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Original Research Article

Isolation and Antimicrobial Susceptibility Pattern of *Vibrio cholerae* from Acute Diarrhoeal Cases at a Tertiary Care Hospital in South Gujarat

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Conflict of interest: Nil

Abstract:

Background: Cholera remains an important public health concern, particularly in regions experiencing seasonal outbreaks. Laboratory surveillance of *Vibrio cholerae*, its serotype distribution and antimicrobial susceptibility patterns is essential for understanding local epidemiology and supporting appropriate clinical management.

Aim: To determine the proportion of *Vibrio cholerae* isolated from acute diarrhoeal cases and describe its serotype distribution and antimicrobial susceptibility pattern.

Methods: A retrospective observational study was conducted in the Department of Microbiology, GMERS Medical College and Hospital, Valsad, Gujarat, from January to December 2025. Laboratory records of stool samples received from patients with acute diarrhoeal illness were reviewed. Samples were investigated by microscopy and culture, including enrichment in alkaline peptone water. Identification was based on colony morphology, Gram staining, oxidase testing and biochemical reactions. Serotyping was performed using specific antisera. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method, with CLSI and EUCAST guidelines.

Results: Of 113 stool samples processed, 13 yielded *V. cholerae*, giving a positivity rate of 11.5%.12 isolates (92.3%) were of the Ogawa serotype and 1 (7.7%) was Inaba. 7 patients (53.8%) were male and 6 (46.2%) were female. 9 cases (69.2%) were from the intensive care unit and 4 (30.8%) from general wards. The most represented age group was 25–34 years (5/13; 38.5%). In this study, monthly isolation was highest in August (5 isolates), followed by September and July (3 each) and October (2). Reported susceptibility was 100% for tetracycline, doxycycline, trimethoprim–sulfamethoxazole, ampicillin and chloramphenicol.

Conclusion: The study demonstrates the continued detection of *V. cholerae* among patients with acute diarrhoeal illness at our tertiary care centre, with predominance of the Ogawa serotype and clustering during the monsoon and post-monsoon period. Continued laboratory surveillance is important for monitoring serotype distribution and antimicrobial susceptibility. Prompt rehydration remains central to cholera management.

Keywords: *Vibrio cholerae*; Cholera; Acute Diarrhoea; antimicrobial susceptibility; Ogawa; South Gujarat.

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Introduction

Acute diarrhoeal diseases remain an important cause of morbidity and mortality, particularly in settings where access to safe water, sanitation and timely healthcare may be limited. *Vibrio cholerae*, a Gram-negative bacterium, causes cholera, which can present with profuse watery diarrhoea and rapidly progressive dehydration. Without timely fluid replacement, severe disease may be fatal. Cholera continues to pose a global public health challenge, with outbreaks requiring coordinated surveillance, laboratory confirmation and public health responses. [1,2] India has experienced recurrent cholera outbreaks, with geographical and temporal variation in their occurrence. A systematic review by Muzembo et al. documented outbreaks across India during 2011–2020 and highlighted the importance of regional epidemiological surveillance. [3] Seasonal clustering, including during monsoon periods, makes local monitoring particularly relevant in vulnerable settings. Antimicrobial therapy is an adjunct to rehydration and may be indicated in selected patients with moderate or severe cholera. However, antimicrobial resistance among *V.*

cholerae isolates can vary across locations and over time. Studies from Gujarat and other parts of India have reported differences in susceptibility profiles, reinforcing the need for locally generated data rather than assuming that findings from other regions apply to every hospital. [4,5]

The Global Task Force on Cholera Control emphasises coordinated surveillance, preparedness and improved access to safe water, sanitation and healthcare as essential components of cholera control. [2] Laboratory identification and antimicrobial susceptibility information can contribute to outbreak investigation and guide treatment decisions when antibiotics are clinically indicated. Standard microbiological procedures and appropriate interpretation of susceptibility results are therefore important components of laboratory surveillance. [6-8] Published Indian studies have described variation in serotypes and antimicrobial susceptibility. Investigations from South India and Mumbai have documented the importance of serotyping and monitoring changing antibiogram patterns. [9,10] Studies from East Delhi and broader reviews of Indian data have also highlighted temporal and regional variation in antimicrobial resistance. [11,12] More recent genomic investigation of outbreak-associated *V. cholerae* in Gujarat has further demonstrated the value of examining resistance determinants and mobile genetic elements alongside conventional epidemiological surveillance. [13] Other investigations from North India and Ahmedabad have contributed to understanding the molecular characteristics and serotype distribution of circulating isolates. [14,15]

Despite these reports, contemporary hospital-based data remain important because local serotype distribution and susceptibility patterns may differ. Information from South Gujarat can help describe the isolates encountered at an individual tertiary care centre and support regional surveillance.

