

# FIRST ISOLATIONS OF VIBRIO PARAHAEMOLYTICUS IN SINGAPORE

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## SYNOPSIS

Four strains of *Vibrio parahaemolyticus* were recently isolated from the stools of gastro-enteritis cases in Singapore. The general symptoms were abdominal pain, vomiting, diarrhoeae and fever. Incubation period was only a few hours and all cases recovered in about three days. All strains were sensitive to Septrin, sulphonamide, chloramphenicol and tetracycline. The *V. parahaemolyticus* is a common cause of food-poisoning in Japan, and propagates rapidly in media containing a high salt content like sea and salted foods. We now have evidence that these organisms are present in Singapore and cause foodborne gastro-enteritis in this country.

## INTRODUCTION

The halophiles are bacteria capable of growing in an environment of high salt concentration, some requiring large quantities of it in the media for growth. These organisms were once thought to be non-pathogenic for man until the appearance of *Vibrio parahaemolyticus*. This pathogen is a Gram-negative bacillus characterised by its obligate halophilic property, and is often found in sea fish, shellfish and marine environments. Food-poisoning due to this organism produces gastro-enteritis in most cases and is of an infectious type.

The *V. parahaemolyticus* was first isolated from autopsy materials by Fujino and his co-workers (1950) during a "Shirasu" (semidried salted sardine) food-poisoning outbreak in Osaka, Japan, and was designated the name, *Pasteurella haemolytica*. About 273 persons fell ill with symptoms of acute gastro-enteritis, and 20 died. In Yokohama, Takikawa (1958) noticed the similarity of his isolates with those of Fujino's during the course of his investigations into a food-poisoning outbreak caused by "Asazuke" (salted cucumber). It was Takikawa who discovered the halophilism of the organisms when he tried to use 4% salt agar medium to recover Staphylococci from the infected materials. He also demonstrated the pathogenicity of the organisms in warm-blooded animals including man, and proposed to name his isolates, *Pseudomonas enteritis*. Since then, these organisms

have received much attention in and outside Japan. From the results of extensive studies to establish the taxonomic position, Sakazaki *et al* (1963) finally placed the organisms in the genus *Vibrio* and gave the species name, *Vibrio parahaemolyticus*, which is now universally accepted. As it is the custom of the Japanese people to eat raw fish, food-poisoning is therefore commonly caused by *V. parahaemolyticus*. Statistics recorded by the Ministry of Welfare revealed that 73% of the food-poisoning cases in 1963 was caused by these vibrios, especially in the summer months. *Vibrio* food-poisoning in Japan is not found in the cold season.

Some Japanese workers in the mid-sixties reported the recovery of the vibrios in the coastal sea waters of the United States, the Philippines, Taiwan, Hong Kong and Singapore. However, the organisms were not isolated from human beings in Singapore until September, 1973, when the first case was detected. Ever since, sporadic cases have occurred. It is probable that many cases of gastro-enteritis prior to this were also caused by *V. parahaemolyticus* but remained undiagnosed.

In this report, we describe the clinical and laboratory findings of 4 cases investigated during the period of September and October, 1973. In all these instances, *V. parahaemolyticus* was isolated from the stools of patients in the Outram Road General Hospital. The identification of these organisms was confirmed by the Colorado State Public Health Laboratory, Colorado, U.S.A.

## MATERIALS AND METHODS

During an investigation to study the presence of *V. parahaemolyticus* in the stools of patients, 76 specimens (watery and some also bloody) were selected and cultured on T.C.B.S. medium and in 1% peptone water containing 3% NaCl.

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Cultures were incubated at 37°C for 24 hours. The first positive case was discovered on the 10th September, 1973. Subsequently, three other cases were detected.

**Culture media**

T.C.B.S.	(B.B.L.)	Thiosulphate Citrate Bile-salt Sucrose agar
S-S	(B.B.L.)	Salmonella-Shigella agar + 3% NaCl
D.C.L.S.	(B.B.L.)	Desoxycholate Citrate Lactose Sucrose agar + 3% NaCl
MacConkey	(B.B.L.)	MaConkey agar + 3% NaCl
Blood agar		Nutrient agar + 5% human blood

**Biochemical reactions**

All media (a—f) were supplemented with 3% NaCl to obtain good growth of the halophilic organisms.

- (a) Indol production was tested in 1% tryptophan water with ether extraction and Ehrlich reagent.
- (b) Methyl red test was performed on a 4 day-old culture in MRVP medium.
- (c) Voges Proskauer test (Barritt's method) for acetylmethylcarbinol was performed on a 4 day-old culture in MRVP medium.
- (d) Utilization of citrate as sole carbon source was demonstrated in Simmons and Christensen's media.
- (e) Kligler's Iron Agar was employed to detect the production of hydrogen sulphide and gas from sugar fermentation.
- (f) Sugar fermentation test was performed in the broth base recommended by Edwards and Ewing (1972), added with sterile sugar solution.
- (g) Salt tolerance was tested in 1% peptone water.
- (h) Kanagawa phenomenon test was performed in Wagatsuma medium with 5% human blood.
- (i) Nitrate reduction was tested on human blood agar plate (Cook's method).
- (j) Oxidase was detected with 1% tetramethyl-p-phenylenediamine aqueous solution.
- (k) Hydrogen sulphide was recorded from the Kligler's and Christensen's citrate agar slants after 24 hours' incubation.

