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RESEARCH ARTICLE

SAFEGUARDING PUBLIC HEALTH FROM MELA-ASSOCIATED CHOLERA OUTBREAKS: EVIDENCE FROM A JHALAWAR DISTRICT STUDY

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ABSTRACT

Aims and introduction: To investigate a recent outbreak of *Vibrio cholerae* during the Mela and inform evidence-based interventions and enhance preparedness efforts. Out of the various infectious diseases, Vibrio cholerae causes a severe diarrheal disease with potentially fatal consequences if left untreated. The emergence and spread of such outbreaks continue to pose a formidable challenge in densely populated areas and during mass gatherings such as religious festivals and pilgrimages. Through this study, we endeavor to contribute valuable insights into the epidemiology of cholera and advance our ability to anticipate, detect, and respond to such outbreaks. **Materials and method:** The study included 253 stool samples collected, transported, and processed according to protocol. The isolates obtained were further subjected to AST and antisera testing. Moreover, water samples from 35 water sources were also analyzed for the presence of pathogenic organisms. **Results:** Out of the total 253 stool samples received 227 were *V.cholerae*. Among patients, the most common age group was 16 to 30 years (84). Males were more infected (121). **Conclusion:** This study will provide valuable insights into the characteristics of the cholera outbreak at pilgrimages.

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INTRODUCTION

In recent decades, the global burden of infectious diseases has remained a persistent challenge, with outbreaks of various pathogens posing significant threats to public health. Among these, *Vibrio cholerae* stands out as a notorious causative agent of cholera, a severe diarrheal disease with potentially fatal consequences if left untreated. The emergence and spread of *V. cholerae* outbreaks continue to pose a formidable challenge, articularly in densely populated areas and during mass gatherings such as religious festivals and pilgrimages.[1] One such event, the Chandrabhaga Mela, held annually along the banks of the Chandrabhaga River in India, serves as a microcosm of the potential risks associated with large-scale gatherings.[2] The convergence of pilgrims, local residents, and vendors in a confined space provides an ideal environment for the transmission and amplification of infectious agents, including *V. cholerae*. Previous studies have documented the occurrence of cholera outbreaks during similar mass gatherings, underscoring the need for vigilant surveillance and proactive measures to mitigate the risk of disease transmission.[3] Drawing upon a wealth of literature documenting the genetic diversity, virulence mechanisms, and antimicrobial resistance patterns of *V. cholerae* strains, this investigation will employ the conventional culture, agglutination assays and antimicrobial sensitivity tools to characterize the isolates associated with the Chandrabhaga Mela outbreak. By integrating genomic data with epidemiological information and environmental sampling results, we aim to delineate the pathways of transmission and identify potential sources of contamination, thereby informing strategies for outbreak control and containment. Through this comprehensive analysis, we endeavor to contribute valuable insights into the molecular epidemiology of cholera and advance our ability to anticipate, detect, and respond to outbreaks in diverse settings. By elucidating the factors driving the emergence and spread of *V. cholerae* during mass gatherings.[4,5]

Aim

Aims: This research aims to investigate a recent outbreak of *Vibrio cholerae* during the Chandrabhaga Mela, employing a multidisciplinary approach to elucidate the underlying epidemiological, microbiological, and environmental factors contributing to the emergence and spread of the pathogen. By leveraging insights from previous studies on cholera epidemiology, microbiological analysis and environmental surveillance, this study seeks to enhance our understanding of the dynamics of cholera transmission in the context of mass gatherings and inform targeted interventions to prevent future outbreaks.

Therefore study aims to inform evidence-based interventions and enhance preparedness efforts aimed at safeguarding public health.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, Jhalawar Medical College, Jhalawar. It included 253 stools samples of patients with cholera-like disease during 28th November to 2nd December 2023. This study is a descriptive type cross-sectional study that focussed on cholera outbreak isolated from diarrhoeal stool specimens obtained from patients who attended Chandrabhaga mela. The specimens were obtained from patients presenting with passage of three or more watery stools with or without vomiting during cholera outbreaks. As soon as the specimens are received in the lab, hanging drop preparation was performed to observe for motility and were inoculated on routine culture media (BA and MacConkey agar) and a selective media (TCBS). Culture were then subjected to aerobic incubation at 37 C for 18-24hrs. The samples received were also enriched in alkaline peptone water and incubated at 37 degrees C for 6hrs.[6,7]

