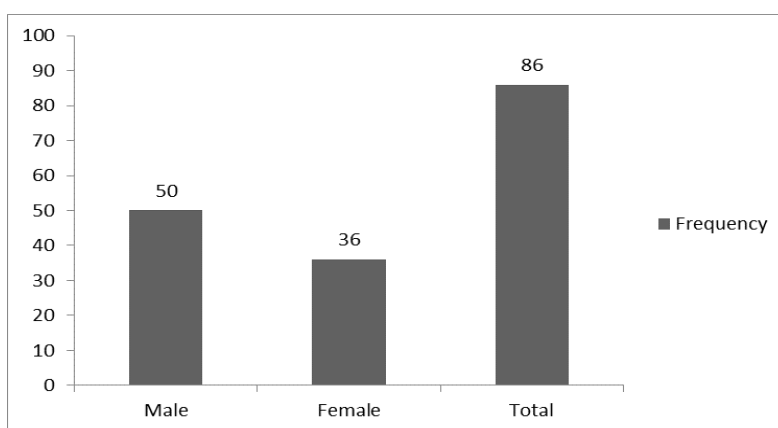


Figure 1. Distribution of patients based on gender

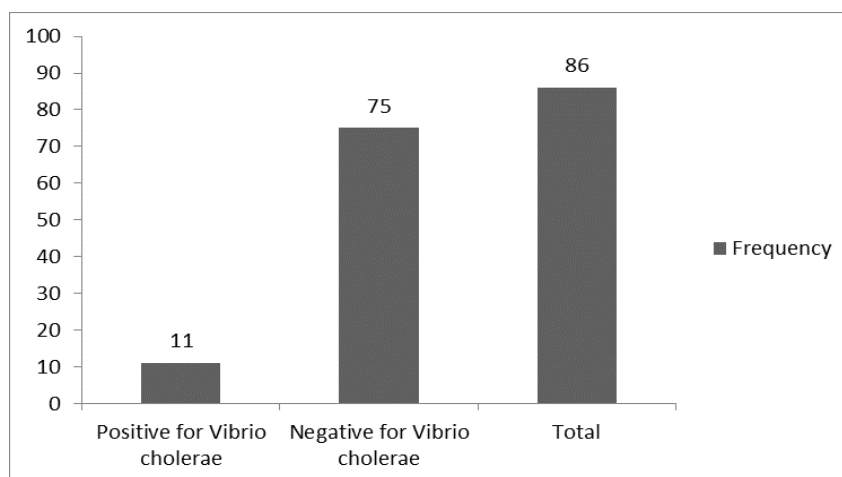


Figure 2. Frequency of *Vibrio cholera* in patients with acute diarrhea

Table 1
Antibiogram assay of *Vibrio cholerae* isolates

	Susceptible (S)	Intermediate resistance (I)	Resistance (R)
Ampicillin	2 (18.18%)	6 (54.55%)	3 (27.27%)
Azithromycin	11 (100%)	00 (00%)	00 (00%)
Ciprofloxacin	10 (90.91)	1 (9.09%)	00 (00%)
Co-Trimoxazole	1 (9.09%)	2 (18.18%)	8 (72.73%)
Doxycycline	4 (36.36%)	3 (27.27%)	4 (36.36%)

Discussion

Currently, resistance to antibiotics has been quickly emerging and spreading in Gram-negative organisms. It is now a global issue to stop the epidemiological spread of these drug-resistant genotypes in the population.¹⁹ *V. cholera* is a serious pathogen that spreads quickly via the feco-oral route in communities, especially in underdeveloped nations with poor sanitary systems. Just a tiny percentage of patients with severe illness need antibiotic treatment; oral and intravenous rehydration therapy is the gold standard of treatment. The prevalence of *V. cholera* that is resistant to antibiotics varies greatly throughout the globe.²⁰ Antimicrobial drugs are freely prescribed in Pakistan, like in many other underdeveloped countries, and antibiotics are frequently employed in animal husbandry, which may be the cause of the issue of the rising spread of antibiotics resistance.

In the current study, totally 86 patients were enrolled. There were 50 (58.14%) males and 36 (41.86%) females' patients in our study. The mean age was 28 (4.22) years. Out of 86 samples, 14 (16.28%) samples were culture positive. Based on identification, 11 (12.79%) samples were positive for *vibrio cholera* while 3 (3.49%) samples were positive for *Salmonella typhi*. A previous study carried out by Ekta Mala et al. reported 1.27% prevalence of *vibrio cholera* amongst patients with acute diarrhea which is not in accordance with our study.²¹ Another study done by Atul Kumar et al. reported 3.8% frequency of *vibrio cholera* amongst patients with acute diarrhea which is also not consistent with our findings.²² Antibiogram assay was done for all the *Vibrio cholera* isolates. Ampicillin was susceptible to 2 (18.18%) isolates while 3 (27.27%) isolates were resistant to ampicillin. The most susceptible antibiotic was Azithromycin, susceptible in 11 (100%) isolates and no isolate was resistant to it. The second most susceptible antibiotic was Ciprofloxacin in 10 (90.91%) cases while one isolate (9.09%) shows Intermediate resistance. Highest resistance was shown to Co-Trimoxazole in 8 (72.73%) isolates while only one isolate (9.09%) was susceptible to it.

According to a research by Atul Kumar et al., the resistance rates for ampicillin, trimethoprim-sulfamethoxazole, and furazolidone were respectively 26.8%, 95.1%, and 70.7%. Throughout the research period, 2% of *Vibrio* isolates exhibited norfloxacin and ciprofloxacin resistance. " Tetracycline, chloramphenicol, amikacin, gentamicin, and third-generation cephalosporin"s did not show any signs of resistance.²² These results were in agreement with those of earlier

investigations where the isolates were shown to be particularly sensitive to tetracyclines and aminoglycosides.^{23, 18}

Another study done by Ekta Mala et al. reported that the organism showed sensitivity to tetracycline, gentamycin, Amikacin and Norfloxacin. Resistant drugs included ampicillin and trimethoprim-sulphamethoxazole.²¹ Hand-washing, adequate food preparation, chlorination of water sources, sewage treatment, and disposal are all important components of prevention. Sanitary measures to avoid fecal-oral transmission are also important. A clean piped supply of drinking water or boiling the water is also essential, as is strict personal cleanliness. Food must not be accessible to flies. An effective kind of protection is provided by parenteral vaccination with a killed suspension of *V. cholera*. There are oral vaccinations that include killed *V. cholera* and the B component of the cholera toxin, but their effectiveness is limited. Public awareness campaigns, water source management, and population control are essential during epidemics. Both tetracycline therapy and widespread single-dose immunization are beneficial. The risk of transmission is reduced by medical staff members carefully cleaning their hands after handling bodily fluids and soiled clothes.

Conclusion

Our study concludes that the prevalence of *Vibrio cholera* is high amongst patients with acute outbreak of diarrhea. Our study also concludes that resistance has been developed by *Vibrio cholera* to many antibiotics like Ampicillin, Co-Trimoxazole and Doxycycline. Our study recommend to do culture and sensitivity before prescription of antibiotics to patients with acute diarrhea.

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