Vibrio mimicus Diarrhea following Ingestion of Raw Turtle Eggs

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Clinical and epidemiological characteristics of diarrhea associated with *Vibrio mimicus* were identified in 33 hospitalized patients referred to the Costa Rican National Diagnostic Laboratory Network between 1991 and 1994. The relevant symptoms presented by patients included abundant watery diarrhea, vomiting, and severe dehydration that required intravenous Dhaka solution in 83% of patients but not fever. Seroconversion against *V. mimicus* was demonstrated in four patients, from whom acute- and convalescent-phase sera were obtained. Those sera did not show cross-reaction when tested against *Vibrio cholerae* O1 strain VC-12. All the *V. mimicus* isolates from these cases produced cholera toxin (CT) and were susceptible to commonly used antibiotics. Attempts to isolate this bacterium from stool samples of 127 healthy persons were not successful. Consumption of raw turtle eggs was recalled by 11 of the 19 (58%) individuals interviewed. All but two *V. mimicus* diarrheal cases were sporadic. These two had a history of a common source of turtle (*Lepidochelys olivacea*) eggs for consumption, and *V. mimicus* was isolated from eggs from the same source (a local market). Among the strains, variations in the antimicrobial susceptibility pattern were observed. None of the strains recovered from market turtle eggs nor the four isolates from river water showed CT production. Further efforts to demonstrate the presence of CT-producing *V. mimicus* strains in turtle eggs were made. Successful results were obtained when nest eggs were tested. In this case, it was possible to isolate CT- and non-CT-producing strains, even from the same egg. For CT detection we used PCR, enzyme-linked immunosorbent assay (ELISA), and Y-1 cell assay, obtaining a 100% correlation between ELISA and PCR results. Primers Col-1 and Col-2, originally described as specific for the *V. cholerae* O1 *ctxA* gene, also amplified a 302-bp segment with an identical restriction map from *V. mimicus*. These results have important implications for epidemiological surveillance in tropical countries where turtle eggs are used for human consumption, serving as potential sources of cholera-like diarrhea.

Aside from *Vibrio cholerae* O1, other bacteria have also been implicated as etiologic agents of cholera-type diarrhea. All of these bacteria have the ability to produce cholera toxin (CT), the main agent responsible for the characteristic clinical presentation of the diarrhea (14, 15). With the exception of *V. cholerae* O1 and *V. cholerae* O139, none of the vibrios have been implicated in large outbreaks or epidemics (4). Among these bacteria, *Vibrio mimicus* is a diarrhea-causing agent (10), able to produce not only CT but also other types of toxins which might also increase its pathogenicity (7, 8, 17, 21).

Literature on the clinical and epidemiological characteristics of *V. mimicus* gastrointestinal infections is scanty. The few clinical studies conducted in Bangladesh and the United States reported that among the strains isolated, only 10 to 35% produced a toxin that was immunobiologically identical to the CT of *V. cholerae* O1 (5, 18, 19). Nonetheless, some investigators believe that the isolation of *V. mimicus* from diarrheal stools should be regarded as potentially significant (18).

V. mimicus has also been isolated from a number of environmental sources, including oysters, prawns, rivers, and brackish waters (2, 5, 9, 10, 18). Nonetheless, few of those studies have investigated the toxigenicity of these environmental strains. In Bangladesh, for example, CT production was demonstrated only in 1% of the *V. mimicus* isolates from aquatic environments (5). The significance of toxigenic environmental strains is unknown, and specific studies are required to elucidate their epidemiological relevance and their role in the pathogenesis of diarrheal diseases.

In Costa Rica, the National Laboratory Network established in 1991 the use of thiosulfate-citrate-bile salts-sucrose agar for the routine surveillance of *V. cholerae* O1 in stool, water, and food samples. Consequently, it was possible for the local laboratories to recognize the presence of other vibrio species from patients with cholera-like diarrhea. From this point on, the National Cholera Reference Center began to receive *V. mimicus* strains and search for environmental sources of this organism.

In this paper, we describe the clinical presentation and epidemiological characteristics of *V. mimicus*-associated diarrhea in Costa Rica. We also report for the first time the isolation of *V. mimicus* from turtle (*Lepidochelys olivacea*) eggs and the role of the eggs as a risk factor for *V. mimicus* diarrhea.