Identification of *Vibrio cholerae* isolates: The isolates which were obtained from routine cultures were inoculated in alkaline peptone water for motility testing [8] and were sub-cultured in thiosulphate-citrate-bile salts sucrose (TCBS) agar and nutrient agar. The plates were then incubated at 37 °C for 18–24 h in aerobic conditions. After 24 h of incubation, bacterial cultures giving growth of pure green and yellow colonies were presumed to be that of *V. cholerae* and subjected to Gram staining, and biochemical and serological identification testing.(Fig 1)

Gram staining and biochemical identification: Thin smear was prepared from the colonies grown on TCBS media, air-dried and heat-fixed. The smear was then stained using Gram staining technique.*V.cholerae*was identified using routine biochemical reactions like oxidase test, triple sugar iron test ,sulphurindole motility test, citrate utilisation and urease test.

Serological identification: The serologic identification was conducted by the slide agglutination technique with polyvalent anti-sera for *V. cholerae* O1 and O139, and monovalents for serotypes Inaba and Ogawa (Denka Seiken Co. Ltd., Japan) according to the manufacturer's instructions and previously published protocols.[10,11,12,13] The confirmed *V. cholerae* isolates were further sub-cultured on nutrient agar and incubated overnight at 37 °C for 18–24 h aerobically. A pure colony was picked aseptically and mixed in a drop of sterile normal saline on a glass slide, making a milky suspension. A drop of antiserum was added onto the drop and mixed. Agglutination reaction was observed. Positive agglutination confirmed serotype and subtype of isolates. In this test, *V. cholerae* O1 biotype El Tor, subtype Ogawa, were used as positive controls, whilst *Escherichia coli* strain ATCC 25922 was used as a negative control.

Antimicrobial susceptibility testing: Susceptibility to antimicrobial agents was assayed by the Kirby Bauer disc diffusion method .[10 11] *Escherichia coli* standard strain, ATCC 25922, was used as internal quality control. The procedure for antimicrobial susceptibility testing was performed for ciprofloxacin, tetracycline, ampicillin, erythromycin, chloramphenicol, nalidixic acid and trimethoprim/sulphamethoxazoleDoxycycline as per the Clinical Laboratory Standards Institute (CLSI) guidelines.[9]

Water samples: Even though *V. cholerae* and other vibrios are inhabitants of water bodies, their numbers remain low for testing purpose. Fig. 2outlines the isolation and identification of *V. cholerae* from water samples. To increase the probability of getting adequate numbers of bacterial cells, large volumes of water sample were tested. Water samples were collected from 35 water sources from in and around the mela premises were collected between 30th November to 5 December and transported to the Department of Bacteriology ,JMC Jhalawar for microbiological analysis. The samples were received in sterile clean sterile containers and were processed immediately (Fig.1).Samples were obtained fromChandrabhaga river, public wells,tube wells, taps, handpumps ,social service stalls of drinking water ,R.O. water from various drinking water suppliers and housewells in the vicinity.

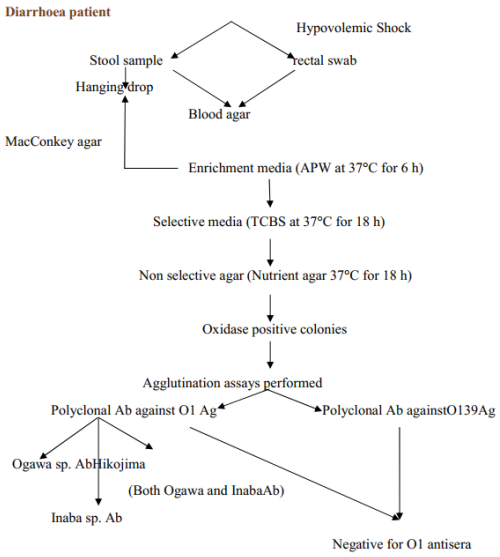


Figure 1. Flowchart indicating isolation and identification of V. cholerae from stool specimens

