UNUSUAL *VIBRIO* SPECIES FOUND IN DIARRHOEAL STOOLS

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SYNOPSIS

Two unusual *Vibrio* species (*Vibrio alginolyticus* and Group-F vibrio) were found in the patients' stools. *Vibrio parahaemolyticus* was also isolated with the *V. alginolyticus* from the same patient who had severe diarrhoea with abdominal colic. The patient from which the Group-F vibrio was isolated had recurrent episodes of diarrhoea. Except for the decarboxylation of amino-acids and salt tolerance, the *V. alginolyticus* and the Group-F vibrio shared many similar properties both culturally and biochemically. In the antibiotic susceptibility testing, the *V. alginolyticus* appeared more resistant than the Group-F vibrio. The enterotoxigenic role of these organisms in causing the diarrhoea has not been known.

INTRODUCTION

Two unusual *Vibrio* species - *Vibrio alginolyticus* and Group-F vibrio, were recently isolated from the stools of patients with diarrhoea.

Many reports of *V. alginolyticus* associated with marine environments (1, 2, 3), shellfish (2, 4, 5, 6), tissue infections (7, 8, 9, 10, 11) and blood of human beings (7, 12) who were constantly exposed to sea water, have been cited. Its appearance in stools was also reported by Sakazaki (13) in 1968 and Wachsmuth (14) in 1980.

Group-F vibrio was identified by Furniss et al. (15) in 1975 when the vibrio was isolated from the diarrhoeal stool of a Bahrain patient. Subsequent isolations were reported in the United States and Bangladesh. In 1977 and 1980, Huq et al (16, 17) described their strains as EF-6 which were identical to the Group-F vibrio of Furniss.

In this paper, we present the first isolations of the two halophilic vibrio species from human stools in Singapore, the laboratory findings and the clinical features of the infections.

CASE REPORT I *(V. alginolyticus)*

The patient was a 49 year-old Chinese male admitted to Middleton Hospital for management of his gastroenteritis. He developed diarrhoea on the 12th October, 1980, accompanied by abdominal colic, but had no vomiting or fever. He passed loose yellowish stools 7 or 8 times a day. None of his stools contained blood or mucus. He was not dehydrated and the past history was unremarkable. The stool specimen sent for culture and sensitivity on the 14th October, 1980, grew *V. alginolyticus* and *V. parahaemolyticus*. He recovered rapidly without chemotherapy.

CASE REPORT II (Group-F vibrio)

The patient was a 83 year-old diabetic Chinese female who was admitted to Changi Hospital on the 8th December, 1980, for gangrene of her right foot. A below-knee amputation was performed on the 12th December. She developed diarrhoea, passing loose stools on the 17th and again on the 21st January, 1981. On the 24th January, her diarrhoea became severe and her stools were watery, yellowish and foul-smelling. The patient appeared weak and dehydrated, but she had no fever, abdominal pain or vomiting. One specimen of her stool sent for culture and sensitivity on the 25th January was positive for Group-F vibrio. No other bacterial pathogens were found in her stools. She was given kaolin et opii only to control the diarrhoea. The patient recovered and was discharged on the 4th February, 1981.

METHODS AND MATERIALS

Culture: Watery stools of the patients were examined for bacterial pathogens. Each specimen was inoculated on solid media such as thiosulphate citrate bile salts sucrose (TCBS), MacConkey and blood agar, and into 2% salted-alkaline peptone water (2% salted-APW) and selenite F broth. The liquid cultures were plated on TCBS and eosin methylene blue (EMB) agar accordingly after overnight incubation at 37°C. The stool was also cultured directly on Thayer-Martin medium and incubated at 42°C under microaerophilic condition for 48 hours to detect Campylobacter species.

Biochemical characteristics: Vibrio-like colonies were identified biochemically as described by Tatum (18). All test media were added with 2% NaCl for growth of the halophilic vibrios.

Antibiotic susceptibility was tested by the method of Bauer et al (19) on Mueller-Hinton agar, except that the bacterial lawn was prepared by streaking the agar surface with a cotton swab saturated with a saline cell suspension (approx. 1 x 10^6 cfu per ml.) in three different planes.

RESULTS

The two *Vibrio* species produced similar reactions on the different culture media after overnight incubation at 37°C. The colonies measured 2-3mm. on the TCBS and blood agar, and 1-2mm. on the MacConkey and EMB agar. The identifications of the vibrios were confirmed by the range of biochemical reactions in Table I. As shown by the results of the antiobiotic susceptibility test in Table II, the *V. alginolyticus* appeared more resistant to the antibiotics tested than the Group-F vibrio.

DISCUSSION

The *V. alginolyticus* and Group-F vibrio strains produced biochemical reactions typical for those described by Tatum (18) and Furniss et al (15). The identification were also confirmed by Takeda (Osaka) and Ohashi (Tokyo). Except for the decarboxylation of the amino-acids and salt tolerance, the vibrios were similar culturally and

TABLE I
Biochemical characteristics of the two *Vibrio* species

	V. alginolyticus	Group-F vibrio
Kligler's iron agar	K/A	K/A
Hydrogen sulphide	–	–
Indole (tryptophane broth)	–	–
Methyl red (48 hours)	–	–
Voges Proskauer ± Barritt's (48 hours)	+	–
Simmons citrate	+	+
Christensen urea	–	–
Decarboxylation (Moeller): lysine	+	–
arginine	–	+
ornithine	+	–
Salt tolerance: 0% NaCl	–	–
3% "	+	+
7% "	+	+
11% "	+	–
Swarming (1.0% agar)	Rapid	–
(1.5% ")	Slow	–
Carbohydrate fermentation: Glucose	Acid	Acid
Lactose	–	–
Sucrose	+	+
Mannitol	+	+
Maltose	+	+
Trehalose	+	+
Rhamnose	–	–
Raffinose	–	–
Cellobiose	+	–
Cytochrome oxidase	+	+

TABLE II
Antibiotic susceptibility tested with BBL Sensi-Discs

		Susceptibility	
	Disc potency	*V. alginolyticus*	Group-F vibrio
Chloramphenicol	30 mcg.	S	S
Ampicillin	10 "	R	S
TM/SMZ	25 "	S	S
Tetracycline	30 "	S	S
Neomycin	30 "	R	S
Triple-sulfa	250 "	S	S
Gentamicin	10 "	S	S
Kanamycin	30 "	R	S
Streptomycin	10 "	R	S
Cephaloridine	30 "	R	R
Penicillin	10 "	R	R
Carbenicillin	100 "	R	S
Nalidixic acid	30 "	S	S

BBL = Baltimore Biological Laboratory
TM/SMZ = trimethoprim 1.25 mcg. : sulphamethoxazole 23.75 mcg.
S = sensitive
R = resistant

biochemically, especially in the essential requirement of salt for growth. The patient from which the *V. alginolyticus* and *V. parahaemolyticus* were isolated had severe diarrhoea with abdominal colic. This was probably caused by the *V. parahaemolyticus*. The patient with the Group-F vibrio had recurrent episodes of diarrhoea suggestive of a low virulence produced by the causative organism. No other bacterial pathogens were isolated in this case. Although the two vibrios were found in the diarrhoeal stools of the patients, they could not be incriminated as the etiological agents of the infections until conclusive data are available.

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