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MATERIALS AND METHODS

Patient samples. The 33 diarrheal patients included in this study were admitted to the emergency units of hospitals or clinics of the Costa Rican National Health Security System from April 1991 to December 1994. All patients had cholera-like diarrhea at the time of admission. The local laboratories performed the routine stool culture, which included media for enteric pathogens, 6-h enrichment in alkaline peptone water (pH 8.9 to 9.1), and then subculture into

thiosulfate-citrate-bile salts-sucrose agar, following standard procedures (14, 15). We tried to obtain acute- and convalescent-phase sera from all patients, but it was possible to recover a second serum sample in only four cases.

Most samples were sent to the National Cholera Reference Center as isolated *V. mimicus* strains for completion of biochemical identification and toxin determination and to monitor the antibiotic susceptibility pattern. Serum samples were also referred for the serological test.

Clinical and epidemiological information was obtained through record files and patient interviews.

Serum analysis. Agglutinating antibodies against *V. mimicus* were titrated by procedures previously described for *V. cholerae* (1). Each patient serum sample was titrated, in duplicate and on two different days, against the strain isolated from its own stool sample, three *V. mimicus* isolates from other patients, and *V. cholerae* O1 strain VC-12. Three acute- and convalescent-phase serum samples from confirmed cholera patients were also included to determine the titer of cross-reacting agglutinating antibodies against *V. mimicus*.

Normal stool samples. Stool specimens were collected from a total of 127 individuals without evidence of diarrhea within the 2 weeks before sampling. Individuals were selected to represent the same sex, age group, and area of residence as the diarrheal patients. These samples were analyzed by the same procedures used for diarrheal stools (14, 15).

***V. mimicus* isolation from turtle eggs.** The first 22 eggs (*L. olivacea*) analyzed were obtained from the same shop in Cartago's market from where two patients with apparently independent but simultaneous cases of *V. mimicus* diarrhea obtained raw eggs for consumption. The isolation of *V. mimicus* from these eggs was done as follows: previously rinsed with sterile distilled water, the eggshells were aseptically opened, and whole egg contents were homogenized in alkaline peptone water, incubated for 6 h at 37°C, and subcultured in thiosulfate-citrate-bile salts-sucrose agar by conventional methods (13). Individual *V. mimicus* colonies were subjected to toxin analysis.

In another experiment, 90 turtle eggs were sampled directly from the turtle nests on Ostional Beach, Guanacaste, Costa Rica, because this area is the source of most of the commercial turtle eggs in the country. An average of five *V. mimicus* colonies per positive egg were independently analyzed for toxin production, as described for the market egg samples above.

Toxigenicity assays. *V. mimicus* strains were studied for heat-labile CT production by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody CT-1 (CT-B), which was kindly provided by M. L. Tamplin, University of Florida (22), and/or by the Y-1 adrenal cell assay (11). Thirty-nine isolated strains were tested for the presence of the *ctxA* gene by PCR using primers Col-1 (5′-CTCAGACGGGATTTGTTAGGCACG-3′, positions 197 to 220) and Col-2 (5′-TCTATCTCTGTAGCCCCTATTACG-3′, positions 475 to 498), provided by L. M. Sánchez, University of Stanford at Mexico, by the procedure described by Shirai et al. (20). Briefly, colonies from an overnight culture on Trypticase soy agar–1% NaCl were resuspended in 0.85% NaCl to a final concentration of microorganisms equivalent to a McFarland no. 2 standard. Of this suspension, 1 ml was boiled for 10 min and centrifuged for 5 min at 13,600 × *g*. Two microliters of the supernatant was used for PCR as described by Shirai et al. with the following modifications: the final volume of the PCR mixture was 50 μl, no hot start was done, the round of amplifications was repeated for 35 cycles, and we included a final extension at 72°C for 7 min. The product was visualized under UV light after electrophoresis with molecular weight markers on a 1% agarose gel (Sigma type IV) stained with ethidium bromide. Twenty-five microliters of the PCR product from *V. cholerae* 2164-78 and 569B and from *V. mimicus* SOS-471-01-94 and SOS-610-11-94 was digested for 1 h with *Hint*I and *Taq*I (Boehringer Manheim) and *Dde*I (Promega), as per the manufactures' recommendations, in a total volume of 30 μl. Electrophoresis was performed on a 2% agarose gel (Boehringer; electrophoresis grade) in Tris-borate-EDTA and visualized with UV light after ethidium bromide staining. The following strains were used as controls: toxigenic *V. cholerae* O1 (strains 2164-78, VC-12, and 569B) and nontoxigenic *V. cholerae* O1 (strain X-316).

