VIBRIO PARAHAEMOLYTICUS ISOLATED FROM BLOOD CULTURES

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SYNOPSIS

Vibrio parahaemolyticus was isolated from three blood cultures taken from a patient admitted to hospital for liver cirrhosis and diarrhoea. The organism was Kanagawa positive and belonged to O group 6 and type K 18. It is possible to mistake lactose-positive halophilic vibrio for V. parahaemolyticus. Despite the halophilic nature of the organism, it is able to grow in the many media containing 0.5% or more of NaCl that are commonly used in clinical laboratories.

INTRODUCTION

Vibrio parahaemolyticus is a gram-negative halophilic marine bacterium and has been recognised in Japan since 1952 to be a major cause of foodborne gastroenteritis (Fujino, T. et al, 1953). Subsequently, workers from other countries reported the isolations of V. parahaemolyticus from the stool of their gastroenteritis cases (Chun, D., 1967; Dadisman, T.A., et al, 1973; Cawley, P. et al, 1973). In Singapore, the first faecal isolation of V. parahaemolyticus from a patient with gastroenteritis was reported in 1974 (Lam, S. et al, 1974).

Besides gastroenteritis, localised tissue infections by V. parahaemolyticus have also been documented (Twedt, R.M., et al, 1969), but there has been no published report in the literature of the isolation of this organism from human blood.

This paper describes the clinical history and laboratory investigations of a patient from whom V. parahaemolyticus had been isolated from his blood cultures.

CASE REPORT

A 39 year-old Chinese male was first admitted to Singapore General Hospital in December 1971 for liver cirrhosis and portal hypertension. He had six further hospital admissions for recurrent episodes of jaundice. Two weeks prior to his last admission on 3.6.77 the patient noticed that his jaundice was

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getting deeper and there was associated intense pruritus. He also had diarrhoea for about a week, passing out loose light yellow stool about eight times a day. Three days prior to admission, he experienced fever associated with chills and rigors.

On examination, the patient was found to be afebrile (37°C). Pulse rate was 88/minute and he was not dyspnoeic. He was drowsy and markedly jaundiced. Spider naevi were present all over his chest. He had palmar erythema, bilateral ankle oedema and a positive liver flap. No abnormalities were found in his heart and lungs. Blood pressure was 120/50. Abdomen was soft. Liver was nonpalpable but spleen was enlarged 6 cm, firm and smooth. The central nervous system was normal except for his drowsiness.

Laboratory findings were as follows: Haemoglobin 11.0 gm/100 ml; Platelet count 40,000 per c. mm; PCV 30%; Prothrombin time 19 seconds (Control, 12 seconds); Partial thromboplastin time 197 seconds (control 82 seconds).

Urinalysis showed no RBCs, no casts, 4-6 WBCs per high power field and 8-10 epithelial cells. Bile and albumin were present in the urine but urobilin and urobilinogen were absent. ESR was 23 mm.

Serum sodium was 113 mmol/L, serum potassium 2.1 mmol/L, serum chloride 71 mmol/L, serum total protein 6.1 gm%, albumin 2.0 gm%, alkaline phosphatase 198 U/L. Serum bilirubin 30 mg%, serum glucose 145 mg%, serum urea 102 mg% and serum creatinine 2.5 mg%. Hepatitis B antigen was negative by C.I.E., alpha-foetoprotein was negative by I.D. and no stone was visualized on X-ray of his abdomen. Three specimens of blood taken the day after his admission were sent for culture. All three grew Vibrio parahaemolyticus. Unfortunately stool specimens were not available for culture.

The electrolyte imbalance was corrected; a high colonic washout was carried out and the patient given syrup neomycin 1 gm stat and 6 hourly.

His general condition was observed to improve during the next few days as he was more rational and less drowsy. The day after his admission he had a small spike of temperature (37.5°C) which subsided within a few hours. Throughout the rest of his hospital stay however, he was afebrile. Twelve days after his admission he went into liver failure after massive bleeding from the oesophageal varices. His blood pressure fell to 90/60. Despite blood transfusion, Vit K and all the other necessary measures, he died on 14.6.77. The day before his death three repeated blood cultures taken were all negative for V. parahaemolyticus. Post mortem was not granted.

RESULTS

Three aliquots of 5 ml of blood were taken at hourly intervals from the patient under aseptic conditions and inoculated into blood culture bottles containing 45 ml of tryticase soy broth (BBL) and 0.05% liquoid. Subcultures were performed after overnight incubation at 37°C on human blood agar and EMB agar plates. Both plate cultures after overnight incubation at 37°C yielded a short gram-negative rod which exhibits pleomorphism. The organism was actively motile and polarly flagellated. Preliminary biochemical investigations showed that it was oxidase positive, able to utilise glucose fermentatively, fermented glucose on Kligler's iron agar slant and produced indole. However, because no growth was observed in the Moeller decarboxylase media, it was suspected that the organism could be halophilic in nature. It was then subcultured on to TCBS agar and biochemical tests were repeated, this time with 3% NaCl incorporated into the various media. The organism was also inoculated into 1% peptone water containing 0% NaCl, 3% NaCl, 7% NaCl, and 10% NaCl. After overnight incubation at 37°C the organism was found to be able to grow only in 1% peptone water with 3% NaCl and 7% NaCl respectively. Colonies on TCBS agar were rather large, smooth and bluish-green in colour. The organism did not ferment lactose and sucrose but fermented glucose and mannitol with the production of acid but not gas. It also gave a negative Voges-Proskauer test.

