

Short Communication

Emergence of *Vibrio cholerae* O1 Biotype El Tor Serotype Inaba in North India

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SUMMARY: All cases of cholera that have occurred at our center in north India have been due to *Vibrio cholerae* O1 serotype Ogawa, including the outbreaks in 2002 and 2004. Here we report the emergence of *V. cholerae* O1 biotype El Tor serotype Inaba for the first time in this region since July 2004. Fifteen Inaba isolates were obtained from 32 patients suffering from cholera-like illness. The patients lived in Chandigarh and the neighboring states of Punjab, Haryana, and Himachal Pradesh. All strains were resistant to nalidixic acid and trimethoprim, and showed moderate sensitivity to amoxicillin. All were sensitive to ciprofloxacin, tetracycline, cefotaxime, amikacin, and gentamicin. All strains were found to be toxigenic when tested with a commercial reverse passive latex agglutination kit. The last reported Inaba isolate dominance in India was observed in Calcutta in 1989. There is a need to closely watch the spread of serotype Inaba, as it may cause outbreaks in other parts of India; molecular studies are warranted to understand the widespread emergence of Inaba in north India.

Vibrio cholerae O1 has two biotypes, classical and El Tor, and two major serotypes, Ogawa and Inaba. Strains of the Ogawa serotype express A and B antigens and a small amount of C antigen, whereas the Inaba strains express only the A and C antigens. A third serotype, which possesses all three antigens, is referred to as Hikojima. The Hikojima serotype is both rare and unstable. Among the three serotypes, the Ogawa serotype is most prevalent. Cholera is known to be endemic in north India. This disease shows seasonal variations that coincide with the monsoon months. Our tertiary care referral center at the Postgraduate Institute of Medical Education and Research in Chandigarh, north India, caters to a population of more than 4 million people in and around Chandigarh. Cases are referred from the four nearby states of Punjab, Haryana, Himachal Pradesh, and Uttar Pradesh. In the past, all cases of cholera diagnosed at our center were due to *V. cholerae* O1 serotype Ogawa, including the outbreaks in July 2002 (1), and a recent focal outbreak in July 2004, which occurred from 6th to 8th July in a temporary labor encampment. One hundred persons were affected among 350 laborers and their families (in press). In the present communication, we report the emergence of *V. cholerae* O1 biotype El Tor serotype Inaba, as the dominant serotype in this region. To the best of our knowledge, this is the first report of the isolation and predominance of the Inaba serotype in this region. To further characterize these Inaba strains, we performed toxigenicity testing and antimicrobial susceptibility testing.

Stool samples were collected in Cary Blair medium and in wide-mouthed sterile containers and were transported within 1 h of collection; the samples were bacteriologically examined within 1 h of arrival at the laboratory. Gross examination was followed by microscopic examination for pus cells, red blood cells, ova, cysts, and trophozoites. A hanging-drop preparation was carried out to test the samples for darting motility.

The culture was performed on MacConkey agar, xylose lysine deoxycholate agar, ampicillin blood agar, and thiosulphate citrate bilesalt sucrose agar (Difco Laboratories, Detroit, Mich., USA) as selective media. Enrichment was carried out on alkaline peptone water and selenite F broth. The bacteriological identification was performed according to the standard methods (2). Biotyping was carried out on the basis of sheep red blood cell hemolysis, chick red cell agglutination, and Polymyxin B (50 U) sensitivity (3). Isolates of *V. cholerae* were confirmed by polyvalent O1 and monovalent Inaba and Ogawa antisera obtained from Denka-Seiken, Tokyo, Japan. The minimum inhibitory concentration (MIC) was determined for amoxicillin, tetracycline, trimethoprim, nalidixic acid, chloramphenicol, amikacin, gentamicin, and cefotaxime for 15 isolates of Inaba according to the agar dilution method of the NCCLS using *Escherichia coli* ATCC 25992 as the control (4). The MIC of ciprofloxacin was determined by using the E-test (AB Biodisk, Solna, Sweden). The strains were also tested for toxigenicity using the reverse passive latex agglutination kit (RPLA-LT, Denka-Seiken) according to the manufacturer's instructions.

The first case of *V. cholerae* O1 serotype Inaba was reported on June 7, 2004 from among a cluster of cases of acute watery diarrhea occurring in a Border Security Force camp in Mohali, in Punjab, near Chandigarh. Six patients were suspected to have cholera, from which *V. cholerae* O1 serotypes Ogawa and Inaba were isolated from three and one cases, respectively. After a gap of 1 month, 32 patients (17 males, 15 females; 6 of which were children) of acute watery diarrhea thought to be due to cholera were admitted to our center during the months of July and August; 19 of these patients were from the state of Punjab, 5 from Haryana, 4 from Himachal Pradesh, and 2 each from Uttar Pradesh and Chandigarh. The ages of these patients ranged from 1 to 73 years, with a median of 27 years.

V. cholerae O1 biotype El Tor serotype Inaba was isolated in 15 patients (9 males, 6 females; 3 of which were children). Seven of these patients were from Punjab, 3 each from Haryana and Himachal Pradesh, and 2 were from Chandigarh. No *V. cholerae* was isolated from the other patients, even

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though they presented with typical cholera-like illness; however, many of these patients were already receiving antibiotic therapy when the stool samples were collected. All of the isolates were found to be toxigenic. The antibiotic susceptibility patterns and the MIC data have been summarized in Tables 1 and 2.

