Pandemic Serovars (O3:K6 and O4:K68) of *Vibrio parahaemolyticus* Associated with Diarrhea in Mozambique: Spread of the Pandemic into the African Continent

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Received 9 November 2004/Returned for modification 5 January 2005/Accepted 24 February 2005

Forty-two episodes of *Vibrio parahaemolyticus* infections were detected in Beira, Mozambique, from January to May 2004. The majority of the isolates (81%) belonged to the pandemic serovars (O3:K6 and O4:K68) of *V. parahaemolyticus*. The pandemic serovars were positive by group-specific PCR (GS-PCR) and a PCR specific for open reading frame ORF8 (ORF8-PCR), which are molecular markers of the pandemic clone, and were positive for *tdh* but negative for *trh*. The remaining 19% of the strains also possessed the *tdh* gene but were GS-PCR and ORF8-PCR negative and did not belong to the pandemic serovars. Patients with *V. parahaemolyticus* infection were older (mean age, 27 years) than patients infected by other diarrheal agents (mean age, 21 years). Ten percent of diarrhea patients from whom no *V. parahaemolyticus* was cultured were severely dehydrated, but none of the *V. parahaemolyticus* cases were severely dehydrated. This is the first report of the isolation of pandemic strains of *V. parahaemolyticus* in sub-Saharan Africa and clearly indicates that the pandemic of *V. parahaemolyticus* has spread into the African continent.

Vibrio parahaemolyticus is a seafood-borne pathogen which can cause gastroenteritis in humans. They are gram-negative, halophilic bacteria that inhabit marine and estuarine environments. *V. parahaemolyticus* was first isolated in Japan in 1950 as a cause of food-borne illness. This organism is associated with bacterial gastroenteritis in the United States (8, 15) and Europe, and it is one of the most important food-borne pathogens in Asia, causing approximately half of the food-poisoning outbreaks in Taiwan, Japan, Vietnam, and southeast Asian countries (3, 5). To date, 75 different combinations of O and K serotypes of *V. parahaemolyticus* are recognized (10, 11). All *V. parahaemolyticus* strains carrying the *tdh* gene, which encodes thermostable direct hemolysin (TDH), or the *trh* gene, which encodes TDH-related hemolysin, or both genes, can cause gastroenteritis in humans (17). Environmental strains of *V. parahaemolyticus* rarely possess either of these genes.

Generally, gastroenteritis caused by *V. parahaemolyticus* is a multiseroovar affliction. However, from February 1996 an abrupt increase in the incidence of *V. parahaemolyticus* was recorded, and all the strains were identified as belonging to serovar O3:K6 (19). This clone carried the *tdh* gene but lacked the *trh* gene and had a unique arbitrarily primed PCR pattern (14, 19). In subsequent years, *V. parahaemolyticus* isolates belonging to this serovar caused several outbreaks in five Asian countries, Canada, the United States, and Russia. The rapid spread of the O3:K6 strains to different countries after 1996

marked the beginning of the first pandemic of *V. parahaemolyticus* (14). This was the first occurrence of a single serotype-associated spread of *V. parahaemolyticus*. The newly recognized serovar also had seven base changes in the *toxRS* operon. This polymorphism in the *toxRS* operon was exploited to develop a group-specific PCR (GS-PCR), which has been used as a molecular marker for the identification of the pandemic clone of *V. parahaemolyticus* (14). The gene coding the filamentous phage f237 is unique for the pandemic *V. parahaemolyticus* clone (4, 16). One of the open reading frames (ORFs) of the f237 phage genome, ORF8, was proposed as a marker of the pandemic clone (10, 16). However, GS-PCR-positive strains lacking ORF8 were recently reported (3, 13) among the pandemic strains.

Increased rates of isolation of the pandemic serovars of *V. parahaemolyticus* in Asia and the United States since 1996 have suggested a *V. parahaemolyticus* pandemic (14, 18, 20, 21). Besides O3:K6, it has been reported that 10 other serotypes (O4:K68, O1:K25, O1:K41, O1:KUT, O1:K56, O3:K75, O4:K8, O4:K12, O4:KUT, and O5:KUT, where UT indicates untypeable) have emerged and have been shown to belong to the pandemic clone by molecular typing techniques (5, 13, 22). Molecular analysis of the pandemic strains indicated that some of these serotypes, especially O4:K68 and O1:KUT, might have diverged from the pandemic strain O3:K6 by alteration of the O and K antigens and by serovar transition (5, 6).

