Case Series

Vibrio Cholerae O1 classical biotype infections among travellers from Kerala A Case Series

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Sri Lankan Journal of Infectious Diseases 2026 Vol.16(1):E103:1-7

DOI: https://doi.org/10.4038/sljid.v16i1.8861

Abstract

Cholera is an acute diarrhoeal disease caused by *Vibrio cholerae* and is transmitted by contaminated food and water. This case series examines three patients from Kerala, India, who travelled together to Andhra Pradesh, India. On their return, they developed severe watery diarrhoea that required hospital admission. All three developed acute kidney injury and were managed with antibiotics and supportive measures. *V. cholerae* O1 Classical biotype, Ogawa serotype was isolated from two of the three cases. All patients and caregivers were informed of the importance of hand hygiene and contact precautions. The contacts were started on chemoprophylaxis with oral doxycycline. This case series discusses the clinical presentation, diagnosis, and management of these patients.

Keywords: *Vibrio cholerae, Diarrhoea, India, Serogroup, Antibacterial agents*

Introduction

Cholera is an acute diarrhoeal disease caused by the motile, Gram-negative bacteria *Vibrio cholerae.* The bacterium is commonly found in marine environments and is transmitted among human hosts by contaminated food and water.[1] *V. cholerae* strains are classified into over 200 serogroups based on the structure of the outer membrane O antigen. O1 and O139 are associated with epidemics of acute watery diarrhea.[2] O1 strains fall into two biotypes (Classical and El Tor) and two serotypes (Ogawa and Inaba). Cholera has caused seven pandemics so far. The seventh pandemic, which is still ongoing, is caused by the El Tor biotype, while the earlier pandemics were caused by the Classical biotype.[2] The disease is endemic and occurs with marked seasonal dynamics, being most prevalent during the hot, humid, and rainy seasons.[3]

Researchers estimate that annually, cholera affects 1.3 to 4.0 million people and causes 21,000 to 143,000 deaths worldwide.[4] Cholera outbreaks are frequent in India, with surveillance data revealing a steady increase in reported cases throughout the country. Despite this, cholera remains grossly under reported and under recognized as a health issue in India.[4,5] Between 2011 and 2020, there were 565

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Received 20 August 2025 and revised version accepted 27 November 2025. Published on 16.1.26

reported outbreaks, resulting in approximately 45,759 cholera cases and 263 (0.6%) deaths.[6] Most infected individuals exhibit mild symptoms and can be effectively treated with oral rehydration solution. Severe cases require immediate treatment with intravenous fluids and antibiotics. Ensuring access to safe water, basic sanitation, and good hygiene practices are essential for preventing cholera. Oral cholera vaccines should be used alongside improvements in water and sanitation to manage outbreaks and prevent the disease in high-risk areas. In 2017, a global strategy called "Ending Cholera: A Global Roadmap to 2030" was launched, aiming to reduce cholera deaths by 90%.[7]

The present case series examines a group of eleven individuals aged 50-70 years who travelled from Kerala, India, to Nellore, Andhra Pradesh, India, for a duration of 10 days. During their stay, they consumed home cooked meals (rava upma, curd rice), ice cream from a local shop, and did not consume untreated water from any public source. On their return, three of the eleven individuals developed severe watery diarrhoea that required hospital admission. This case series discusses the clinical presentation, diagnosis and management of these patients.

Case 1

A 65-year-old male patient presented to Valluvanad Hospital, Ottapalam, Kerala on 1st June 2024, with complaints of multiple episodes of watery loose stools and vomiting. There was no associated fever or chills. He was a known hypertensive on treatment. His pulse rate was 100 beats/minute, blood pressure was 80/50 mmHg and respiratory rate was 18 breaths/minute. The patient's abdomen was soft with mild generalised tenderness. Examination of his central nervous and respiratory systems were clinically uneventful. His blood samples were sent for complete blood count and kidney function tests. His stool samples were sent for hanging drop examination and culture and sensitivity. His blood examination revealed an elevated leucocyte count (14.81 x 10^3/μL) and serum creatinine (5.3 mg/dL). Fluid resuscitation was done in view of severe dehydration and hypotension. He was transferred to the intensive care unit (ICU).

