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Short Report: Emergence of Multidrug-Resistant *Vibrio cholerae* O1 Biotype El Tor in Port Blair, India

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Cholera is a major disease in the developing world. The World Health Organization reported in 2006 that 236,896 cases of cholera occurred in 52 countries, a 79% increase over 2005.[1] During the past decade, the dominance of the O1 Ogawa serotype of *Vibrio cholerae* and a quiescent period during the O139 era was observed.[2] El Tor *V. cholerae* have replaced the classical biotype over the past few decades.[3–5] Cycles of serotype shifting at intervals of 2–8 years have been reported.[6] During the monsoon season, sporadic and small clusters of cases of cholera occur almost every year in Port Blair, India (Bhattgacharya DS and others, unpublished data). Two outbreaks of cholera have been reported from Andaman and Nicobar Islands. The first outbreak, which was caused by *V. cholerae* O1 Ogawa, occurred in Nancowry Islands in 2002.[7] The second outbreak, which was caused by *V. cholerae* O1 Inaba, occurred in Port Blair and its suburbs in 2006.[8] We report the emergence of multidrug-resistant *V. cholerae* O1 cholera in the Andaman Islands.

This study was approved by the institutional ethical committee. During May–June 2010, there was an increase in diarrhea cases in Port Blair, the capital city of the Andaman and Nicobar Islands. Fecal samples were collected from persons with suspected cholera who were admitted to the G.B. Pant Hospital in Port Blair, the only referral hospital in the territory, and a private childcare hospital, and processed according to standard procedures for isolation and identification of *V. cholerae*. Written consent was obtained from the patients or guardians before collection of samples.

The *V. cholerae* strains were serotyped by using polyvalent and monovalent antisera (Denka Seiken Co., Ltd., Tokyo, Japan). Susceptibility to different antimicrobial drugs was tested by using the disk diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) (Wayne, PA) guidelines[9] and commercially available antimicrobial drug disks (Hi-Media, Mumbai, India). The drugs tested were ampicillin (10 μg), carbenicillin (100 μg), imipenem (30 μg), amoxicillin-clavulanic acid (20/10 μg), cefixime (30 μg), cefuroxime (5 μg), cephalothin (30 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), tetracycline (30 μg), co-trimoxazole (20 μg), nalidixic acid (30 μg), ciprofloxacin (30 μg), norfloxacin (10 μg), ofloxacin (5 μg), gatifloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), nitrofurantoin (300 μg), azithromycin (30 μg), and chloramphenicol (30 μg).

Escherichia coli strain ATCC 25922 was used as the quality control strain. The minimum inhibitory concentrations (MICs) of azithromycin, tetracycline, and ciprofloxacin were determined for all strains by using the Etest (AB Biodisk, Solna, Sweden) following CLSI procedures and interpretative standards for *V. cholerae.* Because there is no reference zone size for *V. cholerae* resistance to azithromycin, we considered a zone of inhibition ≥ 18 mm as the cut-off value to determine susceptibility, as followed in other studies on *V. cholerae*.[10]

All *Vibrio cholerae* O1 isolates were screened for virulence genes *ctxA*, *tcpA* (El Tor/Classical), *toxR*, *toxS*, *toxRS*, *VPI*, *toxT*, *ace*, *zot*, and *tcpP* by using a polymerase chain reaction–based detection technique.[11] Random amplified polymorphic DNA fingerprinting was performed for all isolates by using an arbitrary primer M16 (5′-AAAGAAGGACTCAGCGAC-TGCG-3′).[12] Reference strains of *V. cholerae* O139, *V. cholerae* O1 serotype Ogawa, and biotype El Tor were used as controls.[13]

A total of 62 stool samples were collected from patients with suspected cholera who came to or were admitted to the two hospitals in Port Blair. All patients were residents of South Andaman Island. *Vibrio cholerae* was isolated from 19 patients. Six (31.6%) isolates were *V. cholerae* Inaba, one (5.2%) was *V. cholerae* Ogawa, and 12 (63.2%) were non-agglutinating vibrios. The first confirmed case was detected on June 2, 2010, and the last confirmed case was detected on June 23, 2010. The last case-patient apparently had contracted the infection on another island, Little Andaman, and had symptoms develop while he was traveling to Port Blair. The isolate obtained from this patient was *V. cholerae* O1 Ogawa. No deaths caused by cholera were reported during the study period. None of the patients had any recent history of travel to mainland India or other islands, except the patient who contracted the infection on Little Andaman Island.