Antibiotic susceptibility testing. Susceptibility to nalidixic acid, amikacin, ampicillin, chloramphenicol, doxycycline, erythromycin, furazolidone, gentamicin, penicillin G, tetracycline, and trimethoprim-sulfamethoxazole (1.25:23.75) was evaluated by the agar diffusion method (3). The results obtained from 28 clinical *V. mimicus* strains were compared with the susceptibility patterns of 24 strains isolated from raw turtle eggs and four from water environments.

RESULTS

From 1991 to 1994, the National Cholera Reference Center received 33 *V. mimicus* isolates from the National Laboratory Network. The sources of the isolates are shown in Table 1. Clinical strains were obtained pure or as the predominant bacteria from stool samples of patients who required outpatient attention or were hospitalized because of the severity of the diarrhea. The clinical presentation was characterized by typical rice-water diarrhea (70%; one patient also had leukocytes in the stool), other types of diarrhea without blood and/or

TABLE 1. Detection of CT in *V. mimicus* strains by Y-1 cell assay, PCR, and ELISA

Source of strain	No. of CT-positive strains/total no. analyzed by:			
	Y-1 cell assay		*ctxA* PCR	ELISA[a]
	CyT[b]	CyL[c]		
Human (diarrheal stool)	2/2	26/26	15/15	25/25
River water	0/2	2/4	0/4	0/4
Turtle eggs				
Cartago's market	1/11	2/12	0/8	0/12
Ostional Beach	ND[d]	ND	6/12	ND
Reference strains *V. cholerae* O1				
2164-78	+	−	+	+
569B	+	−	ND	+
VC-12	ND	ND	+	ND
X-316	−	−	−	−

[a] Using anti-CT-B class I monoclonal antibody.
[b] CyT, cytotoxic activity. +, more than 10% rounded cells per well. This effect could not be evaluated in 24 human, 2 river, and 7 turtle egg strains because of the presence of a strong cytolytic activity effect.
[c] CyL, cytolytic activity. +, 50% or more dead, lysed, and/or detached cells.
[d] ND, not done.

leukocytes (30%), vomiting (100%), and cramps (93%); three of seven patients made reference to abdominal pain. None of the patients presented fever. Eighty-three percent of them required intravenous Dhaka solution, and 17% required oral rehydration solutions. Among the former patients, one suffered hypovolemic shock and another experienced renal failure. All patients showed a positive response to treatment with the rehydration solutions and antibiotics conventionally used for cholera cases.

Ninety percent of the patients were males, and 90% were adults (above 20 years old). Cases were detected in different geographic regions of Costa Rica; only seven came from counties next to the coast, but none of those patients had a history of recent contact with seawater. Furthermore, *V. mimicus* was not found in any of the stool samples of 127 individuals without symptoms of diarrhea during the previous 2 weeks.

The serological analysis of the acute- and convalescent-phase sera from four *V. mimicus* diarrheal patients demonstrated an increase in the agglutinating antibodies when titrated against the strain isolated from their own fecal samples, while titers against *V. cholerae* O1 strain VC-12 remained <1:40. *V. mimicus* agglutinating antibodies were also measured in four convalescent-phase serum samples from confirmed cholera cases with vibriocidal antibody titers of >1:640 against *V. cholerae* O1 strain VC-12. All of these sera gave titers below 1:40 against *V. mimicus* (data not shown).

We were able to interview 19 *V. mimicus* diarrheal patients. Eleven (58%) recalled eating raw turtle eggs within a week before the onset of the diarrhea. The same question was offered to 74 persons without diarrhea, matched by age group, sex, and area of residence. Among them, only two persons (3%) gave an affirmative answer. Other possible sources of infection were investigated, especially with regard to the ingestion of raw seafood (fish, shrimp, and bivalves). Although these sea products are commonly consumed by Costa Ricans, a clear difference in the frequency of consumption (within 1 week before the interview) was not observed between the group of people who suffered *V. mimicus* diarrhea and the group without diarrhea.

All but two cases of *V. mimicus* diarrhea were sporadic. The two patients involved had history of a common source of turtle

eggs for consumption. In 22 eggs sampled from the same source (Cartago's market), 4 were positive for *V. mimicus* and 12 individual isolates were obtained.

