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Short Communication

Emergence of *Vibrio cholerae* O1 Biotype El Tor Serotype Inaba Causing Outbreaks of Cholera in Orissa, India

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SUMMARY: A total of 431 rectal swabs, collected from acute diarrheal cases at a surveillance site and at different diarrheal outbreak areas of Orissa from May to October 2005, were bacteriologically analyzed. Out of 265 culture-positive samples, *Vibrio cholerae* O1 was isolated in 56 samples (20.8%), of which 37 were the Inaba serotype and 19 were the Ogawa. The antibiogram profile revealed that all the *V. cholerae* O1 Ogawa and Inaba serotypes were uniformly sensitive to ampicillin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacin and tetracycline. The *V. cholerae* O1 Inaba serotypes were resistant to furazolidone and nalidixic acid, while the Ogawa strains were resistant to furazolidone, nalidixic acid and neomycin. The multiplex polymerase chain reaction (PCR) assay on some selected strains of both serotypes revealed that all the strains were positive for *ctxA* and *tcpA* genes showing biotype El Tor. The present study revealed the emergence of *V. cholerae* O1 biotype El Tor serotype Inaba, which caused sporadic outbreaks of cholera in 2005. The outbreaks of diarrheal disorders in one geographical area of the state (in the Pattamundai area, Kendrapara district) in 2005 were due to *V. cholerae* O1 Ogawa, whereas the other outbreaks in other areas (Puri, Khurda and Dhenkanal districts) from August to October 2005 were due to *V. cholerae* O1 serotype Inaba. This is the first report that an emergence of *V. cholerae* O1 serotype Inaba caused sporadic outbreaks of cholera in different parts of Orissa. Switching over of *V. cholerae* O1 Ogawa strains to Inaba, causing diarrheal outbreaks in Orissa, needs close monitoring.

Vibrio cholerae has two toxigenic serogroups, O1 and O139. The O1 serogroup has two biotypes, El Tor and classical, and two major serotypes, Ogawa and Inaba. The 1991 cholera epidemic in Trujillo, Peru, was due to the *V. cholerae* O1 serotype Inaba. The cholera epidemic in the following year was due to serotype Ogawa (1). In 1989, shortly before the 1992 appearance of the *V. cholerae* O139 serogroup in Kolkata, India, there was a predominance of the *V. cholerae* Inaba serotype (2,3). Similar serotypes of *V. cholerae* Inaba strains were isolated in sporadic cases of diarrhea from Delhi and Sevagram in North India in November 1999 (3). Non-toxigenic *V. cholerae* O1 serotype Inaba was reported to be the cause of outbreaks of cholera in the town of Warangal in South India during 1996 (4). The July 2004 emergence of *V. cholerae* O1 biotype El Tor serotype Inaba was reported in North India (5). The state of Orissa extends from 17°49′N to 22°34′N latitude and from 81°28′E to 87°29′E longitude along the eastern coast of India. From May to November, the coastal saline tract of Orissa usually experiences cyclones or floods almost every year. During this period, the area is frequently affected by epidemics and outbreaks of diarrheal disorders, accounting for the state's high morbidity and mortality rates. *V. cholerae* is one of the important enteropathogens responsible for causing frequent diarrheal outbreaks in the state of Orissa, the majority of which were due to *V. cholerae* O1 Ogawa biotype El Tor and the O139 serogroup (6-8). The present study envisages that the emergence of *V. cholerae* O1 serotype Inaba caused the outbreaks of cholera in different parts of Orissa from August to October 2005.

Rectal swabs were collected from patients with acute diarrhea who had been either admitted to hospitals or who had remained in their villages, as well as inpatients with diarrhea of I. D. Hospital, Puri (a surveillance site) in Cary-Blair Transport Medium (CBT). The swabs were transported within 24 h of collection to the Regional Medical Research Centre (RMRC), Bhubaneswar. The rectal swabs were inoculated to selective media such as MacConkey agar, Hektoen enteric agar (HEA) and thiosulphate citrate bile salt sucrose agar (TCBS; Difco, Detroit, Mich., USA). Enrichment was carried out in alkaline peptone water and Selenite F-broth. Significant colonies were picked up and tested biochemically following standard techniques (9,10). Identification of *V. cholerae* was confirmed by polyvalent O1 and monovalent Inaba/Ogawa antisera obtained from Difco. Antibiograms of all *V. cholerae* O1 isolates were obtained following the disc diffusion technique with commercial discs (Hi Media, Mumbai, India) (11). The antibiotics used in this study were ampicillin (A, 10 mcg), chloramphenicol (C, 30 mcg), ciprofloxacin (Cf, 5 mcg), co-trimoxazole (Co, 25 mcg), furazolidone (Fr, 50 mcg), gentamicin (G, 10 mcg), nalidixic acid (Na, 30 mcg), streptomycin (S, 10 mcg), neomycin (N, 30 mcg), norfloxacin (Nx, 10 mcg) and tetracycline (T, 30 mcg). Characterization of the strains as susceptible, intermediately sensitive or resistant was based on the size of the inhibition zones according to the manufacturer's instructions, which matched the interpretive criteria recommended by the World Health Organization (12). In the present study, strains showing intermediate zones of growth inhibition were interpreted as resistant according to Yamamoto et al. (13) and Garg et al. (14). A multiplex PCR assay was employed to determine the presence of A subunit cholera toxin gene (*ctxA*) and to