There is no information on *V. parahaemolyticus* infections in Mozambique or sub-Saharan Africa. During surveillance for cholera in Beira, Mozambique, from December 2003 to January 2004, a large number of *V. parahaemolyticus* isolates were detected. In this study, we describe the microbiological and

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preliminary epidemiological aspects of *V. parahaemolyticus* infections in Beira, Mozambique.

MATERIALS AND METHODS

Study site and population. The port city of Beira, located in Sofala Province, is the second largest city in Mozambique, after Maputo. Beira was built on swampy grounds at the mouth of Pungwe River and has a population of approximately 450,000, divided into 22 neighborhoods (*bairros*). Many areas of Beira are located below sea level. Cholera is endemic in Beira, with cases usually detected from January to June during the rainy season (7). The characteristic marshy areas with brackish water, periodic flooding during the rainy season, the common practice of defecation in open areas, the presence of nontight latrines, and the drainage of municipal waste into the embankments maintain the risk for diarrheal diseases in the area (1). The Cholera Treatment Center is the only site in Beira for the treatment of severe watery diarrhea. Cases presenting to other health care facilities are transferred to the Cholera Treatment Center.

Surveillance. The port city of Beira has a population of approximately 450,000, divided into 22 districts (*bairros*). The source population for the cases and the controls was the residents of Esturro (a district in the center of Beira). Patients who presented to the Cholera Treatment Center with a history of acute, nonbloody diarrhea from 1 January and 31 May 2004 were eligible as a case if they fulfilled the following criteria: (i) consent or, in the case of minors, guardian or parental consent to participate in the study; (ii) a resident of Esturro since 11 December 2003; (iii) not pregnant, if female; and (iv) at least 2 years of age during the time of the mass vaccination. After the initial medical assessment and rehydration were completed, a case report form was completed and a stool specimen was collected from all enrolled cases. The case report form included questions on demographics, medical history, treatment, environmental and socioeconomic factors, and information about oral cholera vaccination. By using the address and directions obtained at the Cholera Treatment Center, each case's household was located to verify residence in Esturro and to check his or her vaccination card (if it was not available during presentation at the Cholera Treatment Center).

Stool specimens or rectal swabs were collected from Esturro residents over 2 years of age presenting to the Cholera Treatment Center with acute nonbloody diarrhea. In addition one specimen was collected daily from a patient residing in a *bairro* other than Esturro. The clinical features of all diarrhea patients were recorded on standardized case report forms. Severe dehydration was defined according to World Health Organization guidelines based on clinical presentation (24).

Stool culture. The nonbloody stool samples or rectal swabs (RS) were collected in Cary-Blair transport medium and transported at ambient temperature to the laboratory within 2 h. The stool samples or RS were plated directly on the thiosulfate citrate bile salt sucrose (TCBS) agar (Eiken, Japan) and taurocholate tellurite gelatine agar (TTGA). The specimens were also enriched in alkaline peptone water for 6 h (pH 8.6, 37°C) (23) and then plated on TCBS agar and TTGA. Sucrose-nonfermenting green colonies on the TCBS agar that were oxidase and gelatinase positive and O/129 sensitive on TTGA were initially suspected to be *V. parahaemolyticus* and were stored in Luria agar slants with 3% salt for biochemical identification. Most of the suspected *V. parahaemolyticus* isolates grew as pure cultures on TTGA and TCBS agar plates with the characteristic colony morphology on the culture plates. Confirmation of the isolates as *V. parahaemolyticus* was done by standard methods (12).

O and K serotyping. The O (somatic) and K (capsular) antigen typing of *V. parahaemolyticus* isolates was done by using commercial antisera, according to the protocol and instructions described by the manufacturer (Toshiba Kagaku Kogyo Co., Ltd., Tokyo, Japan).