**Antibiotic sensitivity**

The medium used was "WELLCO" sensitivity test agar medium (Wellcome Reagents Ltd.) added with 3% NaCl. Plates were pre-dried for 2 hours and then flooded with an overnight broth culture adjusted to a density of 10<sup>6</sup> cells/ml. The antibiotics were tested at the following concentrations per disc:—

Chloramphenicol (Pfizer)	30 mcg.
Septin (B.B.L.)	25 mcg.
Neomycin (B.B.L.)	30 mcg.
Sulphonamide	25 mcg.
Colistin	30 mcg.
Tetracycline	30 mcg.

**RESULTS**

The isolates were Gram-negative rods with rounded ends, occurring singly. Some were long and curved like the Cholera vibrio. The colonies on T.C.B.S. were 2-3 mm. in diameter, raised, shiny and very viscid, often adhering to the agar surface when picked up with a wire-loop. They appeared green with a dark green centre. On a blood agar plate, the colonies produced a greenish-yellow haemolysis after 24 hours. When inoculated on selective media like the D.C.L.S. and S-S agar, no growth was observed even with the addition of 3% salt. The organisms grew well on MacConkey agar containing the additional salt, but failed in the absence of the salt.

All four strains of *V. parahaemolyticus* gave identical biochemical and sensitivity tests as shown in tables I and II.

**CLINICAL FEATURES**

Between 9.9.73 and 3.10.73, four patients were admitted into the Outram Road General Hospital with gastro-enteritis due to *V. parahaemolyticus*.

The first patient was a 15 year-old Chinese male student who went for a swim, ate hawker's food for lunch and 5 hours later, developed diarrhoea, vomiting and generalised abdominal pain. The stools were watery. On admission, he was found to have tenderness in the right iliac Fossa and a provisional diagnosis of gastro-enteritis (? acute appendicitis) was made.

The second patient was a 21 year-old Chinese student nurse who ate "Yeong Tow Foo" (fish preparation) for lunch and developed severe epigastric pain 3 hours later. On the second day of admission, she developed severe diarrhoea and vomiting. The stools were watery but free of blood.

TABLE I  
BIOCHEMICAL CHARACTERISTICS

(a) Indol	..
(b) Methyl red	÷
(c) Voges proskauer	—
(d) Citrate utilization	+
(e) Kligler's iron agar	Alkaline/acid, no gas, no H <sub>2</sub> S
(f) Sugar fermentations	
Glucose	Acid
Lactose	—
Sucrose	—
Mannitol	Acid
Maltose	Acid
Xylose	—
(g) Salt tolerance	
0% NaCl	no growth
3% NaCl	+ve growth
7% NaCl	+ve growth
10% NaCl	no growth
(h) Kanagawa phenomenon	clear haemolysis
(i) Nitrate reduction	+
(j) Oxidase	+
(k) Hydrogen sulphide	—

TABLE II  
ANTIBIOTIC SENSITIVITY

Chloramphenicol	+
Septin	+
Neomycin	weakly
Sulphonamide	+
Colistin	—
Tetracycline	+

The third patient was a 65 year-old Pakistani man who had returned from India recently. He suffered severe diarrhoea and vomiting, and was admitted in severe dehydration with acidosis. His stools were watery. A provisional diagnosis of gastro-enteritis (? Cholera) was made. He required intravenous fluids for resuscitation.

The fourth patient was a 31 year-old Malay man who complained of fever with chills, diarrhoea, vomiting and colicky abdominal pain. His stools were dysenteric containing blood and mucus. A diagnosis of dysentery was made.

All the four patients had low grade fever on admission (37.5—38.5°C) although one patient complained of feeling feverish. All four had a

leucocytosis ranging from 10,700—16,700 with a polymorph count varying from 73%—94%. They were all treated with sulphathalazole for 2—3 days while the first patient received Penicillin and Streptomycin for 1 day and the fourth patient received Chloramphenicol also for 1 day. All became asymptomatic on the 3rd day.

#### DISCUSSION

In our present studies, results obtained from the cultural and biochemical reactions conformed to those of the classical strains. The identification of these isolates was further confirmed by the Colorado State Public Health Laboratory. *Vibrio* food-poisoning generally has an incubation period of about 12 hours, but can be as short as 2 hours or as long as 48 hours. Recovery is between 2 and 5 days. Sakazaki (Riemann, 1969) divided the organisms into two clinical types:

The first clinical type (mimicking Salmonellosis) is characterised by abdominal pain, diarrhoea associated with nausea and vomiting, and mild fever with chills and headache.

The second clinical type (mimicking dysentery) is characterised by fever and stool with mucus and blood.

Diagnosis of gastro-enteritis due to *V. parahaemolyticus* is made by culturing the stools and vomitus of the patients, and from the incriminated food. There is no significant antigenic relationship between *V. parahaemolyticus* and *V. cholerae*, nor is the former as infectious as the Salmonellae or the Shigellae. According to a report by Zen-Yoji *et al* (1965) the infectivity rate is low and a large dose is required to produce infection. Infection due to these organisms should be suspected whenever food-poisoning follows the consumption of sea or salted food. Increasing awareness of this infection will undoubtedly reveal that gastro-enteritis due to *V. parahaemolyticus* is common in Singapore.

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