

## Epidemiological Report

# Multidrug-Resistant *Vibrio cholerae* O1 was Responsible for a Cholera Outbreak in 2013 in Bagalkot, North Karnataka

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**SUMMARY:** Cholera is a major cause of illness in the developing world. During the monsoon season, small sporadic clusters of cholera cases are reported on an annual basis in Karnataka, India. During the monsoons of 2013, there was a cholera outbreak in Badami, a remote area of Bagalkot district in Karnataka. The multi-drug-resistant *Vibrio cholerae* O1 serotype Ogawa was found to be responsible for this outbreak. On 5 August 2013, a 30-year-old woman presented with severe dehydration and watery diarrhea at the Aganwadi Health Centre in Badami. A total of 49 suspected cholera cases were reported, with an attack rate of 3.5%. The *V. cholerae* isolates exhibited resistance to a wide range of drugs, including ampicillin, co-trimoxazole, nitrofurantoin, carbenicillin, and third generation cephalosporins, and showed reduced susceptibility to third generation fluoroquinolones. All of the cephalosporin-resistant *V. cholerae* strains produced extended-spectrum beta-lactamase. All *V. cholerae* O1 isolates harbored virulent genes (*ctxA*, *ctxB*, *tcpA* El Tor, *Tox S*, *VPI*, *ToxT*, *ToxR*, *ToxRS*, *ace*, *zot*, and *tcpP*) and were found to be genetically similar as determined by randomly amplified polymorphic DNA fingerprinting assay. To the best of our knowledge, this is the first report of a cholera outbreak in the district of Bagalkot. The resistance of *V. cholerae* to commonly used antimicrobial drugs is becoming a major public health concern in the region as clinicians are left with a limited choice of antibiotics for the treatment of cholera.

## INTRODUCTION

Cholera caused by toxigenic strains of *Vibrio cholerae* O1 or O139 continues to be a major cause of illness and death, particularly in developing countries. Treatment of these infections involves rehydration through the early administration of appropriate fluids, orally or intravenously. The World Health Organization (WHO) recommends antimicrobial drugs for severely dehydrated cholera patients because it significantly shortens the duration of diarrhea (1). In 2013, 47 countries reported a total of 129,064 cholera cases, with 2,102 deaths, resulting in a case-fatality rate of 1.63%. This represents a 47% decrease in the number of cases reported for 2012, is the second consecutive year in which the number of reported cholera cases declined. The number of reporting countries in 2013 was 47 compared with 48 in 2012 (1).

Sporadic small clusters of cholera cases occur on an almost annual basis in the Indian state of Karnataka, particularly during the monsoon period (unpublished

data). Serious cholera outbreaks have occurred in Karnataka in 2000, 2002, 2005, and 2010 (2–4). The incidence of cholera is relatively rare in the northern districts of the state, although it is common in nearby areas of the adjoining state of Maharashtra. However, in recent years, cholera has emerged in northern districts, with large outbreaks reported from various areas of the Belgaum and Bijapur districts (2–4). So far, there were no reports on cholera cases in the neighbouring Bagalkot district. According to the 2011 census, the population of the Bagalkot district is 1,889,752, with 6 talukas comprising 18 hoblies and 627 villages. Of the 6 talukas, 2 were categorized as “Very Backward” and 1 as “Most Backward.” In this communication, we report the occurrence of a cholera outbreak due to the multi-drug resistant *V. cholerae* O1 Ogawa serotype, in Somanakoppa village (approximate population of 1,400) of Badami, 1 of the 2 “Very Backward” talukas. To the best of our knowledge, this is the first report of a cholera outbreak in this district.

## METHODS

**Samples:** Stool samples were collected from patients with diarrhea, who were admitted to health centres and transported to Belgaum Institute of Medical Sciences (Belgaum), which is the nodal surveillance center for the region.