PCR assays. PCR assays were performed to detect the *toxR*, *tdh*, and *trh* genes of *V. parahaemolyticus* by using the primers described previously (5, 9, 12). GS-PCR and a PCR for ORF8 (ORF8-PCR) were performed to detect molecular markers of the pandemic clone (5, 13, 14). Template DNA was prepared by growing the bacteria in Luria broth (Difco, Detroit, Mich.) with 3% NaCl at 37°C overnight, centrifuging the culture, resuspending the pellet in sterile distilled water, and boiling for 10 min. The PCR products were electrophoresed in a 1% agarose gel stained in ethidium bromide and visualized under UV light with a transilluminator.

Antimicrobial susceptibility test. Antimicrobial susceptibility testing was done by the Kirby-Bauer disk diffusion method (2) with control strain *Escherichia coli* ATCC 25922.

Ethical considerations. This project was approved by the government of Mozambique; the Institutional Review Board of the International Vaccine Institute, Seoul, Korea; and the Secretariat Committee on Research Involving

TABLE 1. Serotypes and genetic traits of *V. parahaemolyticus* strains isolated between 24 February 2004 and 15 May 2004 in Beira, Mozambique

O:K serotype	No. of strains	Presence of the gene and genetic markers				
		Genes			Pandemic markers	
		<i>toxR</i>	<i>tdh</i>	<i>trh</i>	GS-PCR result	ORF8 PCR result
O3:K6	32	+	+	—	+	+
O3:K58	7	+	+	—	—	—
O4:K68	2	+	+	—	+	+
O4:K13	1	+	+	—	—	—

Human Subjects of the World Health Organization, Geneva, Switzerland. Informed consent was obtained verbally at the community level through meetings with community leaders of Beira. Individual written consent was obtained from all cases prior to their participation in the study.

RESULTS

A total of 5,128 diarrhea episodes were treated at the Cholera Treatment Center from 1 January to 31 May 2004. Stool specimens or rectal swabs were collected from 403 diarrhea patients from Esturro. *V. parahaemolyticus* was isolated from 42 stool samples between 24 February and 31 May 2004; 32 of the 42 isolates belonged to serovar O3:K6, and 2 isolates belonged to O4:K68. Seven isolates belonged to serovar O3:K58, and one isolate belonged to O4:K13. All strains of O3:K6 and O4:K68 were positive for *toxR* and *tdh* and were positive by GS-PCR and *orf8*-PCR (Table 1). The serovar O3:K58 and O4:K13 isolates were positive for *toxR* and *tdh* but were negative for *trh*, GS-PCR, and *orf8*-PCR (Table 1).

As shown in Table 2, the *V. parahaemolyticus*-infected patients were older than the other diarrhea patients. None of the 42 patients infected by *V. parahaemolyticus* was under 5 years of age, but 55 of the 361 patients (15%) from whose stool specimens no *V. parahaemolyticus* was isolated were under 5 years of age ($P = 0.007$) (Table 2). Six of 42 of the *V. parahaemolyticus*-infected patients (15%) required intravenous rehydration, whereas 47% (170 of 361) of the patients from whom no *V. parahaemolyticus* was isolated required intravenous rehydration ($P < 0.001$). Similar percentages of *V. parahaemolyticus* patients (81%) and diarrhea patients from whom *V. parahaemolyticus* could not be isolated (74%) presented with vomiting ($P = 0.3$). None of the patients was coinfecting with *V. parahaemolyticus* and *V. cholerae*.

All the strains of serovars O3:K58, O4:K68, and O4:K13 were susceptible to tetracycline, ampicillin, sulfamethoxazole-trimethoprim, nalidixic acid, furazolidone, erythromycin, and ciprofloxacin, whereas all strains of serovar O3:K6 were resistant to ampicillin but susceptible to all other antibiotics. According to both serological and molecular markers, all strains of serovars O3:K6 and O4:K68 belonged to the pandemic genotype (Table 1).

