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Urease-Positive *Vibrio parahaemolyticus* Strain

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An unusual strain of *Vibrio parahaemolyticus* was isolated from the site of a perforated appendix. This was the first reported case in which the vibrios demonstrated a positive urease reaction. In other respects, the strain conformed to the general characteristics of *V. parahaemolyticus*. It was susceptible to chloramphenicol, sulfamethoxazole-trimethoprim, tetracycline, neomycin, triple sulpha, gentamicin, and polymyxin B and produced the "Kanagawa phenomenon." However, its role as a causative pathogen of the diarrhea of the patient was debatable.

Since the isolation of *Vibrio parahaemolyticus* by Fujino et al. in 1951 (9), the halophilic vibrios have now been recognized in Japan as a major cause of food poisoning, especially among people who are in the habit of eating raw seafood (15). During the last decade, the occurrence of these vibrios has been reported in many countries (8). In Singapore, the first human strain of *V. parahaemolyticus* was discovered in 1973 (13). Subsequently, several local strains were isolated from marine sources and the stools of patients who had contracted the infection after the consumption of half-cooked shellfish (12).

This report describes the isolation and identification of an unusual strain of urease-positive *V. parahaemolyticus*.

CASE REPORT

A 35-year-old Chinese male was admitted to the Singapore General Hospital in September 1979 complaining of severe abdominal pain associated with vomiting, diarrhea, and a low-grade fever of 1 day's duration. A provisional diagnosis of acute appendicitis was made. At operation, a perforated gangrenous appendix surrounded by blackish fluid, together with some serous fluid in the pelvic cavity and paracolic gutter, were found. An appendectomy and peritoneal toilet were performed. A swab was taken from the peritoneal cavity and sent for bacteriological investigations. Pathological findings confirmed this as a case of acute suppurative appendicitis.

Postoperatively, the patient was treated with ampicillin (500 mg every 6 h for 7 days) and Flagyl (400 mg every 8 h for 5 days). Recovery was uneventful.

MATERIALS AND METHODS

The infected swab was inoculated on eosin methylene blue blood (human) agar and into Robertson cooked meat broth. After an overnight incubation at 37°C, the direct cultures showed a predominance of *Pseudomonas aeruginosa*, with moderate growth of *Escherichia coli*, *Klebsiella* sp., and *V. parahaemolyticus*. As the *V. parahaemolyticus* presented unusual biochemical reactions, we decided to study it further. A series of biochemical tests, as reported by Sakazaki (18), was performed. The procedures have been previously described (13). Antibiotic susceptibility testing according to the Kirby-Bauer method (2) with Sensi-Disc Microbial Susceptibility Discs (BBL Microbiology Systems, Cockeysville, Md.) was carried out on two sets of Mueller-Hinton agar (normal and 3% salted) in six replicate tests. Serological identification of the K-antigen, as recommended by the Committee on the Serological Typing of *V. parahaemolyticus* (4), was determined, whereas the slide agglutination method of Montague et al. (16) was used for the O-antigen

RESULTS AND DISCUSSION

The results of the biochemical studies in Table 1 show that our strain is similar but not identical to the classical *V. parahemolyticus* strain as defined by Sakazaki (18). Feeley and Balows (7) found that the majority of strains were positive for the methyl red reaction, with less than 1% being negative. Our strain, which was negative for the methyl red reaction, could therefore be included in the minority group of *V. parahaemolyticus*. To our knowledge, however, urease-positive *V. parahaemolyticus* has not been reported before. Our strain was strongly urease positive on both normal and salted Christensen urea agar after overnight incubation.

