Original Article

Isolation and Identification of Vibrio Cholerae from Stool Sample and Antibiotic Susceptibility Pattern in Baluchistan Pakistan

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ABSTRACT

Objective: This study aimed to isolate and identify Vibrio cholerae from stool samples, as well as to analyze the antibiotic sensitivity profile of the isolated strains.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Microbiology Laboratory of the BMCH and Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB) Quetta from January to December 2023.

Methods: A total 273 samples were collected from all over of Balochistan. Out of all the samples, 61 were found positive for V. cholerae and were subsequently analyzed and identified using colony characteristics on thiosulfate-citrate-bile salts-sucrose (TCBS) agar, biochemical tests, serological identification using specific antisera, and antimicrobial susceptibility was assessed using the Kirby-Bauer disc diffusion technique. The inhibition zones were measured and evaluated following the outlined recommended by the Clinical and Laboratory Standards Institute (CLSI).

Results: Among 273 suspected stool samples, 61(22.34%) strains of V. cholerae belonged to the serotype O1, sub-serotype Ogawa. V. cholerae O1 infection was significantly more prevalent 21 (7.69%) among aged 51 years to 60 years. The results showed that the proportion of female patients 38 (13.91%) was higher compared to male patients 23 (8.42%). The incidence of V. cholerae infection peaked during the summer 9.89%, followed by autumn 5.12%, spring 4.02% and winter recorded the lowest incidence 3.29%. All isolates of V. cholerae were 100% sensitive to Levofloxacin, Azithromycin, and Imipenem. Sensitivity was 95% for Kanamycin, 90% for Ciprofloxacin, 83% for Ceftriaxone, 80% for Doxycycline, and 79% for Amikacin. However, all isolates (100%) were completely resistant to Sulfonamides-Trimethoprim, Nalidixic acid, and Ampicillin. Additionally, a resistance rate 71% was observed for Tetracycline.

Conclusion: Continuous and systematic surveillance is essential to monitor the changing patterns of antibiotic susceptibility and the evolving serotypes of pathogens. This will help in detecting emerging resistance trends, guiding appropriate treatment strategies, and informing public health policies to control the spread of resistant strains more effectively.

Key Words: Cholera, Vibrio cholerae, Antibiotic, Baluchistan

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INTRODUCTION

Vibrio cholerae, a Gram-negative bacterium, is responsible for causing cholera, a significant health concern in developing regions. This pathogen naturally inhabits aquatic environments, with the ocean serving as its main reservoir. Given that cholera eradication is not feasible, ongoing environmental surveillance is crucial to detect and control the spread of infections, particularly when V. cholerae is present in its pathogenic form or at elevated concentrations. Although there are over 200 serogroups of this bacterium, only two—O1 and O139—are linked to epidemic outbreaks worldwide**.** V. cholerae includes both pathogenic and nonpathogenic strains, distinguished by variations in their virulence gene content. The bacterium exhibits a diverse range of strains and biotypes, capable of acquiring and exchanging toxins and virulence genes through lateral or horizontal transfer, often facilitated by bacteriophages.[1]

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V. cholerae is found worldwide, with a high prevalence rate. Over time, the increasing waves of cholera pandemics have led to a significant rise in reported deaths. Cholera, in particular, remains a constant threat, causing a large number of deaths annually. Each year, an estimated 3 to 5 million cases of cholera are reported worldwide, leading to approximately 100,000 to 120,000 deaths.[2]

Frequent outbreaks of cholera and gastroenteritis across various Asian countries highlight the urgent need to assess the prevalence of pathogenic species in different regions of Asia. As a developing nation, Pakistan is grappling with a dual burden of diseases, where infectious diseases account for 26% of the total health burden. The most pressing infectious diseases in Pakistan are acute respiratory infections, malaria, viral hepatitis, and diarrheal illnesses, such as diarrhea and dysentery. Cholera has long been an endemic disease in Pakistan, but it was not recognized as a major cause of diarrhea until after 1988. Thirteen recent outbreaks have been linked to inadequate hygiene practices and the consumption of contaminated water and food.[3]

Cholera is a severe diarrheal illness that requires prompt treatment, primarily through rehydration. Antibiotics play a supportive role in the treatment, as they help shorten the disease duration, reduce the shedding of Vibrio bacteria, and assist in preventing the spread of outbreaks.[4]

Every year, outbreaks of severe gastroenteritis occur in different parts of Balochistan, especially during the summer in the southern region where the climate is extremely hot. Balochistan is a remote area, and some regions lack basic medical and transportation facilities, contributing to the recurring outbreaks of severe gastroenteritis. The consumption of contaminated water frequently triggers episodic diarrhea, leading to malnutrition, which further increases vulnerability to disease. In 2004, the village of Badree in Khuzdar district experienced a major cholera outbreak. Within a span of three days, 148 severe gastroenteritis cases were reported, resulting in four mortalities. Laboratory analysis confirmed the presence of V. cholerae serotype Ogawa in both water and stool samples.[5] Balochistan faces significant challenges due to a poor sewage system and widespread water shortages.