Although the outbreak that occurred in the islands in 2002 was caused by *V. cholerae* O1 Ogawa resistant to ampicillin, nalidixic acid and co-trimoxazole, the outbreak of 2006 was caused by *V. cholerae* O1 Inaba that was resistant to nitrofurantoin, in addition to the above three drugs. All *V. cholerae* isolates obtained during June 2010 also were resistant to ampicillin, nalidixic acid, co-trimoxazole, nitrofurantoin, tetracycline, cephalexin, and carbenicillin. Although four of the six *V. cholerae* Inaba isolates obtained during June 2010 were resistant to gentamicin, ciprofloxacin, amikacin, and azithromycin, only one isolate of *V. cholerae* Ogawa was resistant to amikacin and azithromycin. All isolates showed intermediate resistance to norfloxacin and ofloxacin by the disk diffusion method, with a MIC ranging from 0.125 to 1 μg/mL, respectively. The MICs of tetracycline and ciprofloxacin for strains resistant to these two drugs ranged from 16 to 32 μg/mL. The MICs of azithromycin for strains resistant to this drug ranged from 8 to 64 μg/mL (Table 1).

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TABLE 1
Antimicrobial drugs test results for *Vibrio cholerae* strains, Port Blair, India*

Drug	Zone of inhibition (mm) of *V. cholerae* strains							Zone of inhibition (mm) per CLSI		
	BC-289	BC-293	BC-297	BC-301	BC-317	DS-593	DS597	Resistant	Intermediate	Sensitive
Amikacin	14	0	14	14	17	16	14	< 14	15–16	> 17
Gentamicin	12	12	0	12	16	17	16	< 12	13–14	> 15
Imipenem	23	21	24	18	22	18	24	< 13	14–15	> 16
Azithromycin	11	10	10	13	17	19	0	< 13	14–17	> 18
Cephalothin (cephalexin)	10	10	12	13	13	13	12	< 14	15–17	> 18
Cefuroxime	18	17	17	16	17	22	18	< 14	15–17	> 18
Cefixime	20	19	19	21	19	19	20	< 15	16–18	> 19
Ampicillin	0	0	0	0	0	0	0	< 13	14–16	> 17
Carbenicillin	0	10	0	17	13	16	0	< 19	20–22	> 23
Nalidixic acid	13	0	0	0	0	0	0	< 13	14–18	> 19
Ciprofloxacin	14	12	10	10	22	21	21	< 15	16–20	> 21
Norfloxacin	15	15	15	13	17	17	17	< 12	13–16	> 17
Ofloxacin	14	13	14	15	17	17	18	< 12	13–15	> 16
Gatifloxacin	16	18	22	18	20	21	20	< 14	15–17	> 18
Co-trimoxazole	0	5	0	0	0	0	0	< 10	11–15	> 16
Tetracycline	12	13	0	10	14	12	10	< 14	15–18	> 19
Chloramphenicol	20	20	19	18	20	20	18	< 12	13–17	> 18
Nitrofurantoin	10	0	12	10	14	10	10	< 14	15–16	> 17
Ceftriaxone	23	23	21	21	22	21	23	< 13	14–20	> 21
Cefotaxime	23	24	23	25	23	23	23	< 14	15–22	> 23
Ceftazidime	20	18	18	18	20	19	18	< 14	15–17	> 18
Amoxicillin/ clavulanic acid	20	18	19	18	20	19	19	< 13	14–17	> 18

	MIC (mg/mL)							MIC breakpoint (mg/mL) per CLSI		
	BC-289	BC-293	BC-297	BC-301	BC-317	DS-593	DS597	Resistant	Intermediate	Sensitive
Azithromycin	64	64	8	8	8	8	64	ND	ND	ND
Ciprofloxacin	32	32	32	24	32	16	32	≥ 4	1 (0.25–1)†	≤ 1
Tetracycline	32	32	24	32	16	16	16	≥ 16	8	≤ 4

*MIC = minimum inhibitory concentration, CLSI = Clinical and Laboratory Standards Institute; ND = not described.
†Value in parentheses is range.