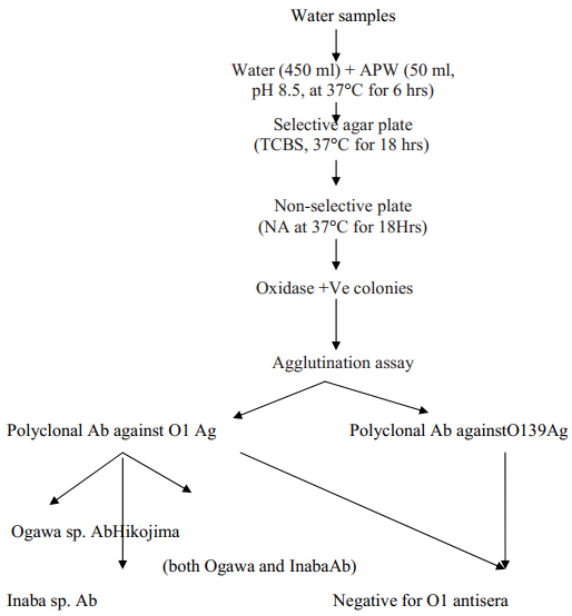


Figure 2. Flowchart for of isolation and identification of *V. cholerae* from environmental samples

RESULTS

Out of the total 253 stool samples received from 28 November to 2nd December 2023 at Department of Microbiology , JMC Jhalawar , 227 isolates were confirmed as *V.cholerae*. All the patients had similar history of visiting the Chandrabhaga mela and presented at the hospital with cholera-like illness. Majority of patients belong to 16 to 30 yrs of age group (84). (FIGURE 3).

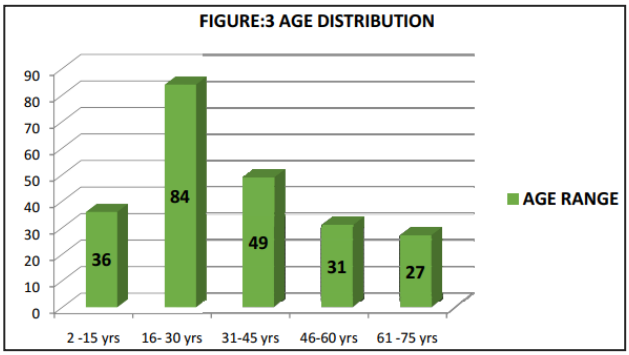
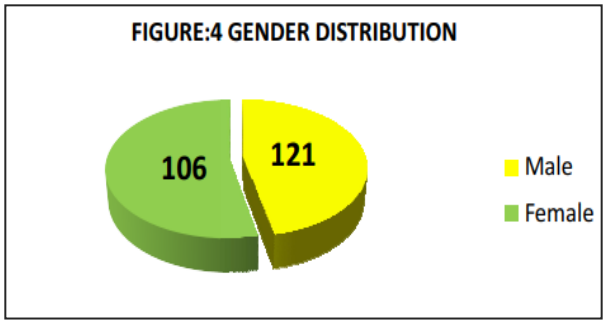
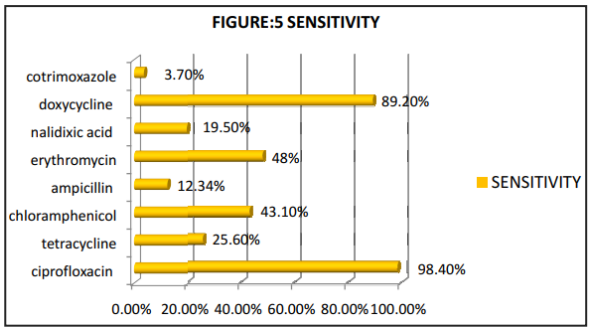


Table 1. Distribution of cases according to locality

S.no	Urban	Rural	Total
Male	54(23.8%)	67(29.5%)	121(53.3%)
Female	41(18.1%)	65(28.6%)	106(46.7%)
Total	95(41.9%)	132(58.1%)	





In terms of gender males (121) were more infected than females (106).(FIGURE 4).95 cases belong to urban population ,rest (132) were from rural areas.(Table 1). All the 227 isolates were identified as *Inaba* serotype in agglutination studies performed with Denka Seiken Co. Ltd., Japan kits. The positive isolates for *V. cholerae* were screened for their susceptibility to various antibiotics commonly used to manage cholera cases. Antimicrobial sensitivity pattern was performed with the available antibiotics .Of which Ciprofloxacin (98.4%) and doxycycline (89.2%) had the highest effectiveness, followed by erythromycin (48%) and chloramphenicol (43.1%).