METHODS

The current study, conducted from January to December 2023, aimed to characterize V. cholerae and was carried out at the Microbiology Laboratory of the BMCH and Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan Quetta. A total of 273 stool samples were obtained from patients admitted to various district hospitals across Balochistan, all of whom had a history of untreated diarrheal symptoms. Patients who had previously received antibiotic treatment were excluded from the study. The collected samples were promptly transported to the Microbiology laboratory for further analysis in an insulated foam box containing ice packs, with the temperature carefully maintained between 4°C and 6°C to preserve the integrity of the samples and prevent contamination. Initially, stool samples were enriched in alkaline peptone water, followed by inoculation onto Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar and MacConkey agar. The inoculated plates were then incubated for 24 hours at 37°C. After incubation, colonies produced yellow pigmentation on TCBS agar and showing lactose negativity or a slight pink on MacConkey agar were considered potential V. cholerae. Suspected colonies were further analyzed through Gram staining and morphological examination based on standard methods. Pure cultures were obtained by transferring single colony onto Nutrient agar. The biochemical tests were performed such as Oxidase test, Indol test, Catalase test, urease test and motility tests were performed. Carbohydrate fermentation (glucose, mannitol, inositol, sucrose, arabinose, mannose) and salt tolerance were evaluated using broths containing 0%, 6.5%, and 8% NaCl concentrations.

The serological identification of the samples was performed using a slide agglutination test with polyvalent antisera (Murex Diagnostic Limited) specifically targeting serogroup O1. The isolates that showed a positive reaction with anti-O1 antisera underwent further subtyping using specific antisera targeting the Ogawa, Inaba, and Hikojima strains, as well as serogroup O139. These subtyping procedures were carried out using high-quality reagents obtained from Dienka Sieken Co. Limited, Japan,

Antibiotic testing resistance profile of V. cholerae isolates was assessed using Mueller-Hinton agar (MHA) against a various antimicrobial agent calibrated to a 0.5 McFarland standards and tested with 12 commercially available discs (Oxoid, UK) by the Agar-based disk diffusion assay. Afterward the incubation, inhibition zones were measured and evaluated following the outlined recommended by the Clinical and Laboratory Standards Institute (CLSI).[6]

The statistical analysis included frequency analysis of the antibiotic susceptibility test. Furthermore, the paper provides visual representations, including pie charts and bar charts, to enhance comprehension. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS). Ethical approval for the research was obtained from the Ethics Review Committee of the Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta (Approval No. Supdt: /CASVAB /869/101/2022). The study was conducted in accordance with the Helsinki Declaration.

RESULTS

Out of 273 suspected stool samples, 61 (22.34%) V. cholerae strains were isolated, confirmed through morphological, biochemical, and serological analyses, all 61 isolates were identified as serotype O1, sub-serotype Ogawa.

A significant difference was observed in the association between age and V. cholerae O1, sub serotype Ogawa infection. Most cases 21 (7.69%) occurred in individual aged 51- ≥ 60 years, while those aged 31- 40 years had the lowest infection rate 5 (1.83%) as shown in Figure - 1. Furthermore, notable number of isolates was found in female individuals, totaling 38 (13.91%), which is higher than that observed in male individuals23 (8.42%). The incidence of V. cholerae infection was observed to be notably higher during the summer months, with 27cases (9.89%) reported, compared to autumn with 14 cases (5.12%), spring with 11 cases (4.02%), and winter, which recorded the lowest number of cases at 9 (3.29%).