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biotype *V. cholerae* strains by targeting *tcpA* (encoding the major structural subunit of the toxin-co-regulated pilus), which is specific to classical and El Tor strains (15).

In the present study, a total of 431 rectal swabs were bacteriologically analyzed from May to October 2005, of which 265 (61.5%) were culture-positive. Of these 265 cases, 203 (76.6%) were *Escherichia coli*, 56 (20.8%) were *V. cholerae* and 6 (2.3%) were *Shigella* spp. Of the 56 *V. cholerae* O1 isolates, 19 were Ogawa and 37 were Inaba. Among the *Shigella* spp. isolated, most of them were *S. flexneri* type 6 (n = 4) followed by *S. boydii* (n = 1) and *S. dysenteriae* type 1 (n = 1). During this 6-month period, not a single strain of *V. cholerae* O139 serogroup was isolated. The sporadic outbreaks of diarrhea occurred mostly in the coastal districts of Orissa, as shown in Figure 1. There were a total of four outbreaks at different times in the study period. The attack rate (incidence) was observed to be 3.4, 4, 2.7 and 4.16% in the Kendrapara, Puri, Khurda and Dhenkanal districts, respectively. From the Pattamundai area of the Kendrapara district, 10 rectal swabs were bacteriologically analyzed from 31 patients (male, 25; female, 6) who were suffering from acute watery diarrhea, from which *V. cholerae* O1 Ogawa were isolated from 3 cases. *V. cholerae* O1 serotype Inaba was isolated for the first time in the Gaudabada Sahi of the coastal town of Puri, where 32 persons (male, 22; female, 10) were suffering from watery diarrhea. All four of the rectal swabs analyzed were positive for *V. cholerae* O1 serotype Inaba. During the month of September 2005, 10 people (male, 7; female, 3) suffered from acute attack of diarrheal disorder in the Bhubaneswar municipality of the Khurda district. Here, too, all four of the rectal swabs analyzed were culture-positive for the *V. cholerae* O1 Inaba serotype. Further, during the month of October 2005, another outbreak of diarrheal disorder was reported from Chasapada village of Baltikiri PHC, Dhenkanal district. Forty-four persons (male, 25; female, 19) were suffering from severe watery diarrhea. Five rectal swabs were culture-positive for *V. cholerae* O1 Inaba serotype. Apart from these sporadic outbreaks, continuous surveillance activities were carried out in the I. D. hospital at Puri, where patients from different parts of the district were admitted for diarrheal disorder. During this period, 308 diarrhea patients were admitted and their rectal swabs were bacteriologically analyzed, revealing 25 *V. cholerae* O1 Inaba and 16 Ogawa serotypes (Table 1). This indicates that the present cholera outbreaks were confined to most of the coastal districts of Orissa (Fig. 1). In the present study all the *V. cholerae* O1 Inaba and Ogawa serotypes were uniformly sensitive to ampicillin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacin and tetracycline. The Inaba serotypes were 100% resistant to furazolidone and nalidixic acid and were about 50% resistant to neomycin, cotrimoxazole and streptomycin. On the other hand, the Ogawa serotypes were 100% resistant to furazolidone, nalidixic acid and neomycin and were about 50% resistant to co-trimoxazole and streptomycin. The multiplex PCR assay on representative strains of *V. cholerae* O1 Ogawa and Inaba revealed that all were *ctxA*- and *tcpA*-positive, showing biotype El Tor.

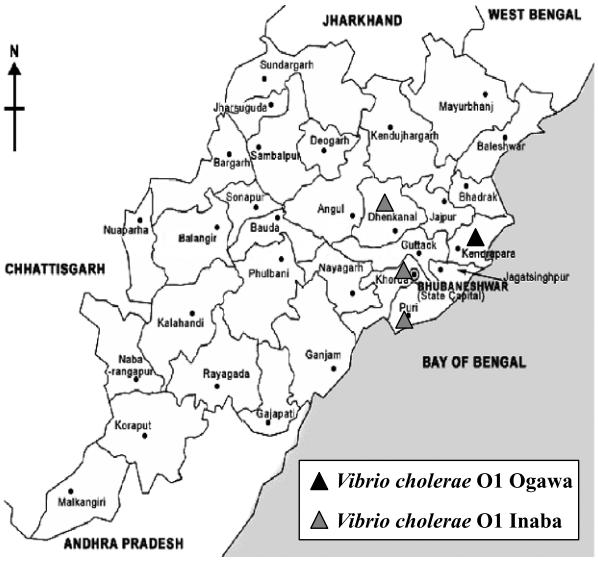

Fig. 1. District map of Orissa showing the outbreaks of cholera in 2005.

