Outbreak of cholera by multidrug resistant *Vibrio cholerae* O1 in a backward taluka of Bagalkot, North Karnataka

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Received: June 11, 2014. Accepted: November 18, 2014
Published online: March 13, 2015
DOI: 10.7883/yoken.JJID.2014.257
Outbreak of cholera by multidrug resistant *Vibrio cholerae* O1 in a backward taluka of Bagalkot, North Karnataka

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Running Title : Cholera outbreak with MDR strains in Badami, India

Keywords : Cholera, outbreak, multi-drug resistant, diarrhoea
Abstract:
Cholera is a major cause of illness in the developing world. During the monsoon, sporadic and small clusters of cases of cholera are encountered almost every year in Karnataka, India. During the monsoons in 2013, there was emergence of cholera in Badami, a remote backward area of Bagalkot district in Karnataka, by multi drug resistant *V. cholerae* O1 serotype Ogawa. The outbreak of diarrhoea started on 5th August, 2013, when a 30 year old woman reported with severe dehydration and watery diarrhoea at the Aganwadi Health Centre in Somanakoppa village of Badami. A total of 49 cases suspected of cholera reported during this period in the local health centre/ anganwadi with an attack rate 3.5%. The *V. cholerae* isolates presented a wide spectrum of resistance involving 4-5 groups of drugs which involves ampicillin, co-trimoxazole, nitrofurantoin, carbenicillin, third generation cephalosporins and showed reduced susceptibility to third generation fluoroquinolones. All the cephalosporin resistant strains were confirmed to produce extended spectrum beta lactamase. All the *V. cholerae* O1 isolates harboured virulent genes ctxA, ctxB, tcpA El Tor, Tox S, VPI, ToxT, ToxR, ToxRS, ace, zot and tcpP and were found to be genetically similar to each other by RAPD fingerprinting assay.

The present study, to the best of our knowledge, is the first report of cholera outbreak from the district of Bagalkot. Resistance to commonly used antimicrobial drugs in *V. cholerae* is becoming a major public health concern in the region as clinicians are left with limited choice of antibiotics for treatment, when necessary.
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 consists of early administration of rehydration therapy with appropriate oral or intravenous fluids. The World Health Organization recommends antimicrobial drug treatment for severely dehydrated patients with suspected cholera because it substantially shortens the duration of diarrhoea [1]. In 2013, 47 countries reported a total of 129,064 cases of cholera including 2,102 deaths, giving a case-fatality rate (CFR) of 1.63%. This represents a decrease of 47% in the number of cases reported compared to 2012 and this is the second consecutive year in which reported cholera cases declined. The number of reporting countries in 2013 was 47 compared to 48 in 2012 [1].

Sporadic and small clusters of cases of cholera occur almost every year in the state of Karnataka, the second largest state of South India by area, particularly in the monsoon period (unpublished data). Outbreaks of cholera occurred in the state in 2000, 2002, 2005, and 2010 [2,3,4]. Cholera was relatively rare in the northern districts of the state, although common in nearby areas of the adjoining state of Maharashtra. However in the recent years, cholera seem to be emerging in this part of the state as well with large outbreaks being reported from various areas of Belgaum and Bijapur districts [2,3,4]. However there has as yet been no report of cholera from neighbouring Bagalkot district so far. Bagalkot district, which, according to the 2011 Census has a population of 1,889,752 and has 6 talukas, comprising of a total of 18 hobbies and 627 villages. Of the 6 taluks, two are categorised as "Very Backward Taluka" and one as "Most Backward Taluk". In this communication, we report the occurrence of an outbreak of cholera due to multi-drug resistant *V. cholerae* O1 Ogawa in Somanakoppa village of Badami, one of the two “Very Backward Talukas” of the district (population~1400), which, to the best of our knowledge, is the first report of cholera outbreak from the district.
Methods

Samples

Stool samples were collected from the diarrhoeic patients who were admitted in the health centres and transported to Belgaum Institute of Medical Sciences (BIMS), Belgaum which is the nodal surveillance centre for the region.