A stool sample was received in the microbiology laboratory for culture and hanging drop examination. On macroscopic examination, the stool resembled rice water. The hanging drop examination revealed actively motile bacilli. The faecal sample was enriched in alkaline peptone water for 6 hours, followed by inoculation onto blood agar, MacConkey agar, and thiosulfate citrate bile salts sucrose (TCBS) agar and incubated at 37 °C for 18-24 hours. Faecal culture tested positive for *Vibrio cholerae*, which was identified based on colony morphology on culture media (yellow colonies on TCBS agar, non-lactose fermenting colonies on MacConkey agar, haemolytic colonies on blood agar) and confirmed by standard biochemical tests (oxidase positive, string test positive and cholera red reaction positive). The isolate was found to be susceptible to ampicillin, azithromycin, ciprofloxacin, tetracycline, doxycycline and ceftriaxone, but resistant to cotrimoxazole by Kirby Bauer disc diffusion technique.

The patient was initially started empirically on ceftriaxone, metronidazole and levofloxacin, as well as probiotics, antiemetics, intravenous fluids, renoprotective drugs and electrolyte supplements. Once the definitive diagnosis of cholera was confirmed, therapy was switched to doxycycline. The treatment was given for 5 days. The patient's clinical condition improved, and he was discharged on 6th June 2024.

Case 2

A 55-year-old man presented to Ramdas Clinic and Nursing Home, Perinthalmanna, Kerala, with multiple episodes of watery stools and vomiting on 1st June 2024. There was no associated fever, chills, or abdominal pain. His pulse rate was 92 beats/minute, blood pressure was 110/80 mmHg and

respiratory rate was 22 breaths/minute. His abdomen was soft and non-tender, with no organomegaly. Examination of his central nervous and respiratory systems were clinically uneventful. His blood samples were sent for complete blood count and kidney function tests. His stool samples were sent for hanging drop examination and culture and sensitivity. The patient was given ofloxacin, doxycycline and metronidazole during his hospital stay. His serum creatinine was 4.4 mg/dL, and he was diagnosed with acute gastroenteritis with acute kidney injury.

A stool sample was received in the microbiology laboratory for culture and hanging drop examination. Macroscopic examination showed that the sample was clear and watery. The hanging drop examination revealed actively motile bacilli showing darting motility. While faecal culture is the gold standard for confirming cholera, facilities for culture were not available in this resource-limited setting. The diagnosis of probable cholera was therefore based on classical clinical features, including multiple episodes of profuse watery stools, supported by the demonstration of actively motile bacilli showing darting motility observed on the hanging drop preparation, which reinforced the clinical impression. No further laboratory investigations were carried out. A clinical diagnosis of cholera was made, and the patient was managed with doxycycline and other supportive measures. He was discharged on 7th June 2024 in a stable condition.

Case 3

A 51-year-old male developed watery diarrhoea on 1st June 2024. He was prescribed an oral antibiotic (cefixime) from a local clinic for 5 days. As his symptoms persisted, he presented to PK Das Institute of Medical Sciences, Ottappalam, Kerala on 5th June 2024.

On admission, he complained of fever with chills, nausea, and vomiting. He passed ~50 episodes of loose stools per day. The stool was watery with no mucus or blood. He was a known hypertensive on treatment. His vital signs were normal with pulse rate of 91 beats/minute, respiratory rate of 18 breaths/minute, and blood pressure of 140/110 mm Hg. The patient's abdomen was soft and non-tender and there was no organomegaly. Examination of his central nervous system and respiratory system were clinically uneventful. His blood samples were sent for kidney function tests. Stool samples were sent for hanging drop examination and culture and sensitivity. He was given intravenous azithromycin 1 gram stat and put on IV rehydration. The patient was diagnosed with acute gastroenteritis with acute kidney injury (serum creatinine 2.9 mg/dL, anuria) and was transferred to the ICU.