**Microbiological assays:** Samples were processed for enrichment and identification of bacterial enteric pathogens by plating on thiosulfate-citrate-bile salts-sucrose (TCBS) agar, MacConkey (MAC) agar, and Hektoen

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enteric agar. After 18 h of incubation at 37°C, yellow sucrose-fermenting colonies resembling *V. cholerae* on TCBS plates, and non-lactose-fermenting colonies on MAC plates were subjected to standard biochemical tests, including sugar fermentation in triple sugar iron agar, and oxidase production. Isolates were serotyped using polyvalent and monovalent antisera (Denka Seiken Co., Ltd., Tokyo, Japan) at the Belgaum Regional Medical Research Centre (RMRC).

Teams consisting of doctors and scientists from the State Surveillance Unit and RMRC visited the area to investigate the outbreak and initiate control measures. Information was collected by verbal autopsy of patients and relatives, and from examination of Aganwadi, Primary Health Centre (PHC), and Bagalkot Government Hospital records. The sites and sources of drinking water, usage patterns and defecation sites were examined. There were 2 bore wells in the area, of which 1 was in operation before and during the outbreak. This particular well was close to an open field where many of the villagers, including the index case, routinely defecated. This water source was temporarily closed and the other bore well was made operational from 12 August 2013. Water samples were collected from both water sources and sent to RMRC for determination of fecal contamination levels. Information, Education, and Communication (IEC) activities were initiated, and the supply of oral and intravenous rehydrants and drugs to the affected area was ensured. All water sources and storage tanks were subjected to chlorination.

At RMRC, a rapid H<sub>2</sub>S strip test (Hi-Media Laboratories, Mumbai, India) and Most Probable Number (MPN) method was used to determine contamination levels by coliform count, as described previously (5).

**Antibiotic sensitivity testing:** Susceptibility of the *V. cholerae* isolates to different antibiotics were tested at RMRC by disk diffusion, following standard guidelines (6) and using commercially available disks (Hi-Media, Mumbai, India). The disks used included ampicillin (AMP; 10 µg), carbenicillin (CAR; 100 µg), imipenem (IMP; 30 µg), amoxicillin-clavulanic acid (AMC; 20/10 µg), cefuroxime (CXM; 5 µg), cephalothin (CEF; 30 µg), cefixime (CFM; 30 µg), ceftriaxone (CRO; 30 µg), cefotaxime (CTX; 30 µg), ceftazidime (CAZ; 30 µg), tetracycline (TET; 30 µg), co-trimoxazole (CoT; 20 µg), nalidixic acid (NAL; 30 µg), ciprofloxacin (CIP; 30 µg), norfloxacin (NOR; 10 µg), ofloxacin (OFX; 5 µg), gatifloxacin (GAT; 5 µg), gentamicin (GEN; 10 µg), amikacin (AMK; 30 µg), nitrofurantoin (NIT; 300 µg), azithromycin (AZM; 30 µg), and chloramphenicol (CHL; 30 µg). *Escherichia coli* ATCC 25922 was used as a quality control strain. The minimum inhibitory concentrations (MICs) of third generation cephalosporins (CAZ, CTX, and CRO), CAR, NAL, CIP, and CoT were determined for all *V. cholerae* strains using the E-test (AB Biodisk, Solna, Sweden), following Clinical and Laboratory Standards Institute procedures and interpretative standards for *V. cholerae*. Extended spectrum beta-lactamase (ESBL) production was detected using the double-disk synergy method with ceftazidime-clavulanic acid (CAZ; 30/10 µg) and ceftriaxone-clavulanic acid (CAC; 30/10 µg) (7). In the absence of a reference zone size for *V. cholerae* resistance to AZM, we used the zone size for other organ-

isms to determine susceptibility (zone of inhibition ≥18 mm for AZM) of the *V. cholerae* strains to AZM (5,8).

**PCR analysis and random amplified polymorphic DNA (RAPD) assays:** All *V. cholerae* O1 isolates were subjected to PCR-based detection of various virulent genes (*ctxA*, *ctxB*, *tcpA* [El Tor/Classical], *toxR*, *toxS*, *toxRS*, *VPI*, *toxT*, *ace*, *zot*, and *tcpP*) (9). In addition, all isolates were subjected to DNA fingerprinting by RAPD assays, using primers M16 (5'-AAA GAA GGA CTCA GCG ACT GCG-3') and PB1 (5'-GCG CTG GCT CAG-3') as described previously (4,5).

