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. During the past decade, the dominance of the O1 Ogawa serotype of Vibrio cholerae and a quiescent period during the O139 era was observed. El Tor V. cholerae have replaced the classical biotype over the past few decades. Cycles of serotype shifting at intervals of 2–8 years have been reported. 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and small clusters of cases of cholera occur almost every year in Port Blair, India (Bhattgacharya DS and others, unpublished data).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. The second outbreak, which was caused by V. cholerae O1 Inaba, occurred in Port Blair and its suburbs in 2006. 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 diarrhoea 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 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.

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. Random amplified polymorphic DNA fingerprinting was performed for all isolates by using an arbitrary primer M16 (5'-AAAGAAGGACTCAGCGAC-TGGC-3'). Reference strains of V. cholerae O139, V. cholerae O1 serotype Ogawa, and biotype El Tor were used as controls.

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, cephalaxin, and carbencillin. 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).
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* isolates, which were similar to fingerprints of *V. cholerae* strains isolated during the outbreak of cholera in Port Blair in 2006. The fingerprinting profile of the sole *V. cholerae* Inaba isolate, which was similar to fingerprints of *V. cholerae* O1 isolates had the virulence genes *ctxA*, *tcpA* (El Tor), *toxR*, *toxS*, *toxRS*, *VPI*, *toxT*, *ace*, *zot*, and *tcpP*. During the quiescent period, survival of *V. cholerae* in water bodies might have enabled dissipation of drug resistance to different serotypes or strains. 

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*. 

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. 

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. 

Emergence of resistance to multiple drugs has been reported in other diarrheal pathogens in these islands. This finding is not unique because many investigations conducted in different areas have demonstrated an increase in the antimicrobial resistance spectrum among epidemiologically significant *V. cholerae* over time.

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. 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.

Received May 20, 2011. Accepted for publication March 12, 2012.

Acknowledgments: The authors are thankful to the Indian Council of Medical Research for providing financial grant for the study and to Dr. P. Vijayachari, Director, RMRC, for administrative support. The authors are also thankful to the Directorate of Health service (Andaman & Nicobar Islands) for their extensive support and help during the work. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

Financial support: This study was supported by the Indian Council of Medical Research (Project no. 5/8-1(209)/D/2006/ECD-II).

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

Authors' addresses: Debdutta Bhattacharya, D. S. Sayi, R Thamizhmani, Haimanti Bhattacharjee, and A. P. Sugunan, Regional Medical Research Centre, Indian Council of Medical Research, Port Blair, Andaman and Nicobar Islands, India, E-mails: debdutta_0408@yahoo.co.in, sayidev4u@yahoo.co.in, mailmethamizh@rediffmail.com, haimanti0408@gmail.com, and sugunanap@icmr.org.in or pblicmr@yahoo.co.in, Avijit Roy, Directorate of Health Service, Andaman and Nicobar Administration, Port Blair, Andaman and Nicobar Islands, India, E-mail: bharadwaj_haimanti0408@gmail.com, A. P. Bharadwaj, Chirayu Child Care Centre, Pediatric, Port Blair, Andaman and Nicobar Islands, India, E-mail: bharadwaj_ap@yahoo.com. Avijit Roy, Directorate of Health Service, Andaman and Nicobar Administration, Port Blair, Andaman and Nicobar Islands, India, E-mail: deputydirectorhealth@gmail.com.

REFERENCES