A stool sample was received by the microbiology laboratory for culture and hanging drop examination. On macroscopic examination, the sample appeared clear and watery (Figure 1). Hanging drop examination revealed actively motile bacilli showing darting motility. The faecal sample was enriched in alkaline peptone water for 6 hours, followed by inoculation on blood agar, MacConkey agar and TCBS agar, and incubated at 37 °C for 18-24 hours. His faecal culture was found to be positive for *V. cholerae* by standard biochemical tests and colony characteristics (Figure 2, 3). String test with 0.5% sodium deoxycholate was positive. Cholera red reaction[8] of the isolate was positive. Identification was confirmed by VITEK® 2 compact system (bioMérieux, Marcy-l'Étoile, France). Antibiotic susceptibility testing was performed by disc diffusion (antibiotic discs from HiMedia Laboratories Pvt. Ltd Mumbai, India) and automated method using VITEK® 2 compact system (bioMérieux, Marcy-l'Étoile, France), according to CLSI M45 (3rd edition, 2015).[9] The isolate was found to be susceptible to ampicillin, azithromycin, ciprofloxacin, tetracycline, doxycycline and resistant to cotrimoxazole (Figure 4 and Table 4).

During the hospital stay, the patient was treated with a single dose of intravenous azithromycin and other supportive medications. The patient's clinical condition improved and he was discharged on 6th June 2024 on oral doxycycline for two weeks.

Figure 1
Stool sample of case 3

Figure 2
Colonies of *V. cholerae* on TCBS agar

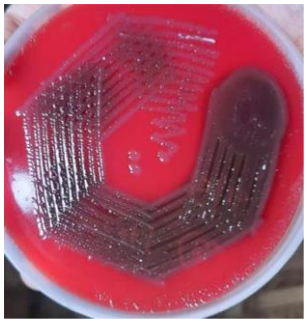

Figure 3
Colonies of *V. cholerae* on blood agar

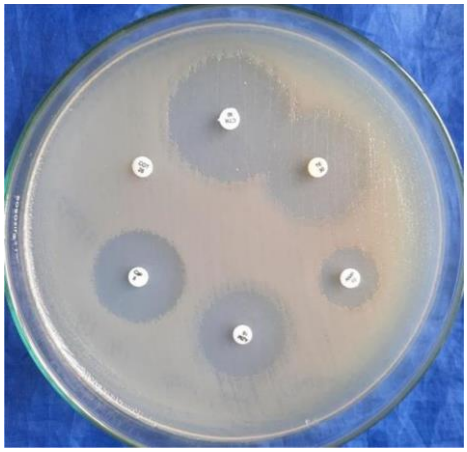

Figure 4 : Antibiotic susceptibility testing of *V. cholerae* on Mueller Hinton Agar.

Antibiotic	Observed Value	Zone diameter (mm) interpretive criteria by CLSI M45			Result
		S	I	R	
Ampicillin (10 g)	18mm	≥17	14–16	≤13	S
Ciprofloxacin (5µ g)	28mm	≥21	16–20	≤15	S
Tetracycline (30 µg)	32mm	≥15	12–14	≤11	S
Cotrimoxazole (1.25/23.75 µg)	6mm	≥16	11–15	≤10	R

Table 4: Antibiotic and their zone diameter values.
S - sensitive R – resistant I- intermediate resistant

The isolates from case 1 and case 3 were sent to the State Public Health and Clinical Laboratory, Thiruvananthapuram, Kerala for typing. Both isolates were found to be *V. cholerae* O1 serogroup, Classical biotype, Ogawa serotype.