Aim & Objectives: The aim was to estimate the proportion of *Vibrio cholerae* from Acute Diarrheal cases and to assess their Antimicrobial Susceptibility Pattern.

The objectives were to:

1. To isolate *Vibrio cholerae* from stool samples of patients with acute diarrhea.
2. To determine the antimicrobial susceptibility pattern of *V. cholerae* isolates.
3. To determine serotype distribution.

Materials & Methods

Study Design and Setting: This retrospective observational study used secondary laboratory data from the Department of Microbiology, GMERS Medical College and Hospital, Valsad, Gujarat, India. The study period extended from 1 January 2025 to 31 December 2025.

Study Population and Sample Selection: The study population comprised patients with acute diarrhoeal illness whose stool samples were submitted to the microbiology laboratory for culture and sensitivity testing. All eligible samples received during the study period were considered for inclusion.

Stool samples were collected in clean, wide-mouthed, labelled containers without preservatives. Unlabelled, mislabelled or leaking containers were excluded. A total of 113 stool samples were included in the analysis.

Microbiological Examination: Microscopic examination included saline and iodine wet mounts and hanging-drop preparation. Samples were cultured on alkaline peptone water (APW) for enrichment, along with MacConkey agar, nutrient agar and thiosulfate citrate bile salts sucrose (TCBS) agar. Cultures were processed using standard laboratory procedures. The microbiological approach was consistent with established medical microbiology methods. [6]

Identification of presumptive isolates was based on colony morphology, Gram staining, oxidase testing and biochemical reactions. Confirmed isolates were tested using specific antisera for *V. cholerae* O1 and O139. O1 isolates were further differentiated into Ogawa, Inaba and Hikojima serotypes using specific antisera.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method. The study records identified the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) as the interpretive references. [7,8] The antimicrobial agents included in the reported results were tetracycline, doxycycline, trimethoprim–sulfamethoxazole, ampicillin and chloramphenicol.

Quality Control: Quality control of culture media, reagents, biochemical tests, serological reagents, and antimicrobial susceptibility testing (AST) was performed using appropriate quality assurance procedures. Each batch of culture media was assessed for sterility, growth-supporting ability, selectivity, and differential characteristics using appropriate reference strains.

Biochemical tests and reagents were checked for functionality, pH, storage conditions, and expiry. AST quality control was performed using *Escherichia coli* ATCC 25922 as the reference strain, and results were accepted only when quality-control values fell within the acceptable ranges specified by the applicable CLSI guidelines.

Study variables and outcome measures: Variables included the proportion of samples positive for *V. cholerae*, age group, sex, location of admission (intensive care unit or general ward), month of isolation, serotype distribution and antimicrobial susceptibility pattern.

The principal outcome measures were the proportion of *V. cholerae*-positive samples and the distribution of isolates by demographic characteristics, month, serotype and antimicrobial susceptibility.

Statistical Analysis: Data were entered into Microsoft Excel and WHONET and was analysed descriptively and expressed as frequencies, percentages and proportions. No inferential statistical analysis or correlation testing was performed. The analysis was intended to describe the findings within the study population rather than establish causal relationships or statistically significant associations. Missing information was treated as unavailable rather than as a negative finding.

Ethical Considerations: Ethical approval was obtained from the Institutional Human Ethics Committee, GMERS Medical College and Hospital, Valsad. As this was a retrospective review of existing laboratory records without direct patient contact, informed consent was waived. Patient confidentiality was maintained, and personal identifiers were not included in the analysis.

Results

Proportion of *Vibrio cholerae* Isolation: A total of 113 stool samples from patients with acute diarrhoeal illness were processed during the study period. *V. cholerae* was isolated from 13 samples, giving a positivity rate of 11.5%. Figure 1

Serotype Distribution: Of the 13 isolates, 12 (92.3%) were identified as Ogawa and 1 (7.7%) as Inaba. Ogawa was therefore the predominant serotype in the study population. No Hikojima isolate was reported. Figure 1.

Distribution by Admission Location: 9 cases (69.2%) were recorded among patients admitted to the intensive care unit, while 4 (30.8%) were from general wards. This describes the location of admission among identified cases. Figure 2.