The predominance of cholera due to *V. cholerae* O1 serotype Inaba was observed in Kolkata in 1989; in Delhi in 1998; in Sevagram in 1999 and in North India in 2004 (2,3,5). The present study revealed that most of the *V. cholerae* O1 reported up to July 2005 were serotype Ogawa, causing localized diarrheal outbreaks in the Patamundai area of the Kendrapada district of Orissa. In the Puri area, the first cholera outbreak due to *V. cholerae* O1 Inaba occurred in August 2005. Subsequently, the same serotypes were reported from the Bhubaneswar and Dhenkanal areas as having caused cholera outbreaks during September and October 2005, respectively. Isolation of *V. cholerae* O1 Inaba, which caused diarrheal outbreaks in different parts of Orissa from August to October 2005, has demonstrated that the Inaba serotype is widespread in this region.

It is assumed that the present Inaba serotype might have evolved from pre-existing *V. cholerae* O1 Ogawa isolates. *V. cholerae* O1 strains are known to interconvert and to switch

Table 1. Distribution of different serotypes of *V. cholerae* (May - October 2005)

Month	Samples collected	No. positive for *V. cholerae*			Serogroup		Place of isolation
		M	F	Total	Ogawa	Inaba	
May	63	0	0	0	0	0	–
June	81	0	1	1	1	0	Puri Sadar (Puri District)
July	150	2	6	8	8	0	Pattamundai (Kendrapara District); Puri Sadar (Puri District)
August	64	7	3	10	3	7	Pipili, Puri Sadar (Puri District)
September	38	5	11	16	1	15	Puri Sadar, Brahmagiri (Puri District); Rangamatia (Khurda District)
October	35	12	9	21	6	15	Gop, Chandanpur, Sunder, Bramhagiri (Puri District); Chasapada (Dhenkanal District)
Total	431	26	30	56	19	37	

between the two known serotypes, Ogawa and Inaba (3,16-20). Serotype switching may be related to immune pressure on the prevailing serotype, as suggested by the observation of an epidemic in Latin America in 1991 (21). In vivo sero conversion has been reported to correlate well with the host immune response, and this finding has been supported by observations using germ-free mice (22) as well as by the results of a study by Sheehy et al. (23). *V. cholerae* O1 Ogawa biotype El Tor was isolated from various diarrhea outbreaks from 1995-2004 in Orissa. After the super cyclone of 1999 in Orissa, there was a codominance of the O139 serogroup, which gradually receded along with the O1 Ogawa serogroups. Suddenly, however, diarrheal outbreaks in 2005 by *V. cholerae* O1 serotype Inaba were noticed, showing dominance over Ogawa strains.

Ciprofloxacin resistance has been reported in India by Garg et al. (3). However, in the present study all of the *V. cholerae* O1 biotype El Tor serotype Ogawa isolates were found to be susceptible to ciprofloxacin, including the Inaba isolates. The resistance profiles of the *V. cholerae* O1 serotypes Inaba and Ogawa were FrNa and FrNaN, respectively. In the present study, both the Ogawa and Inaba serotypes were 100% sensitive to ampicillin and 50% sensitive to streptomycin and co-trimoxazole; whereas in our earlier study, in the 1999 post-cyclone outbreak, the *V. cholerae* O1 Ogawa strains exhibited 100% resistance to ampicillin, co-trimoxazole and streptomycin (7). This indicates that the *V. cholerae* O1 Ogawa strains are changing their antibiogram pattern. This may be due to irrational use of antibiotics and changing patterns of the environment, which need close monitoring. The multiplex PCR assay on some representative strains of *V. cholerae*, including both Ogawa and Inaba isolates, revealed that all the strains were *ctxA*- and *tcpA*-positive, showing biotype El Tor, whereas an outbreak of non-toxigenic *V. cholerae* O1 Inaba was reported in Warangal, Maharastra, in 1996 (4). Further molecular analysis, such as PFGE and ribotyping, will be helpful for finding the clonality of the *V. cholerae* O1 isolates and are in progress. These data will serve as a warning to public health authorities in this region, who will need to increase their preparedness for all impending epidemics due to *V. cholerae* biotype El Tor serotype Inaba in the next cholera season.

This is the first report of the emergence of the Inaba serotype causing cholera outbreaks during August to October 2005 in Orissa. Molecular analysis will be helpful to understand the widespread emergence of *V. cholerae* O1 serotype Inaba in different parts of the country, including Orissa.

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