Microbiological assays

The samples were processed for enrichment and identification of bacterial enteric pathogens by plating directly on Thiosulphate Bile Salt Sucrose (TCBS) agar, MacConkey (MAC) agar and Hektoen Enteric Agar. Sucrose fermenting yellow colored colonies resembling *V. cholerae* that appeared on the TCBS plates and non-lactose fermenting colonies that appeared on MAC plates after 18 hour incubation at 37°C were further subjected to standard biochemical tests including sugar fermentation in triple sugar iron (TSI) agar and oxidase production. The isolates were subsequently serotyped using polyvalent and monovalent antisera (Denka Seiken Co., Ltd., Tokyo, Japan) at Regional Medical Research Centre (RMRC), Belgaum.

Meanwhile, teams consisting of doctors and scientists of 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 examination of Aganwadi, PHC and Govt Hospital records. The sites and sources of drinking water, usage pattern and defecation sites were examined. There were two borewells in the area of which one was in operation during and before the outbreak began. It was near to an open field where many villagers including the index case reported to defecate routinely. This water source was temporarily closed and the other borewell was made operational from 12th August 2013. Water samples were collected from both the water sources in 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. Chlorination of all water sources and storage tanks was carried out.

At RMRC, a rapid H₂S strip test (Hi-Media Laboratories, Mumbai) and Most Probable Number (MPN) method was employed to determine contamination levels by coliform count as carried out previously [5].

**Antibiotic sensitivity testing**

Susceptibility of the *V. cholerae* isolates to different antibiotics were tested at RMRC by disk diffusion technique following to standard guidelines [6] using commercially available disk (Hi-Media, Mumbai) which include 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). The *Escherichia coli* ATCC 25922 was used as the quality control strain. MICs of 3rd generation cephalosporins (CAZ, CTX and CRO), CAR, NAL, CIP and CoT were determined for all the strains by using E-test (AB Biodisk, Solna, Sweden) following CLSI procedures and interpretative standards for *V. cholerae*. 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 azithromycin, we used the zone size for other organisms to determine the susceptibility (zone of inhibition ≥18 mm for azithromycin) of the *V. cholerae* strains to the drug [5,8].

**PCR analysis**
All the *Vibrio cholerae* O1 isolates were subjected to PCR based detection of various virulent genes *ctxA, ctxB, tcpA (El Tor/Classical), toxR, toxS, VPI, toxT, ace, zot* and *tcpP* [9].

All the isolates were subjected to DNA fingerprinting by Random Amplified Polymorphic DNA (RAPD) fingerprinting assay using arbitrary primers M16 (5′ AAA GGA GGA CTCA GCG ACT GCG ’3) and PB1 (5′ GCG CTG GCT CAG 3’) as reported previously [4,5].

**Results**

The outbreak of diarrhoea started on 5th August, 2013, when a 30 year old woman reported with severe dehydration and watery diarrhoea at the Aganwadi Health Centre in Somanakoppa village of Badami. She was admitted to Bagalkot Govt. Hospital on 6th August and was put on IV fluid and treated with NOR (400mg), OFX (200mg) and Ranitidine (150mg). She recovered and was discharged on 8th August, 2013. However two individuals from her neighbourhood developed symptoms of diarrhoea on 7th August, and in addition her son and husband also developed symptoms on 8th August, 2013. All the cases were put on treatment with NOR (400mg), OFX (200mg) and Ranitidine (150mg). The outbreak lasted till 16th August and a total of 49 cases were reported during this period in the local health centre/anganwadi and Primary Health Centre with an attack rate 3.5 % (Figure 1). Presumptive *V. cholerae* isolated from 4 of the 6 samples were confirmed as *V. cholerae* O1 Ogawa by serology.

A wide spectrum of resistance was observed among the *V. cholerae* isolates (Table 1). The MIC of CAR and CoT for strains resistant to the respective drugs ranged from 64-128 μg/ml and 4/76 μg/ml respectively (Table 1). The MIC of the resistant strains to quinolones NAL and CIP ranged between >256 μg/ml and 0.75-1 μg/ml respectively. The MIC for CIP showed that the isolates had reduced susceptibility to CIP. The MICs of third generation cephalosporin resistant isolates ranged between 32 μg/ml for CRO, 4 to 32 μg/ml for CTX, 4 to 16 μg/ml for CAZ. All the cephalosporin resistant strains were confirmed to produce ESBL using the combination disc test. The drugs to which all the
isolates were sensitive include AZM, OFX, GAT, IMP and TET and showed intermediate resistance to NOR, AMK and CHL.

