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Urea-Hydrolyzing *Vibrio parahaemolyticus* Associated with Acute Gastroenteritis

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A case of gastroenteritis caused by a urea-hydrolyzing strain of *Vibrio parahaemolyticus* is presented. Urea-hydrolyzing strains of *Vibrio parahaemolyticus* have rarely been reported and have not been described previously as a cause of gastroenteritis in the United States. With the exception of urea hydrolysis and the methyl red test, the isolate had all the characteristics of *V. parahaemolyticus*. The need to screen suspicious non-lactose-fermenting colonies from stool specimens with the oxidase test is emphasized.

Vibrio parahaemolyticus produces a self-limiting gastroenteritis, usually after consumption of raw or partially cooked seafood. Reports in the literature describing urea-hydrolyzing *V. parahaemolyticus* strains and their etiological role in disease, however, are rare. Lam and Yeo (4) in Singapore isolated such an organism from the site of a perforated appendix but doubted its causative role in diarrhea. Huq et al. (3) isolated urea-hydrolyzing strains from patients during a *V. parahaemolyticus* epidemic occurring simultaneously with a *Vibrio cholera* epidemic in Bangladesh in 1975. The origin of the outbreak was not specified. This report describes the first account in the United States of a urea-hydrolyzing strain of *V. parahaemolyticus* causing diarrhea after the consumption of raw shellfish.

In October 1981, a 61-year-old previously healthy male developed an abrupt onset of nausea, abdominal cramps, chills, and diarrhea lasting for 48 h. The onset of symptoms occurred 30 h after the patient consumed raw oysters harvested by a commercial processing company in southwestern Washington. The man continued to have abdominal cramping and watery diarrhea and attended an outpatient medical clinic on day 3 of the illness, at which time stool cultures were obtained and an antiperistaltic medication (Lomotil [atropine sulfate-diphenoxylate hydrochloride]) was prescribed. Antibiotic therapy was not instituted. The symptoms persisted for 9 days with a gradual complete resolution. Repeat stool cultures obtained 2 weeks after the onset of illness were negative for *V. parahaemolyticus*.

A sample of stool was streaked onto 5% sheep blood agar, MacConkey agar, salmonella-shigella agar (Difco Laboratories, Detroit, Mich.), and Hektoen-enteric agar (BBL Microbiology Systems, Cockeysville, Md.) plates for isolation of pathogenic enteric bacteria. After overnight incubation at 35°C, heavy growth of *V. parahaemolyticus* was present on all plates, with little accompanying normal enteric flora. Colonies on blood and MacConkey agar were 1.0 mm in diameter at 24 h, enlarging to 5.0 mm with continued incubation at 35°C. Colonies taken from blood agar after 24 h were spot-indole and spot-oxidase positive. The cultures were identified as *V. parahaemolyticus* by using the API 20E system (code 4356106; Analytab Products, Plainview, N.Y.) and confirmed by standard biochemical procedures (1), using purple broth base (Difco) and 1% Seitz filtered carbohydrates (6). The inoculum for the standard biochemical tests consisted of a 4- to 5-h broth culture (Trypticase soy broth, BBL Microbiology Systems) of selected colonies taken from MacConkey agar. Salt tolerance was tested by using 6.5% sodium chloride broth and mannitol salt agar (7.5% sodium chloride). Two drops (0.1 ml) of 20% sodium chloride were added to 3 ml of the methyl red (MR)-Voges Proskauer (VP) broth, nitrate broth, the decarboxylase broths, and nutrient gelatin. All tests were incubated at 35°C and examined daily for 4 days. Esculin hydrolysis and β-D-galactosidase activity were determined by using Minitek disks (BBL Microbiology Systems) and inoculated with four drops (0.2 ml) of the Trypticase soy broth inoculum. The test for the Kanagawa reaction was performed at the Centers for Disease Control, Atlanta, Ga. Antibiotic susceptibility tests were determined by the standardized disk diffusion method (5). Minimal inhibitory concentrations (MICs) were determined by using commercially prepared antibiotic trays (Prepared Media Laboratory, Tualatin, Oreg.). Plates and trays were

TABLE 1. Characteristics of a urea-hydrolyzing strain of *V. parahaemolyticus* at 35°C