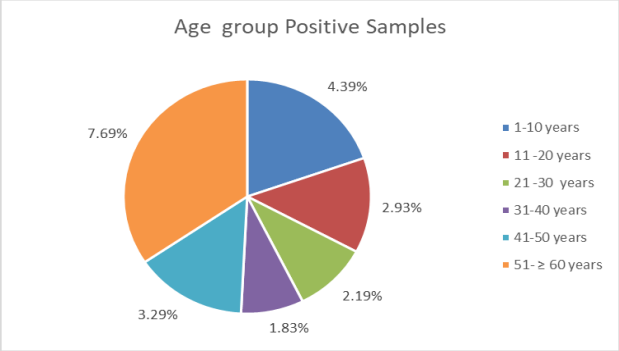
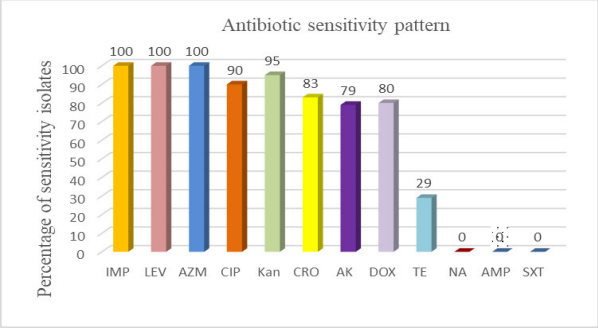


Figure No.1: Cholera Incidence in different age groups



IMP= Imipenem, LEV = Levofloxacin, AZM = Azithromycin, CIP = Ciprofloxacin, KAN= kanamycin, CAP= chloramphenicol, CRO = Ceftriaxone, AK = Amikacin DO = Doxycycline, TE= Tetracycline, NA = Nalaxic acid, AMP = Ampicillin, SXT= Sulfonamides Trimethoprim.

Figure No. 2. Antibiotic resistance against V. cholerae isolates.

The antimicrobial susceptibility testing of V. cholerae O1 isolates demonstrated that all strains were fully (100%) sensitive to Levofloxacin, Azithromycin, and Imipenem. Sensitivity was also observed in 95% of isolates for Kanamycin, 90 % for Ciprofloxacin,83% for Ceftriaxone, 79% for Amikacin, and 80% for Doxycycline. However, all isolates (100%) were completely resistant to, Sulfonamides-Trimethoprim, Nalidixic acid and Ampicillin. Additionally, a resistance rate (71%) was noted for Tetracycline as shown in Figure-2.

DISCUSSION

Cholera is a highly aggressive disease capable of causing intense, sudden watery diarrhea. Despite this, the actual global incidence of cholera is significantly higher than the reported figures.[7,8] Environmental influences are significant in the epidemiology of cholera. Cholera rates surge significantly during floods compared to times without flooding. Meanwhile, natural disasters like cyclones and earthquakes not only contribute to cholera outbreaks but also disrupt public health services.[9]

This study aimed to determine the prevalence of V. cholerae O1 infection across the Balochistan province in patients suspected of having cholera. Findings revealed that 22.34% of samples tested positive from various districts throughout Balochistan.

Our study found a link between the prevalence of V. cholerae O1 and different age groups. Older adults, aged 51 to ≥ 60 years, had a higher risk of contracting V. cholerae O1 (7.69%), possibly due to lower stomach acid production in this group. This contrasts with the findings of previous study.[10] The result of current study showed that incidence of female with V. cholerae O1 was (13.91%) while males had (8.42 %). However, a previous study by [11,12] contrasts with our findings, reporting higher infection rates in male patients. This study evaluated the infectivity and antimicrobial susceptibility of a V. cholerae isolate against a panel of 12 different antibiotics. In present study, all V. cholerae O1 isolates showed 100% sensitivity towards Levofloxacin, Azithromycin, which support the result of [13,14]. All isolates in our study exhibited 100% sensitivity to Imipenem, aligning with similar findings reported by other researchers.[15]. In our study, 90% of the isolates demonstrated sensitivity to Ciprofloxacin. This supports the results with the findings of [16]. The majority of the isolates exhibited sensitivity to Doxycycline and Kanamycin, with sensitivity rates of approximately 80% and 95%, respectively, while 29% were sensitive to Tetracycline. These results are consistent with the findings of [11].

A present study reported that V. cholerae serotype O1 strains exhibited 100% resistance to, Ampicillin, Sulfonamides-Trimethoprim and Nalidixic acid similar with[16].

CONCLUSION

Continuous and systematic surveillance is crucial for monitoring the changing patterns of antibiotic susceptibility and the evolving serotypes of pathogens. Such efforts are vital for detecting emerging resistance

trends, guiding appropriate treatment strategies, and shaping public health policies to effectively control the spread of resistant strains. Furthermore, robust surveillance data enable the prediction of outbreaks and the implementation of region-specific interventions. It also plays a pivotal role in designing targeted vaccination programs and optimizing antimicrobial use. Addressing these challenges is essential to mitigating the global impact of antimicrobial resistance and preserving the efficacy of treatments for future generations.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ashiq Hussain, Yasmeen Lashari, Muhammad Mazhar
Drafting or Revising Critically:	Fazal ur Rahman, Iftikhar ul Haq, Shahid Ali Magsi
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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