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

RAPD fingerprinting with primers M16 and PB1 carried out separately revealed that all the strains were genetically similar to each other and the fingerprint pattern was found to match with the *V. cholerae* O1 strain isolated from Belgaum during 2010.

While the water sample from the second borewell source (used since 12th August 2013) was found to be fit for drinking with a fecal coliform count of less than 10 cfu/ml, the water from the borewell that was used before 12th August 2013 (before the start of and in the initial phase of the outbreak) was found to be grossly contaminated with a MPN value of more than 180 cfu/ml.

Stepped up surveillance, change of water source and IEC activities carried out in the region by the state health authorities helped in containment of the outbreak and it subsided by 16th August 2013.

The epidemic curve is shown in Fig 1.

**Discussion and conclusions**

Although the source of drinking water was common to the village, the points of collection of this water were different for different people. During the course of discussion with the index case and her family and with the other affected persons and neighbours, it was found that the family of the index case and the neighbours who had diarrhoea early in the outbreak shared a common open field for defecation which had a tap nearby. This tap received water from a storage tank which in turn received water from borewell which was in operation earlier. These patients reported having used this tap either for ablution or drinking earlier. It is possible that the family members and neighbours of the index case contracted the infection from the water near the site of open defecation and the tap. It is also quite possible that the borewell source and/or groundwater got
contaminated because of open defecation that triggered the outbreak. Apparently, the location of the second borewell source which was some distance away from the site of open defecation was either not contaminated or the water was devoid of faecal coliforms because of regular chlorination that started since 12th August 2013. We were however unable to ascertain how the index case contracted cholera as she did not report travelling to other villages or any friend or relatives visiting them from elsewhere recently.

In accordance with earlier reports [4,10] the present study detected *V. cholerae* O1 Ogawa to be predominant serotype in this part of Karnataka. Although the number of stool samples collected is few, considering the remoteness of the location, limited infrastructure and transportation facilities available in this “Very Backward District”, it is commendable that the authorities were able to transport the samples to Belgaum for analysis. The long time it takes to reach Belgaum may account for non-isolation of any pathogenic organism from 2 samples. However, within these limitations, it is evident from the knowledge obtained that *V. cholerae* strains circulating in the region are gradually acquiring resistance to wider spectrum of antibiotics. Multidrug resistance in *Vibrio cholerae* and variant strains of *V. cholerae* has been reported from elsewhere in India and neighbouring countries [5,10,11-14]. However it has not been reported from Bijapur district of North Karnataka earlier. While the World Health Organization recommends the use of either DOX or CIP as treatment of choice for cholera [15,16], the detection of reduced susceptibility to CIP together with the increasing use of fluoroquinolones might create an opportunity for the emergence of highly quinolone-resistant strains associated with multi-drug resistance [17]. Since the treatment of severe cases of diarrhoea is important, the emergence of resistance in *V. cholerae* to most of the 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 may develop mechanisms to render these antimicrobials
ineffective, there is a need to monitor the resistance pattern of this organism over time and space. Although a National Antibiotic Policy has been framed, there is immediate need for implementation and monitoring the guidelines. Information obtained in this study will be instrumental in identifying emerging antimicrobial resistance, for developing treatment guidelines appropriate for the region, and to provide baseline data with which to compare outbreak strains in the future.
Acknowledgement:
The authors are thankful to the Department of Medical Education, Govt of Karnataka, IDSP, and Indian Council of Medical Research for financial support. The authors are also thankful to the technical staffs of the District Surveillance Unit, Bagalkot and Ms. Swapnali Kadam, Ms. Shanta Kalal and Mr. Jyotiba of RMRC, Belgaum for technical support.

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.

Funding:
The study was supported by the intramural funds of Department of Medical Education, Department of Health and Family Welfare Services, Govt of Karnataka and the Indian Council of Medical Research, Department of Health Research, Govt of India.

Conflict of Interest:
The authors do not have any commercial or other associations that may pose a conflict of interest.

Transparency Declaration:
None to declare.
Reference:


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

<table>
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<th>Sl No.</th>
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<th>Lab No.</th>
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<td></td>
<td></td>
<td></td>
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</tr>
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Figure 1. Epidemic curve of the cholera outbreak at Somanakoppa village, Badami