**Ethics approval:** The study was carried out as part of public health response and therefore prior approval of the Institutional Ethics Committees were not taken.

## RESULTS

The cholera outbreak commenced on the 5th of August 2013, when a 30-year-old woman presented with severe dehydration and watery diarrhea at the Aganwadi Health Centre in Somanakoppa village. She was admitted to Bagalkot Government Hospital on 6 August, given fluids intravenously, and treated with NOR (400 mg), OFX (200 mg), and ranitidine (150 mg). The patient recovered and was discharged on the 8th of August 2013; however, 2 individuals from her neighborhood developed diarrhea on the 7th of August, followed by her son and husband on the 8th of August. All patients were treated with NOR (400 mg), OFX (200 mg), and ranitidine (150 mg). The outbreak lasted until 16 August, with 49 cases reported and an attack rate of 3.5% (Figure 1). Isolates of *V. cholerae* were obtained from 4 of the 6 samples and were confirmed as *V. cholerae* O1 Ogawa by serology.

The *V. cholerae* isolates were resistant to a wide spectrum of drugs (Table 1). The MIC of CAR and CoT ranged from 64–128 µg/ml and 4/76 µg/ml, respectively (Table 1). The MIC of the resistant strains to NAL and CIP ranged were >256 µg/ml and 0.75–1 µg/ml, respectively. The MIC for CIP revealed that the isolates had reduced susceptibility to CIP. The MICs of third-generation cephalosporin-resistant isolates were 32, 4–32, and 4–16 µg/ml for CRO, CTX, and CAZ, respectively. All the cephalosporin-resistant strains were confirmed to produce ESBL. All isolates were sensitive to AZM, OFX, GAT, IMP, and TET, and exhibited moderate levels of resistance to NOR, AMK, and CHL.

The PCR assays revealed that all the *V. cholerae* O1 isolates harbored the virulence genes *ctxA*, *ctxB*, *tcpA* El Tor, *Tox S*, *VPI*, *ToxT*, *ToxR*, *ToxRS*, *ace*, *zot*, and *tcpP*.

RAPD fingerprinting assays revealed that all the strains were genetically similar, with the fingerprint pattern found to match the *V. cholerae* O1 strain isolated from Belgaum in 2010.

Water from the second bore well was found to be fit for drinking, with a fecal coliform count of less than 10 cfu/ml. However, water from the bore well that was used before 12 August 2013 was found to be contaminated, with an MPN value of more than 180 cfu/ml.

An increase in surveillance, use of an alternative water source, and the institution of IEC activities helped to contain the outbreak, which had subsided by 16 Au-

Cholera Outbreak with MDR Strains in Badami, India

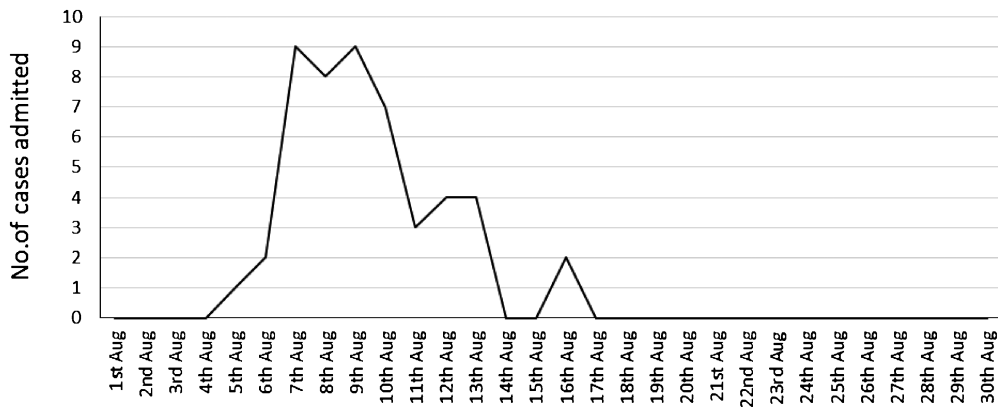


Fig. 1. Epidemic curve of the cholera outbreak at Somanakoppa village, Badami.