Monthly Distribution: In our study, isolation was distributed across 4 months: August 5 isolates; September 3; July 3; and October 2. August had the highest number of isolates. These findings describe the monthly distribution among the 13 identified cases and do not establish a statistically tested seasonal association. Figure 2.

Demographic Distribution: 7 patients (53.8%) were male and 6 (46.2%) were female. The most represented age group was 25–34 years, accounting for 5 cases (38.5%). Figure 3.

Antimicrobial Susceptibility Pattern: The results indicated 100% susceptibility to each of the 5 tested antimicrobial agents: tetracycline, doxycycline, trimethoprim–sulfamethoxazole, ampicillin and chloramphenicol.

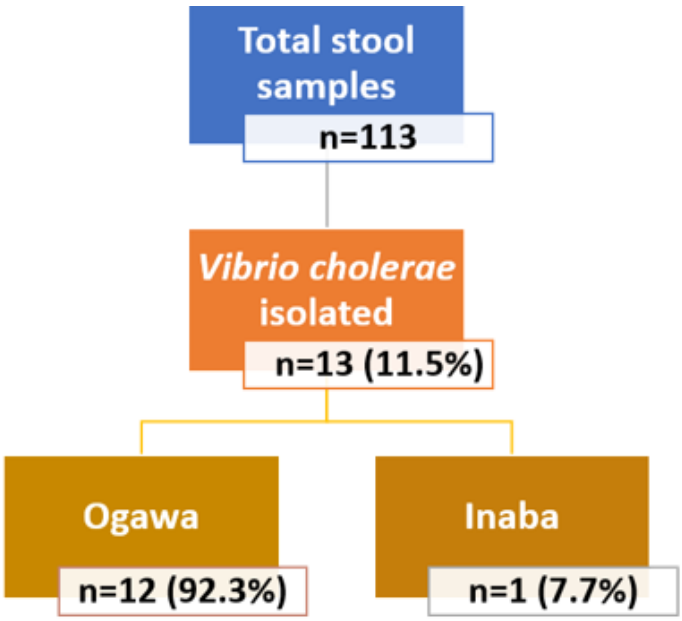


Figure 1: Distribution of serotypes of isolated *V. cholerae*

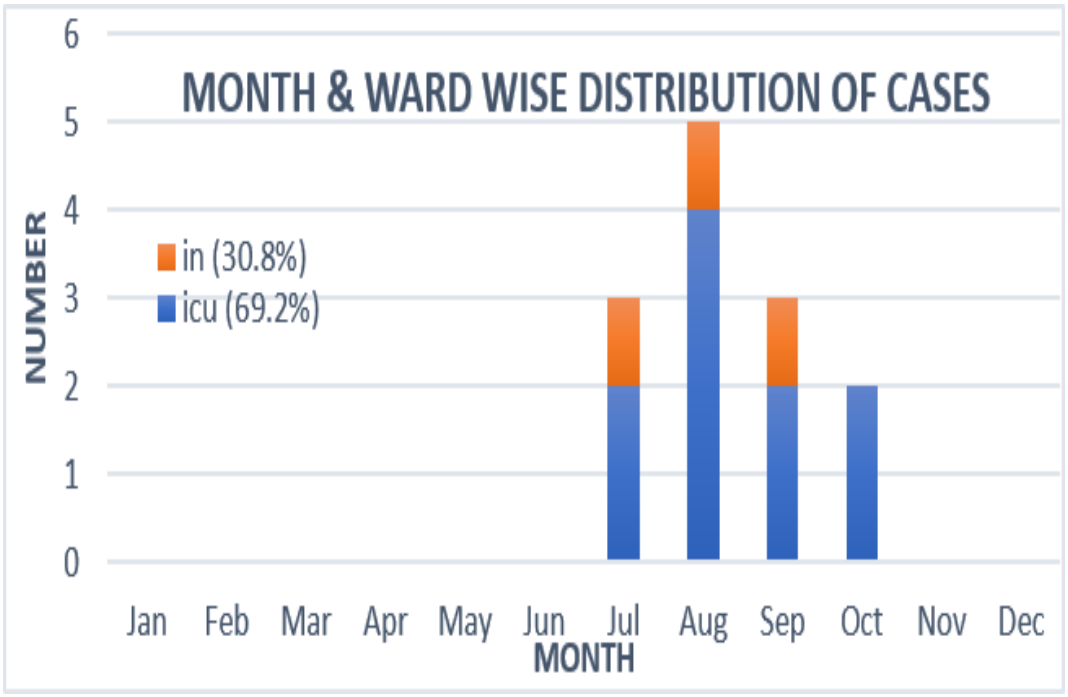


Figure 2: Month & ward wise distribution of cases

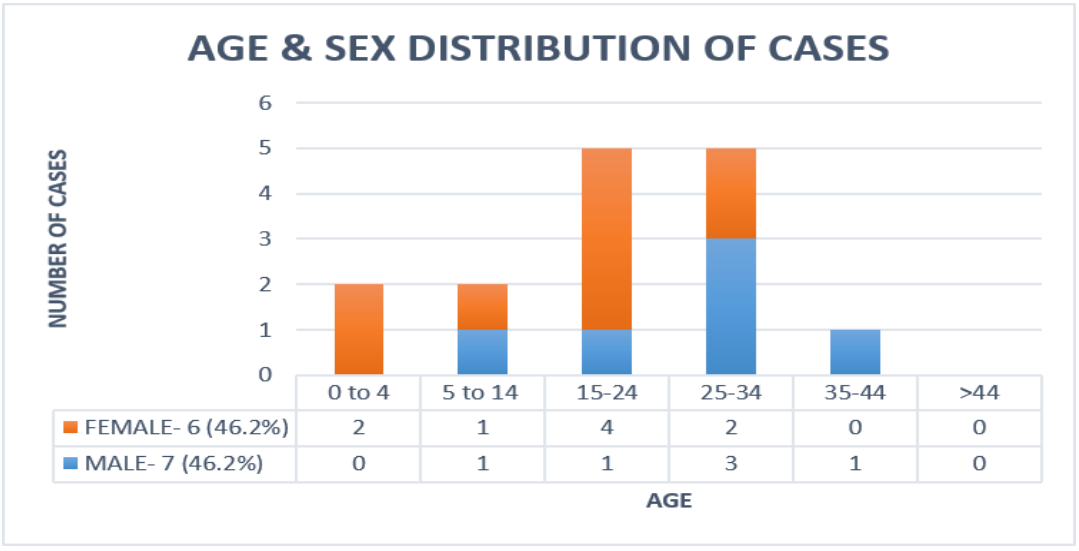


Figure 3: Age & sex distribution of cases

Discussion

This study assessed the isolation, serotype distribution and reported antimicrobial susceptibility of *V. cholerae* among stool samples submitted from patients with acute diarrhoeal illness at a tertiary care hospital in South Gujarat.

The principal findings were a positivity rate of 11.5%, predominance of the Ogawa serotype, clustering of isolates during July–October and reported susceptibility to the 5 tested antimicrobial agents.

Proportion of Isolation and Epidemiological Relevance: The isolation of *V. cholerae* from 13 of 113 samples demonstrates that cholera was present among the acute diarrhoeal cases investigated during the study period. The finding is relevant to local surveillance, but it should not be interpreted as the prevalence of cholera in the general population. The denominator comprised samples submitted to a single hospital laboratory, and the likelihood of testing may have depended on