In general, diarrhea caused by *V. parahaemolyticus* is self-limiting. However, it is interesting to study the in vitro antibiotic susceptibility pattern of our strain. Le Clair et al. (14) reported that a high concentration of NaCl had little or no effect on most of the commonly used antibiotics, whereas Stokes and Waterworth (19) in 1972 found that salt might have an adverse effect on some antibiotics belonging to the aminoglycoside group and that inoculum size also affected the zones of inhibition. Hollis et al. (11) tested the susceptibility on normal Mueller-Hinton medium. As shown in Table 2, we observed a sig-

nificant difference in the zone sizes between antibiotic susceptibility tests in the presence and absence of added salt in the medium. The difference indicated a change in susceptibility for kanamycin, streptomycin, and cephaloridine. It may be necessary, therefore, to standardize a method for testing the antibiotic susceptibility of halophilic vibrios. The urease-positive strain was susceptible to chloramphenicol, sulfamethoxazole-trimethoprim, tetracycline, neomycin, triple sulpha, gentamicin, and polymyxin B (300 IU) and resistant to ampicillin and carbenicillin.

Serological analysis showed that the *V. parahaemolyticus* strain belonged to the 01:K1 type.

In 1976, Cook demonstrated that *Streptococcus faecium* could be induced to grow and produce urease in the rumen of sheep, but this property became unstable after isolation (5).

TABLE 1. *Biochemical characteristics of V. parahaemolyticus*[a]

Test	Result
Indole	+
Methyl red	−
Voges-Proskauer	−
Simmons citrate	+
Urease	+
Motility	+
Oxidation-fermentation	F[b]
Hydrogen sulfide	−
Lysine decarboxylase	+
Arginine dihydrolase	−
Ornithine decarboxylase	+
Phenylalanine	−
Malonate	−
Catalase	+
Acid production by:	
Glucose	+
Lactose	−
Sucrose	−
Maltose	+
Mannitol	+
Arabinose	+
Rhamnose	−
Trehalose	+
Xylose	−
Adonitol	−
Dulcitol	−
Inositol	−
Sorbitol	−
Salicin	−
Growth in 1% peptone water plus:	
0% NaCl	−
3% NaCl	+
7% NaCl	+
10% NaCl	−
Kanagawa phenomenon	+

[a] All tubed media for the biochemical tests were added with 3% NaCl.

[b] F, Fermentation.

TABLE 2. *Antibiotic susceptibility of V. parahaemolyticus*[a]

Antibiotic	Disk potency (μg)	Susceptibility[b]	
		0% NaCl	3% NaCl
Chloramphenicol	30	S	S
Ampicillin	10	R	R
Sulfamethoxazole-trimethoprim	25[c]	S	S
Tetracycline	30	S	S
Neomycin	30	S	S
Triple sulpha	250	S	S
Gentamicin	10	S	S
Kanamycin	30	S	I
Streptomycin	10	S	I
Carbenicillin	50	R	R
Cephaloridine	30	S	I
Polymyxin B	300[d]	S	S
Polymyxin B	50[d]	R	R

[a] Growth on the salted Mueller-Hinton agar for antibiotic susceptibility testing was much heavier than on the normal agar after overnight incubation and demonstrated a higher resistance to certain antibiotics.

[b] S, Sensitive; R, resistant; I, intermediate.

[c] Sulfamethasoxazole, 23.75 μg; trimethoprim, 1.25 μg.

[d] In international units.

Collective experimental results, such as the loss of activity in vitro (5) during aerobic growth (6) of plasmid-mediated genetic traits by growth under certain physical conditions and in the presence of curing agents (3, 17), provided evidence that urease in *S. faecium* might be coded on a plasmid. Similarly, the plasmid-borne ability to use citrate was found in *Salmonella typhi* (20). Bacterial resistance to antibiotics also appeared to be coded on plasmids (1). As there is now increasing evidence that there can be other plasmid-coded bacterial characters, it is possible, therefore, that the unusual urease-positive property of our *V. parahaemolyticus* strain may involve a plasmid.

Although *V. parahaemolyticus* was cultured from the peritoneal cavity in a case of acute appendicitis, its role as a causative pathogen for the initial diarrheal condition of the patient was not proven because the patient's stools were not examined for the presence of enteric pathogens and because symptomless carriers of *V. parahaemolyticus* had been detected among our people during an epidemiological survey of a cholera outbreak in September 1978 (10).

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