Lower susceptibility was observed for tetracycline (25.6%), ampicillin (12.34%), nalidixic acid (19.5%), and trimethoprim/sulfamethoxazole(cotrimoxazole)(3.7%).(FIG.5)
In our study we also tried to figure out the association of the severity of disease with certain variables like age and gender. We found that there is no statistically significant association between the Age (Chi square =8.524 p-value = 0.56) and Gender (Chi square =1.23 p-value = 0.59) and severity of cholera patients in our study(Table no 2). This means that any observed differences in cholera severity across different age groups or genders could be due to random variation rather than a true underlying effect or this may be attributed to small sample size. These findings suggest that interventions to reduce cholera severity should not necessarily prioritize based on age or gender but should focus on other factors such as access to clean water, sanitation, and timely medical treatment. This can guide public health strategies and interventions by suggesting that age and gender alone may not be critical factors in determining the severity of cholera. The non-significant p-value indicates that you might need to explore other variables that could be influencing cholera severity, such as:

- Socioeconomic Status: Access to clean water and sanitation.
- Health Infrastructure: Availability and quality of medical care.
- Pre-existing Conditions: Other health conditions that might exacerbate cholera severity.

Understanding that age and gender do not significantly affect cholera severity in your study population can help focus resources on more impactful factors. For example, improving water quality and sanitation or ensuring timely medical treatment might be more effective interventions.

Table 2. Severity of cholera in relation to different variables

Dehydration	Mild	Moderate	Severe
No. of cases	122(53.7%)	84(37%)	21(9.3%)
Age			
<2 yrs			
2-15yrs	0	0	0
16-30yrs	18(7.9%)	14(6.16%)	4(1.7%)
31-45yrs	52(22.9%)	23(10.1%)	9(3.9%)
46 -60yrs	21(9.3%)	19(8.4%)	4(1.8%)
61-75yrs	17(7.5%)	11(4.8%)	3(1.3%)
	14(6.2%)	17(7.5%)	1(0.5%)
Gender			
Male	66(29%)	42(18.5%)	13(5.7%)
Female	56(24.7%)	42(18.5%)	8(3.5%)

WATER SAMPLE ANALYSIS :Out of the 35 water samples received from various sites R.O water collected from 11 different water suppliers and majority of other sources were found to be sterile and safe for drinking purposes except for the water samples from the Chandrabhaga river , a local well ,a tubewell and a handpump were showed the presence of microorganisms (Table no.3) and were promptly reported to mela authorities for needful action.

Study limitations: The study has several limitations, including a relatively small sample size, inability to take into consideration other variables that could be influencing cholera severity.

Table 3. Water sample analysis report of the samples obtained from various sources

S.no	Sample	Microbiological analysis
1	Chandrabhaga river	Coliforms
2	Local well	Coliforms and *Vibrio cholerae 01 serovar inaba*
3	Tubewell	Coliforms and *Vibrio cholerae 01serovar inaba*
4	Handpump	Coliforms and *Vibrio cholerae 01serovar inaba*

CONCLUSION

The cholera outbreak at the Chandrabhagamela underscores the vulnerability of mass gatherings in India to Cholera. By acknowledging the public health burden these outbreaks cause and implementing effective preparedness measures, authorities can significantly reduce the risk of future outbreaks and protect the health of pilgrims. Further research is warranted to refine preventative strategies and ensure the safety of public gatherings. Overall, the results of this study provide valuable insights into the characteristics of the cholera outbreak at Chandrabhagamela. These findings can inform public health interventions, such as:

- Vaccinating populations at risk with Inaba-specific cholera vaccines.
- Promoting best practices for hygiene and sanitation at future melas.
- Emphasizing the importance of early diagnosis and appropriate antibiotic treatment based on local susceptibility patterns.

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Author contributions

Conceptualization, Methodology, Validation, Investigation, Writing– Original Draft Preparation, Writing – Review & Editing was done by Dr Sadhana Joshi and Dr Ruby Naz Data Curation, Formal Analysis statsitics was done by Sadhana Joshi. Visualization, Resources generation and overall Supervision was performed by Dr Ruby Naz.

Conflicts of interest: This study has no conflict of interest.

Ethical consideration

Confidentiality and privacy were strictly adhered to and no names of individuals were recorded or made known in the collection or reporting of information. The study was granted ethical clearance DHR(GOI) Permanent registration No.EC/NEW/INST/2022/RJ/0134 Dated 30august 2022 and ethical approval to conduct the study was sought from the Institutional Ethical committee Jhalawar medical college, Jhalawar

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