Regardless of origin of isolation, all *V. mimicus* strains were resistant to penicillin G and susceptible to nalidixic acid, chloramphenicol, furazolidone, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole. None of the isolates from humans showed resistance to doxycycline, amikacin, ampicillin, or erythromycin; of the 24 isolates from eggs, 25% were ampicillin resistant and 4% were erythromycin resistant; and of the four strains from water, three were doxycycline resistant, two were ampicillin resistant, and one was amikacin resistant.

All *V. mimicus* clinical strains exhibit CT production. In contrast, none of the 4 isolates from river water nor any of the 12 isolates from turtle eggs from Cartago's market produced CT (Table 1). From the turtle eggs obtained directly from the nests at Ostional Beach, toxigenic and nontoxigenic *V. mimicus* strains were successfully recovered. As shown in Table 1, CT detection by the Y-1 cell assay was not always possible because of the strong cytolytic effects of the supernatants. This effect was more commonly observed among the isolates from humans.

PCR of *V. mimicus* and *V. cholerae* DNA with Col-1 and Col-2 primers amplified products of identical size (302 bp). The restriction patterns of these products were also identical. *Taq*I gave fragments of 241 and 60 bp, *Hin*fI gave fragments of 211 and 90 bp, and *Dde*I gave fragments of 233 and 68 bp (data not shown). There were no discrepancies between PCR and ELISA results for the 20 *V. mimicus* strains analyzed by both techniques.

DISCUSSION

Even though *V. mimicus* has been documented as responsible for various types of gastrointestinal and extraintestinal human illness, little is known about its clinical presentation (10, 18, 19). There is epidemiological evidence pointing to an environmental origin for these infections, but no previous studies have been able to isolate toxigenic *V. mimicus* from the suspected foods (19). Investigations searching for possible sources of *V. mimicus* infection have been conducted in different countries, including Costa Rica, and have found this bacterium in a variety of aquatic environments, such as seafood (like fish, shrimp, and bivalves), freshwater, brackish water, seawater, mud samples, and water plants (2, 9, 10, 23).

It has been shown that some *V. mimicus* strains are able to produce a heat-labile enterotoxin, functionally and immunobiologically related to the heat-labile CT (12, 22), as well as other toxins and toxic substances that might contribute to its pathogenesis (5, 7, 8, 12). Nevertheless, as in the case of *V. cholerae* O1, CT is probably the main factor responsible for the severity of this diarrhea.

Publications from Bangladesh, Calcutta (India), and the United States reported that only 10 to 35% of the *V. mimicus* found in clinical specimens produced CT. These data may explain why most of the patients studied (60 to 80%) presented a mild form of diarrhea and were attended as outpatients, treated only with oral rehydration solutions (18, 19). In contrast, all the patients that we studied were hospitalized as suspected cholera cases, 83% of them requiring intravenous rehydration. All the strains derived from these patients were CT producers. This high occurrence of CT-producing strains is similar to the results obtained from a collection of eight clinical isolates referred from Bangladesh (12).

The investigation of CT production from clinical strains was difficult using the Y-1 assay, because all cell-free supernatants had cytolytic activity. Therefore, we used the PCR and ELISA techniques to identify the CT-positive strains. For PCR, we used the Col-1 and Col-2 primers, because although they were originally described as specific for *V. cholerae* O1 *ctxA* (20), we could obtain a 302-bp product from *V. mimicus* which had a restriction pattern identical to the *ctxA* product of *V. cholerae*, according to the sequence published by Mekalanos et al. (16). Even more, the results from ELISA and PCR were always in agreement (Table 1). Therefore, PCR using Col-1 and Col-2 is not specific for *V. cholerae* CT, as originally described by Shirai et al. (20), and should not be used as the only means for *V. cholerae* O1 detection in food and environmental samples.

In Costa Rica, the majority of the patients identified with *V. mimicus* diarrhea were adult males, differing from the pattern in Bangladesh, where no specific sex or age distribution was observed (18). As described in the literature, these diarrheas appeared sporadically and no secondary cases were observed (18, 19).