A multiplex polymerase chain reaction showed that all *V. cholerae* O1 isolates had the virulence genes *ctxA*, *tcpA* (El Tor), *toxR*, *toxS*, *toxRS*, *VPI*, *toxT*, *ace*, *zot*, and *tcpP*. Random amplified polymorphic DNA RAPD analysis with primer M16 generated identical fingerprints for all the *V. cholerae* Inaba isolates, which were similar to fingerprints of *V. cholerae* O1 strains isolated during the outbreak of cholera in Port Blair in 2006. The fingerprinting profile of the sole *V. cholerae* Ogawa isolate, DS-597, was similar to that of *V. cholerae* O1 Ogawa strains isolated during 2002 outbreak.

In the present study, all isolates showed multidrug resistance for 7–11 drugs. Three drug resistance patterns were observed among the seven *V. cholerae* isolates. Although multidrug resistance in *V. cholerae* isolates has been reported from elsewhere in India and neighboring countries,[2,14–16] it has not been reported from Andaman and Nicobar Islands. It is likely that *V. cholerae* O1 Inaba has been circulating in the environment of South Andaman, probably in some nonpathogenic/non-cultivable form since the outbreak in 2006. The re-emergence after a quiescent period, when apparently no cholera occurred, might be caused by acquisition of virulence genes by non-pathogenic *V. cholerae*[17,18] or by an increase in contamination of the environment by *V. cholerae* to a level adequate for successful transmission of infection. During the quiescent period, survival of *V. cholerae* in water bodies might have enabled dissipation of drug resistance to different serotypes or strains.[14]

During the last two outbreaks in 2002 and 2006 caused by *V. cholerae* O1 Ogawa and Inaba, respectively, all strains isolated were sensitive to tetracycline, gentamicin, amikacin, azithromycin, and cephalexin. Many of the *V. cholerae* strains isolated during the recent outbreak were resistant to these drugs. Ciprofloxacin and azithromycin resistance has already emerged in *V. cholerae*.[19] Resistance to quinolones is generally associated with amino acid substitutions in portions of GyrA and ParC proteins, which are caused by mutations in the quinolone resistance–determining region.[20]

The presence of an integron, an integrative and conjugative element, and active efflux also adds to the factors conferring resistance to a wide range of antimicrobial drugs. Azithromycin resistance can be mediated by various mechanisms, including overexpression of efflux pumps, production of methylases, and mutations in the drug target, the 23S ribosomal RNA gene (A2059G). The mechanism of this high-level resistance could be novel or a combination of known mechanisms. However, the possibility of the strain being introduced into the environment of the islands by persons with undetected cholera who traveled to the islands from mainland India cannot be ruled out.[14] Emergence of resistance to multiple drugs has been reported in other diarrheal pathogens in these islands.[21] This finding is not unique because many investigations conducted in different areas have demonstrated an increase in the antimicrobial resistance spectrum among epidemically significant *V. cholerae* over time.[20]

Resistance to commonly used antimicrobial drugs is becoming a major public health concern because it complicates treatment and may result in longer hospital stays for patients. Spread of antimicrobial drug resistance has been recognized by the World Health Organization as an extremely serious problem. *Vibrio cholerae* possesses a number of mechanisms

to evade the effects of antimicrobial drugs and a stage may come when the commonly used antimicrobial drugs are no longer effective.[20] However, we are not yet stranded because strains are still sensitive to some of the newer quinolones and cephalosporins. Nonetheless, the expanding spectrum of drug resistance among these *V. cholerae* isolates is a cause for serious concern.

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