clinical suspicion and referral patterns. Muzembo et al. described recurrent cholera outbreaks and regional variation across India, supporting the importance of local data for interpreting the epidemiology of cholera. [3] The WHO situation report and the Global Task Force on Cholera Control (GTFCC) roadmap similarly underscore the importance of continued surveillance, early detection and coordinated outbreak response. [1,2]

Serotype Distribution and Comparison with Previous Studies: Ogawa accounted for 92.3% of isolates in the present study, while Inaba accounted for 7.7%. This indicates a marked predominance of Ogawa among the isolates identified at this centre during the study period. Serotype distribution, however, can change between outbreaks and geographical locations; the present findings should not be generalised beyond the study setting.

Barve et al. described phenotypic characteristics and antimicrobial susceptibility among *V. cholerae* isolates from a tertiary care hospital in Vadodara, Gujarat, providing a relevant regional comparison. [4] Patil et al. also examined serotyping and antibiogram patterns at a tertiary care centre in South India. [9] Torane et al. reported phenotypic and antibiogram patterns among isolates from Mumbai over an extended period, illustrating the value of examining changes over time. [10] Studies by Kingston et al. and Leuva et al. add evidence concerning molecular characteristics and serotype distribution in North India and Ahmedabad, respectively. [14,15] Differences between these studies and the present findings may reflect variations in location, outbreak period, study design and the number of isolates examined.

Monthly Distribution and Seasonal Context: The largest number of isolates was recorded in August, with additional cases in July, September and October. This distribution suggests clustering during the monsoon and post-monsoon period in the study setting.