Test or substrate[a]	Result[b]	Expected value (%) for *V. parahaemolyticus*[c]
TSI	K/A	K/A
Oxidase	+	100
Motility	+	100
Indole peptone broth	+	98
Indole spot test	+	NT[d]
MR		
0% NaCl	−	NT
1% NaCl	−	NT
3% NaCl	+	NT
VP	−	0
Citrate (Simmons)	+	100
Urease (Christensens)	+	0
Phenylalanine deaminase	−	0
Lysine decarboxylase	+	97
Arginine dihydrolyase	−	0
Ornithine decarboxylase	+	95
Nitrite production	+	NT
Gelatin liquefaction	+	100
Litmus milk peptonization	+	NT
Malonate utilization	−	0
Esculin hydrolysis	−	NT
Starch hydrolysis	+	100
DNase	+	NT
β-D-galactosidase (ONPG)	−	NT
NaCl for growth		
0%	−	0
1%	+	NT
3%	+	100
6.5%	+	NT
7.5%	+	100
Glucose fermentation	+	100
Lactose	−	0
Sucrose	−	0
Maltose	+	100
Mannitol	+	100
Xylose	−	NT
Fructose	+	100
Salicin	−	0
Dulcitol	−	0
Adonitol	−	0
Inositol	−	0
Arabinose	+	78
Raffinose	−	0
Rhamnose	−	0
Sorbitol	−	3
Trehalose	+	100
Cellobiose	+	63

[a] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.
[b] Symbols: +, positive reaction in 1 to 2 days; −, negative reaction at 5 days; K/A, alkaline slant/acid butt with no H_2S.
[c] See reference 2. Based on 100 human isolates.
[d] NT, Not tested.

not supplemented with sodium chloride since the agar and broth media contained adequate salt to sustain growth.

Table 1 shows the results of standard biochemical tests. The organisms failed to grow in the MR-VP broth, nitrate broth, decarboxylase broths, and gelatin medium without added salt. In TSI (BBL Microbiology Systems) agar the slant was alkaline and the butt was acid, with no gas or hydrogen sulfide evident. The citrate utilization test was negative with a fresh isolate but became positive after subculture. The biochemical reactions conformed to the characteristics of *V. parahaemolyticus*. This strain was significantly different, however, in two important aspects. (i) Urea hydrolysis in unsupplemented agar (BBL) was rapid and intense, with the whole of the tube having changed within 24 h of incubation. (ii) The MR test, with and without supplement, was negative before extensive subculture, which contrasts with the expected findings with *V. parahaemolyticus*. The results of the MR test support the findings of Lam and Yeo (4), who reported that their urea-hydrolyzing strain also was unable to lower the pH of the medium to 5.4. Our *V. parahaemolyticus* strain was positive for the Kanagawa phenomenon.

TABLE 2. Susceptibility of a urea-hydrolyzing strain of *V. parahaemolyticus* to 13 antibiotics

Antibiotic	Zone size (mm)	MIC (μg/ml)
Cephalothin	14	8.0
Cephoxitin	25	4.0
Cephataxime	25	NT[a]
Gentamicin	19	<0.5
Kanamycin	17	4.0
Tobramycin	19	1.0
Amikacin	19	2.0
Ampicillin	17	1.0
Carbenicillin	NT	<8.0
Mezlocillin	27	NT
Colistin	10	<4.0
Chloramphenicol	23	<0.5
Tetracycline	19	0.5

[a] NT, Not tested.

The susceptibility pattern of the urea-hydrolyzing strain is shown in Table 2. Mueller-Hinton agar and broth medium for MIC determinations without added salt supported abundant growth of the test organism. The standards of the National Committee for Clinical Laboratory Standards for *Enterobacteriaceae* were used for evaluation (5). The organism was susceptible to all antibiotics tested except cephalothin, which was intermediate in susceptibility. The MICs correlated well with those of the disk diffusion test.

Since urea-hydrolyzing vibrios may easily be confused with other urea-hydrolyzing organisms recovered from stool cultures, the utilization of an oxidase screen of all suspicious non-lactose-fermenting colonies is extremely important. The necessity of prompt and reliable identification of

these strains of *V. parahaemolyticus* is underscored by the fact that this organism was directly associated with severe gastroenteritis.

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