Prognosis and outcome

Since cholera is a notifiable disease in India, the District Medical Officer, Palakkad, Kerala was informed of these cases. All patients and caregivers were given an explanation on the importance of hand hygiene and contact precautions. The contacts were started on chemoprophylaxis with oral doxycycline. Earlier studies have demonstrated the effectiveness of doxycycline in reducing transmission of *V cholerae* among household contacts of cholera patients. A study conducted in Calcutta by Gupta *et al*.[10] reported a significant decrease in secondary cholera cases among contacts who received doxycycline prophylaxis. All three patients were discharged and were asymptomatic during their follow-up visits.

Discussion

We describe 3 cases diagnosed with cholera among patients who travelled together to Nellore, Andhra Pradesh. All three patients presented with similar symptoms of profuse watery diarrhoea (rice water stools) and dehydration typical of cholera. All stool samples showed motile bacilli, but *V cholerae* was isolated only from two of the three cases. This was due to the limited laboratory capacities at the second facility.

A systematic review by Muzembo *et al* on cholera outbreaks in India from 2011 to 2020 also reported a majority of cases from southern and eastern India. There was an increase in cholera outbreaks in the monsoon season (June to September) similar to our study (June). Muzembo B A *et al.*[11] also reported that most diagnoses of cholera were based on clinical symptoms, and laboratory confirmed cases ranged from 4.7% to 71.4%. The Global Task Force on Cholera Control (GTFCC) recommends that all suspected cholera cases are tested using the Rapid Diagnostic Test (RDT) followed by culture or polymerase chain reaction (PCR) on RDT positive samples.[12] In the absence of RDTs, as in our study, the GTFCC recommends culture/PCR on all suspected samples.

Both isolates of *V. cholerae* were found to be of O1 serogroup, Classical biotype, Ogawa serotype. O1 and O139 have historically been associated with cholera epidemics.[13] Another study from India also reported a majority of O1 (83.4%) and 3.8% of O139 serogroups.[14]

The isolates in this study showed similar antibiotic susceptibility patterns, i.e., they were resistant to cotrimoxazole and susceptible to ampicillin, azithromycin, ciprofloxacin, tetracycline and doxycycline. A study by Chatterjee *et al* (2020) records a recent increase in resistance to cotrimoxazole in India. Resistance against ciprofloxacin, even though low previously, has also been showing a rising trend.[15]

The mainstay of treatment of cholera is early fluid and electrolyte replenishment using oral rehydration salts (ORS). Doxycycline, azithromycin, ciprofloxacin and tetracycline are effective antibiotics to treat cholera.[16,17] Administration of a single dose of azithromycin or doxycycline is effective in reducing diarrhoeal symptoms in less than 48 hours.[18]

The Advisory Committee on Immunization Practices (ACIP) recommends a single-dose oral cholera vaccine CVD 103-HgR (Vaxchora) for travellers aged 2–64 years visiting areas with active cholera transmission.[19]

Conclusion

These cases highlight the importance of early recognition, diagnosis and management of cholera. The ability to make accurate clinical judgments is vital for suspecting cholera in patients from non-endemic areas, and obtaining a thorough travel history is crucial.

Declaration

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this article.
Acknowledgements: The authors thank the staff and colleagues of PK DAS Institute of Medical Sciences for their support in the conduct of this study and preparation of the manuscript.
Funding source: No external funding was received.
Ethics Statement: The study was approved by the Institutional Ethics Committee of PK DAS Institute of Medical Sciences, Kerala, India. The approval number is IEC No: 20/104/24.
Author contributions:
Nivedita Mohan and Shafeedha Rashbi K –contributed equally to the study design, data acquisition, data analysis, and drafting of the manuscript.
Sathyajith Ramvihar-involved in the critical revision of the manuscript, provided technical support, and supervised the study.
Madasseri Deepti Sukumar, Arun S Menon, and Dhan raj Saboo -data acquisition.

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