DISCUSSION

The large number of *V. parahaemolyticus* infections during a diarrhea surveillance study in Beira, Mozambique, was a serendipitous finding. The majority (81%) of the strains isolated

TABLE 2. Comparison of nonclinical data and clinical symptoms of patients infected by *V. parahaemolyticus* and diarrheal agents other than *V. parahaemolyticus* in Beira, Mozambique

Type	Subject	Patients infected with:		Total	P value
		Species other than <i>V. parahaemolyticus</i>	<i>V. parahaemolyticus</i>		
Nonclinical data	Enrolled cases	361	42	403	
	No. (%) male	171 (47)	19 (46)	190 (47)	0.7
	Mean age (yrs)	23	27		0.1
	No. (%) of patients 2–5 years of age ^a	55 (15)	0	55 (14)	0.007
Clinical symptoms	No. (%) of patients with diarrhea and vomiting	266 (74)	33 (81)	299 (74)	0.3
	No. (%) of patients with severe dehydration	36 (10)	0	36 (9)	0.03
	No. (%) of patients for whom IV rehydration was required	170 (47)	6 (15)	176 (47)	<0.0001

^a No children under 2 years of age were included.

in the study belonged to serovars O3:K6 and O4:K68 and therefore belonged to the pandemic serogroup. In Asia and the United States, the pandemic strains of *V. parahaemolyticus* have been detected since 1996. This is the first report on the isolation of pandemic serovars of *V. parahaemolyticus* in the African continent. There is therefore a need to consider *V. parahaemolyticus* in the differential diagnosis of etiologic agents for watery diarrhea in individuals residing in Africa or coming from Africa.

Our study had several limitations: children under 2 years were not tested, and surveillance was treatment center based and thereby tended to reflect more severe diarrheal cases in the community. The Cholera Treatment Center was known to the community as a place for the treatment of watery diarrhea; thus, our surveillance may have missed diarrhea cases with nonwatery presentations. The only other enteropathogen tested for in the surveillance was *V. cholerae*; thus, coinfections with *V. parahaemolyticus* and diarrheal agents other than *V. cholerae* may have been more common than our data would suggest. A similar number of patients with *V. parahaemolyticus* and diarrhea caused by other agents presented with vomiting, but severe dehydration was less frequently seen in the *V. parahaemolyticus*-infected patients. This finding may be related to the fact that *V. parahaemolyticus*-infected patients were older than patients with other causes of diarrhea. None of the *V. parahaemolyticus* patients was under 5 years of age. The absence of *V. parahaemolyticus* in patients under 5 years of age is striking and may account for the significant differences in rehydration, and we plan to study this in the future.

This finding may point toward a potential route of transmission. Tuyet and coworkers (21) have described the potential role of the consumption of uncooked or partially cooked seafood in the transmission of *V. parahaemolyticus* in Vietnam. It seems possible that older individuals are at higher risk than younger children for *V. parahaemolyticus* infection because of different exposures. In contrast to Far East Asia, raw seafood is not considered a delicacy in Beira. However, dried shrimp is frequently eaten uncooked, especially in the absence of cooking facilities. Shellfish are a popular source of protein but tend to be eaten cooked. Secondary contamination of foods in kitchens is likely to be a potential route of transmission in settings like this where raw seafood is not consumed.

The focus of our surveillance was on Esturro, a *bairro* with a

mixed labor force. People from rural areas moved into this slum during the civil war, which ended in 1992, because of the higher level of safety in urban areas than in isolated areas. The infrastructure, including the water supply and sanitation, has remained at a minimal level. Outside Esturro, many residents frequently earn a living through agricultural activities in the countryside. Little if any care is paid to food safety. The water supply and sanitation have remained rudimentary in many households. It seems likely that increased attention to hygiene and food safety should reduce the incidence of *V. parahaemolyticus* infections. However, before recommendations on the means of decreasing the risk of *V. parahaemolyticus* infections can be made, a better understanding of the risk factors is needed. Such studies should include individuals from all parts of Beira, as the risk of infection may differ between *bairros*.

ACKNOWLEDGMENTS

We are grateful for the enthusiastic support that we received from our Mozambiquean collaborators. We thank Catarina Mondlane and Raul Vaz for laboratory work.

This work was supported by the Diseases of the Most Impoverished Program, funded by the Bill and Melinda Gates Foundation and coordinated by the International Vaccine Institute. ICDDR,B is supported by the aid agencies of the governments of Australia, Bangladesh, Belgium, Canada, Japan, Kingdom of Saudi Arabia, The Netherlands, Sweden, Sri Lanka, Switzerland, and the United States.

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