When patients in this study were asked about suspected seafood consumption, 58% recalled the ingestion of raw turtle eggs within the week before the onset of illness. In Costa Rica, there are several beach areas for turtle nesting sites and turtle eggs are traditionally served in cantinas as a complement of alcoholic beverages. This practice is more common among adult males and could explain, at least in part, the sex and age distribution that we found. For these reasons, we also investigated the frequency of consumption of raw turtle eggs among healthy people with similar characteristics. We found that although this is a traditional food, only 3% of the informants without diarrhea consumed this product during the week before the interview. Also, none of 127 stool samples from adults without diarrhea was positive for *V. mimicus*. On the basis of these observations and the occurrence of two simultaneous *V. mimicus* cases in which the patients recalled consumption of turtle eggs from the same market shop, turtle eggs were sampled, and from them it was possible to demonstrate the presence of *V. mimicus*. Furthermore, it was also interesting that among *V. mimicus* diarrheal cases, three were children. From two of them there was no information about ingestion of suspected foods. Parents from the third child recalled that although she did not eat turtle eggs, she was playing with them within the week before the onset of diarrhea.

When we studied the toxigenicity of strains obtained from turtle eggs, we isolated CT- and non-CT-producing strains, even from the same egg. In contrast to clinical isolates, it was rare to find cytolytic activity in the cell-free supernatants. Four isolates from river water showed cytolytic activity, but none produced CT. Previous studies have also shown that very few (1%) of the environmental *V. mimicus* isolates were CT positive (5, 23). Although CT is probably the main toxin responsible for the cholera-type diarrhea, the significance of isolating non-CT-producing strains from diarrheal patients in other studies merits further investigation concerning the presence of other toxins.

With regard to antimicrobial susceptibility patterns, the clinical isolates of *V. mimicus* were all susceptible to conventional antibiotics used for cholera patients, in accord with observations made in other countries (6, 18). Although the excretion of viable cells was not evaluated after the onset of therapy, some patients continued with diarrhea 2 to 3 days after initiation of the specific chemotherapy, suggesting a behavior similar to the CT effect in cholera. Among environmental strains, the ones isolated from river water presented variations in the antibiotic susceptibility patterns, similar to the findings of Chowdhury et al. (6).

Since there have been no previous studies on the serological

reactions of patients with *V. mimicus* diarrhea, the microagglutination technique for *V. cholerae* O1 was adapted and used to evaluate seroconversion in four patients. With this technique it was possible to demonstrate specific seroconversion against *V. mimicus*, and no significant cross-reaction with *V. cholerae* O1 strain VC-12 was observed. This seroconversion supports the role of the *V. mimicus* isolates as etiologic agents of diarrhea. Further serological and toxigenic characterization of the clinical and environmental isolates might contribute to the understanding of the possible association of those features with the clinical presentation, source of isolation, and geographical distribution of this pathogen.

This is the first report of the isolation of *V. mimicus* from turtle eggs. Our findings strongly suggest the role of these eggs as vehicles for acquiring *V. mimicus* diarrhea. The sources of *V. mimicus* turtle egg contamination and the kinds of toxins present in these bacterial strains are under current investigation.

REFERENCES

1. **Ahmed, A., A. K. Bhattacharjee, and W. H. Mosley.** 1970. Characteristics of the serum vibriocidal and agglutinating antibodies in cholera cases and in normal residents of the endemic and non-endemic cholera areas. J. Immunol. **105:**431–441.

2. **Antillón, F., and E. Rodríguez.** 1992. Aislamiento de *Vibrio cholerae* no-O1 en el Golfo de Nicoya, Costa Rica. Rev. Biol. Trop. **40:**231–232.

3. **Barry, A. L., and C. Thornsberry.** 1991. Susceptibility tests: diffusion test procedures, p. 1117–1125. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

4. **Cholera Working Group, International Centre for Diarrhoeal Diseases Research, Bangladesh.** 1993. Large epidemic of cholera-like disease in Bangladesh caused by *Vibrio cholerae* O139 synonym Bengal. Lancet **342:**387–390.

5. **Chowdhury, M. A. R., K. M. S. Aziz, B. A. Kay, and Z. Rahim.** 1987. Toxin production by *Vibrio mimicus* strains isolated from human and environmental sources in Bangladesh. J. Clin. Microbiol. **25:**2200–2203.

6. **Chowdhury, M. A. R., K. M. S. Aziz, Z. Rahim, and B. A. Kay.** 1986. Antibiotic resistance patterns of *Vibrio mimicus* isolated from human and environmental sources in Bangladesh. Antimicrob. Agents Chemother. **30:**622–623.