Table 1. Details of the *Vibrio cholerae* strains isolated from the outbreak at Somanakoppa village, Badami

SI No.	Date	Lab No.	Antibiotic Resistant pattern	MIC ( $\mu\text{g/ml}$ )						
				CAR	NAL	CIP	CoT	CTX	CAZ	CRO
1	14/08/2013	IDST13	AMP, CAR, NAL, CoT, NIT, CET, CXM, CFM, CRO, CTX, CAZ, AMC	64	>256	0.75	4/76	4	4	32
2	14/08/2013	IDST14	AMP, GEN, CAR, NAL, CoT, NIT, CET, CXM, CFM, CRO, CTX, CAZ, AMC	128	>256	1	4/76	32	4	32
3	14/08/2013	IDST15	AMP, GEN, CAR, NAL, CoT, NIT, CET, CXM, CFM, CRO, CTX, CAZ, AMC	128	>256	1	4/76	32	16	32
4	14/08/2013	IDST17	AMP, CAR, NAL, CoT, NIT, CET, CXM, CFM, CRO, CTX, CAZ, AMC	128	>256	1	4/76	32	4	32

gust 2013. The epidemic curve is presented in Figure 1.

**DISCUSSION AND CONCLUSIONS**

Although the source of drinking water was common to the village, the point of water collection differed for individuals. During the course of discussions with the index case, her family, and other affected people, it was found that the family of the index case and the neighbors who had diarrhea early in the outbreak shared a common open field for defecation, with a tap located nearby. The water source for this tap was a storage tank, which in turn received water from the first bore well that was in operation prior to the outbreak. These patients reported having used this tap for either ablution or drinking. It is possible that the family members and neighbors of the index case contracted cholera from the water near the site of open defecation and the tap. It is also possible that the source of the bore well and/or groundwater became contaminated because of open defecation, thereby triggering the outbreak. The source of the second bore well was some distance away from the site of open defecation, and was either not contaminated or the water was devoid of fecal coliform because of regular chlorination that commenced on 12 August 2013. Unfortunately, we were unable to ascertain how the index case contracted cholera as she did not report travelling to other villages, and had not been visited by friends or relatives from other locations prior to the outbreak.

In accordance with previous reports (4,10), we found that *V. cholerae* O1 Ogawa was the predominant serotype in this part of Karnataka. Although the number of

stool samples collected was low, and considering the remoteness of the location, and the limited infrastructure and transportation facilities available in this area, it is commendable that the authorities were able to transport the samples to Belgaum for analysis. The extended period it takes to reach Belgaum may account for the inability to isolate any pathogenic organisms from 2 samples. However, aside from these limitations, it is clear that the *V. cholerae* strains circulating in the region are gradually acquiring resistance to a wider spectrum of antibiotics. Multidrug resistance of *V. cholerae* and variant strains of *V. cholerae* have been reported in other regions of India, and in neighboring countries (5,10-14). However, multidrug resistance has not been reported from the Bijapur district of North Karnataka. The WHO recommends the use of either DOX or CIP as the treatment of choice for cholera (15,16); however, the reduced susceptibility of *V. cholerae* to CIP, together with the increased use of fluoroquinolones might create an opportunity for the emergence of highly quinolone-resistant strains of *V. cholerae* (17). Given that the treatment of severe diarrhea is important in combating cholera, the emergence of *V. cholerae* resistance to the most common antimicrobial agents is a matter of concern, particularly considering the epidemic potential of the organism. In the wake of excessive use and misuse of antimicrobials that create selection pressure on microbes, which in turn might develop mechanisms to render these antimicrobials ineffective, there is a need to monitor the resistance of *V. cholerae*. Although a National Antibiotic Policy has been framed, there is an immediate need for the implementation and monitoring of guidelines. The

results obtained from this study will be instrumental in identifying emerging antimicrobial resistance, and in developing treatment guidelines appropriate for the region, thereby providing the baseline data to compare outbreak-associated strains of *V. cholerae* in the future.

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**Conflict of interest** None to declare.

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