V. cholerae O1 strains have been demonstrated to interconvert and to undergo serotype switching between the two known serotypes (5-10) and Hikojima strains, which undergo conversion at an elevated frequency. At the molecular level, a mutation in the *wbeT* region, which codes for O1 antigen biosynthesis, has been observed in cases involving serotype switching (7,9). Serotype switching may be related to immune pressure on the prevailing serotype, as was suggested by observations of an epidemic in Latin America in 1991 (11). In vivo seroconversion has been reported to correlate well with the host immune response, and this finding has been supported by observations using germ-free mice (12), as well as by the results of a study carried out by Sheehy et al. (13).

With the advent of the O139 serogroup in 1992, the Inaba serotype of *V. cholerae* O1 was displaced in Calcutta and other parts of India by the O139 serogroup, and the last Inaba predominance in Calcutta was observed in 1989 (10,14). An outbreak of non-toxicogenic *V. cholerae* O1 Inaba was reported

in Warangal in Maharashtra in 1996 (15). Since then, only sporadic isolations from Delhi in 1998 and Sevagram in November 1999 have been reported in this region (10). These Inaba strains that emerged in different parts of the country in 1998 to 1999 were demonstrated to have evolved from the prevailing *V. cholerae* O1 Ogawa El Tor biotype (10). Serotype Ogawa had been the prevalent serotype at our center until July 9, 2004. Isolation of Inaba from different states in north India has demonstrated that this serotype is widespread in this region.

This serotype has recently caused a huge outbreak of cholera in Delhi, which affected a large number of people in April 2004 (personal communication with Dr. N.C. Sharma, Junior bacteriologist, Infectious Disease Hospital, Delhi, India). In their study, Longini et al. (16) revealed that epidemics of the Inaba serotype were caused after intervals of 12 months by a previously circulating Inaba serotype. These data should serve as a warning to public health officials, who will need to increase their preparedness for an impending epidemic due to *V. cholerae* O1 biotype El Tor serotype Inaba in the next cholera season. The predominance of adults (80%) affected by the new serotype shows a lack of exposure is suggestive of changes occurring at the genetic level.

Although ciprofloxacin resistance has been reported in India (10) in the case of *V. cholerae* O1 biotype El Tor serotype Ogawa, the present Inaba strains were found to be susceptible to ciprofloxacin therapy. The levels of susceptibility to other agents were similar to those to the prevailing Ogawa strains in our region. Thus no change in the treatment protocol is expected. Although cases have been reported in the literature that have been caused by nontoxicogenic strains of *V. cholerae* O1 serotype Inaba (15), the strains isolated at our centre were found to be toxigenic by in vitro testing.

Although some cross-protection between the two serotypes is known to exist due to the shared A antigen, it is nonetheless highly desirable to immunize against both serotypes. Two of the licensed vaccines (old parenteral whole-cell vaccine and the whole-cell recombinant B-subunit vaccine) both have Ogawa and Inaba serotypes. However, some cholera vaccines are based on live-attenuated Ogawa isolates, which requires further consideration.

In conclusion, such sudden and complete serotype switches as reported here should not be ignored; the lessons from previous similar phenomena should be implemented in this case, i.e., future epidemics due to this serotype should be anticipated, and the antigens of Inaba should be strengthened in current vaccine strategies. Lastly, utmost emphasis should be laid on the reporting of all *V. cholerae* O1 cases, with the ultimate goal of understanding the constantly changing and obscure epidemiology of cholera vibrio.

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Table 1. The MIC values of the 15 isolates of *Vibrio cholerae* O1 serotype Inaba

Drug	Range	MIC ₅₀	MIC ₉₀	No. of isolates		
				S	MS	R
Nalidixic acid	2-128	>128	>128	0	0	15
Tetracycline	0.25-2	<0.25	<0.25	15	0	0
Chloramphenicol	0.25-128	8	16	12	3	0
Amoxycillin	2-128	16	16	0	15	0
Trimethoprim	0.5-128	64	64	0	0	15
Amikacin	0.25-4	4	4	15	0	0
Gentamicin	0.25-4	1	2	15	0	0
Cefotaxime	0.25-2	<0.25	<0.25	15	0	0
Ciprofloxacin	0.25-0.5	0.25	0.5	15	0	0

S, susceptible; MS, moderately susceptible; R, resistant.

Table 2. The antibiotic resistance pattern of Inaba isolates

Isolate no.	NA	Tet	Chlor	Amox	Tmp	Ak	G	Cef	Cipro
1	R	S	S	MS	R	S	S	S	S
2	R	S	S	MS	R	S	S	S	S
3	R	S	S	MS	R	S	S	S	S
4	R	S	S	MS	R	S	S	S	S
5	R	S	S	MS	R	S	S	S	S
6	R	S	S	MS	R	S	S	S	S
7	R	S	S	MS	R	S	S	S	S
8	R	S	S	MS	R	S	S	S	S
9	R	S	S	MS	R	S	S	S	S
10	R	S	S	MS	R	S	S	S	S
11	R	S	S	MS	R	S	S	S	S
12	R	S	S	MS	R	S	S	S	S
13	R	S	MS	MS	R	S	S	S	S
14	R	S	MS	MS	R	S	S	S	S
15	R	S	MS	MS	R	S	S	S	S

NA, nalidixic acid; Tet, tetracycline; Chlor, chloramphenicol; Amox, amoxycillin; Tmp, trimethoprim; Ak, amikacin; G, gentamicin; Cef, cefotaxime; Cipro, ciprofloxacin.

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