7. **Chowdhury, M. A. R., R. T. Hill, and R. R. Colwell.** 1994. A gene for the enterotoxin zonula occludens toxin in *Vibrio mimicus* and *Vibrio cholerae* O139. FEMS Microbiol. Lett. **119:**377–380.

8. **Chowdhury, M. A. R., S. Miyoshi, H. Yamanaka, and S. Shinoda.** 1991. Virulence potentials of *Vibrio mimicus* in aquatic environments. Biomed. Lett. **46:**97–101.

9. **Chowdhury, M. A. R., Y. Yamanaka, S. Miyoshi, K. M. S. Aziz, and S. Shinoda.** 1989. Ecology of *Vibrio mimicus* in aquatic environments. Appl. Environ. Microbiol. **55:**2073–2078.

10. **Davis, B. R., G. R. Fanning, J. M. Madden, A. G. Steigerwalt, H. B. Bradford, J. R. H. Smith, Jr., and D. J. Brenner.** 1981. Characterization of biochemically atypical *Vibrio cholerae* strains and designation of a new pathogenic species, *Vibrio mimicus*. J. Clin. Microbiol. **14:**631–639.

11. **Donta, S. T., H. W. Moon, and S. C. Whipp.** 1974. Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. Science **183:**334–336.

12. **Dotevall, H., G. Jonson-Strömberg, S. Sanyal, and J. Holmgren.** 1985. Characterization of enterotoxin and soluble hemagglutinin from *Vibrio mimicus*: identity with *V. cholerae* O1 toxin and hemagglutinin. FEMS Microbiol. Lett. **27:**17–22.

13. **Elliot, E. L., C. A. Kaysner, and M. L. Tamplin.** 1992. *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and other *Vibrio* spp., p. 111–140. *In* Food and Drug Administration (ed.), Bacteriological analytical manual. AOAC International, Arlington, Va.

14. **Farmer, J. J., and M. T. Kelly.** 1991. *Enterobacteriaceae*, p. 360–383. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

15. **Kelly, M. T., F. W. Hickman-Brenner, and J. J. Farmer III.** 1991. *Vibrio*, p. 384–395. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

16. **Mekalanos, J. J., D. J. Swartz, and G. D. N. Pearson.** 1983. Cholera toxin gene: nucleotide sequence, deletion analysis and vaccine development. Nature (London) **306:**551–557.

17. **Ramamurthy, T., M. J. Albert, A. Huq, R. R. Colwell, Y. Takeda, T. Takeda, T. Shimada, B. K. Mandal, and G. B. Nair.** 1994. *Vibrio mimicus* with multiple toxin types isolated from human and environmental sources. J. Med. Microbiol. **40:**194–196.

18. **Sanyal, S. C., M. I. Huq, P. K. B. Neogi, L. Alam, M. I. Kabir, and A. S. M. H. Rahaman.** 1983. *Vibrio mimicus* as an aetiologic agent of diarrhoea and its pathogenesis. Indian J. Med. Microbiol. **1:**1–12.

19. **Shandera, W. X., J. M. Johnston, B. R. Davis, and P. A. Blake.** 1983. Disease from infection with *Vibrio mimicus*, a newly recognized *Vibrio* species. Ann. Intern. Med. **99:**169–171.

20. **Shirai, H., M. Nishibuchi, T. Ramamurthy, S. K. Bhattacharya, S. C. Pal, and Y. Takeda.** 1991. Polymerase chain reaction for detection of cholera enterotoxin operon of *Vibrio cholerae*. J. Clin. Microbiol. **29:**2517–2521.

21. **Spira, W. M., and P. J. Fedorka-Cray.** 1984. Purification of enterotoxins from *Vibrio mimicus* that appear to be identical to cholera toxin. Infect. Immun. **45:**679–684.

22. **Tamplin, M. L., R. Jalali, M. K. Ahmed, and R. R. Colwell.** 1990. Variation in epitopes of the B subunit of *Vibrio cholerae* non-O1 and *Vibrio mimicus* cholera toxins. Can. J. Microbiol. **36:**409–413.

23. **Venkateswaran, K., C. Kiiyukia, M. Takaki, H. Nakano, H. Matsuda, H. Kawakami, and H. Hashimoto.** 1989. Characterization of toxigenic vibrios isolated from the freshwater environment of Hiroshima, Japan. Appl. Environ. Microbiol. **55:**2613–2618.