The recurring and geographically variable nature of cholera outbreaks described in the Indian systematic review supports the importance of continuing month-wise local surveillance. [3] WHO and GTFCC guidance also emphasises preparedness and surveillance to support timely public health responses. [1,2]

Demographic and Clinical Distribution: Male patients accounted for 53.8% of cases, while female patients accounted for 46.2%. The 25–34-year age group represented the largest proportion of confirmed cases. The predominance of ICU admissions among the identified cases highlights that many isolates were associated with patients managed in an intensive care setting, but admission location alone cannot establish disease severity, complications or treatment outcomes.

Antimicrobial Susceptibility and Comparison With Published Evidence: The study reported 100% susceptibility to tetracycline, doxycycline, trimethoprim–sulfamethoxazole, ampicillin and chloramphenicol. The results should not be taken to establish that these antibiotics will be effective in all patients or in future outbreaks.

Earlier studies have reported variation in antimicrobial susceptibility among *V. cholerae* isolates. Uppal et al. investigated susceptibility among isolates from a tertiary care medical centre in New Delhi, while Shukla Das et al. examined trends in antibiotic resistance in East Delhi. [5,11] Chatterjee et al., in a scoping review of existing Indian data, also highlighted the need to map cholera outbreaks alongside antimicrobial resistance information. [12] Patil et al. and Torane et al. provide further hospital-based comparisons of antibiogram patterns. [9.10] These studies reinforce that susceptibility results should be interpreted according to the location, period, isolate population and antimicrobial agents tested rather than extrapolated without qualification.

The present study reported susceptibility findings differ from the resistance patterns described in some earlier investigations. This difference may reflect temporal or geographical variation, differences in the antimicrobial panels and testing methods, or the small number of isolates. It cannot be attributed to any single factor from the available data.

CLSI and EUCAST guidelines were used for interpretation in our study. [7,8]

Recent genomic investigation of outbreak-associated *V. cholerae* in Gujarat by Bhure et al. examined antimicrobial resistance genes, virulence determinants and mobile genetic elements. [13]

Such work illustrates the value of complementing conventional phenotypic surveillance with molecular investigation where resources and study objectives permit. Our study did not include molecular testing and therefore cannot comment on the genetic basis of the reported susceptibility pattern.

Clinical and Public Health Implications: The findings support the continued role of routine laboratory identification and serotyping in detecting cholera cases and describing the strains circulating locally. Susceptibility data may contribute to treatment decisions when antimicrobial therapy is indicated, provided the results are interpreted using appropriate standards and alongside current clinical guidance. Rehydration remains the cornerstone of cholera management. Antibiotics are adjunctive and should be used according to clinical indications and applicable treatment guidance, rather than on the basis of a small single-centre antibiogram alone. 2] At the public health level, local surveillance can support outbreak recognition, serotype monitoring, antimicrobial stewardship and preparedness. These findings can be integrated with broader epidemiological information and, where available, environmental and genomic surveillance. [1-3,13]

Strengths and Limitations: A strength of this study is that it provides contemporary, single-centre laboratory data from South Gujarat, including serotype distribution and reported susceptibility to 5 antimicrobial agents. The inclusion of all eligible samples received during the study period reduced the need for selective sampling among the available laboratory records.

Several limitations should be acknowledged. First, the retrospective design depended on the completeness and accuracy of existing records. Second, this was a single-centre study with only 13 positive isolates, limiting generalisability and the precision of percentage estimates.

Third, clinical details, disease severity, treatment received, complications and patient outcomes were not assessed. Fourth, the antimicrobial panel was limited. Finally, our study did not include molecular characterisation, environmental sampling or inferential statistical analysis. The observed monthly distribution therefore cannot establish seasonality or causation.

Conclusion

In this retrospective study at a tertiary care hospital in South Gujarat, *V. cholerae* was isolated from 13 of 113 stool samples (11.5%). Ogawa was the predominant serotype, accounting for 92.3% of isolates. Cases were clustered between July and October, with the highest number recorded in August. Our study reported 100% susceptibility to tetracycline, doxycycline, trimethoprim–sulfamethoxazole, ampicillin and chloramphenicol.

These findings provide a local snapshot of cholera-associated laboratory isolates and reinforce the importance of continued surveillance. Prompt rehydration, appropriate laboratory confirmation, judicious use of antimicrobials and coordinated public health surveillance remain essential components of cholera control.

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