The organism was identified as Vibrio parahaemolyticus and sent to Dr. M. Ohasi, Chief Bacteriologist of Metropolitan Research Laboratory of Public Health in Tokyo, Japan for serotyping. It was found to belong to 0 group 6 and type K 18. The strain was also found to be Kanagawa positive. Antibiotic susceptibility test using the method of Bauer, A.W., et al (1966) was performed using tryticase soy broth (pH7.2) and Mueller-Hinton agar with no extra NaCl added. The organism was found to be sensitive to tetracycline, chloramphenicol, gentamicin, cephalosporin, trimethoprim-sulfamethoxazole, and Kanamycin. It was resistant to ampicillin.

DISCUSSION

No worker has reported on the isolation of V. parahaemolyticus from human blood except for one article in which Hollis, D.G., et al (1976) mentioned having received a culture of V. parahaemolyticus isolated from blood. However no clinical history was available.

V. parahaemolyticus was considered to be responsible for a case of serious localised infection with impending shock and a case of fulminating septicaemia in 2 previous reports (Roland, F.P., 1970; Zide, N. et al, 1974). However it was subsequently pointed out that the organism in question was in actual fact a lactose-positive halophillic vibrio (Hollies, D.G. et al, 1976). These workers were able to differentiate L + vibrios from V. parahaemolyticus by their abilities to ferment lactose, produce β-D-galactoside and tolerate lower NaCl concentration. The sources of isolation of L+ vibrio group and clinical histories available indicate that the organism appeared to be more likely to produce serious localized or systemic illnesses than V. parahaemolyticus.

Food poisoning due to V. parahaemolyticus is quite common in Singapore. Since the first isolation of the organism in 1973, the number of gastroenteritis cases recorded up to October 1977 was 167. Most of them presented as sporadic cases and more males seemed to be affected than females. The popular habit of eating raw or partially cooked shellfish most of which harboured a certain amount of V. parahaemolyticus has accounted for quite a number of food poisoning cases (Lam, S., et al, 1977).

This patient was a male and he gave a history of having had diarrhoea for about a week prior to admission to hospital. Unfortunately no stool specimen had been sent for culture. The cause of his diarrhoea was presumably V. parahaemolyticus as a result of his consuming probably shellfish contaminated with the organism.

Although little is known about the mechanism of the development of diarrhoea in gastroenteritis due to V. parahaemolyticus, it is a well-known fact that the characteristics of the vibrio to show a positive Kanagawa phenomenon correspond relatively well with its gastroenteritis producing ability (Ohasi, M., et al, 1973). The V. parahaemolyticus isolated from this patient was also found to show a positive Kanagawa phenomenon.

Septicaemia with unusual enteric pathogens has been described in persons with hemochromatosis (Yamashiro, K.M., et al, 1971). Conn suggested that cirrhotic livers may permit bacteria to bypass the liver's reticuloendothelial system, hence causing a bacteraemia (Conn, HO, 1964). The presence of liver cirrhosis in this patient could have been the prime predisposing factor that accounted for the presence of V. parahaemolyticus in his blood.

Hollis, D.G., et al (1976) found that the V. parahaemolyticus cultures received in their laboratory were able to grow readily on heart infusion agar to which no extra NaCl was added. They noted that heart infusion agar and many media contain 0.5% or more NaCl. The V. parahaemolyticus isolate from this patient's blood was able to grow readily on/ in nutrient agar with 10% human blood, Kligler's iron agar, tryticase soy broth, trytone broth, oxidation-fermentation medium, MacConkey agar, EMB agar, Simmons citrate and Christensen's urea agar with no extra NaCl being added. Colonies on EMB agar after overnight incubation however were small. All of these media contain 0.5% NaCl. Hence, despite the halophilic nature of the organism, there should be no difficulty in recovering it from clinical materials other than stool, as it can grow on the many media containing 0.5% or more of NaCl that are being commonly used in clinical laboratories.

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REFERENCES

1. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., & Turck, M: Antibiotic Susceptibility Testing by a Standardise Single Disk Method. American J. Clin. Path. 45:496. 1966.
2. Cawley, P., et al: Isolation of Vibrio parahaemolyticus in New Zealand. New Zealand Med. J. 78:107, Aug. 1973.
3. Chun, D: Isolation of Vibrio parahaemolyticus in Korea. Modern Med. 6:105-108, 1967 (in Korean).
4. Conn, HO: Spontaneous enteritis and bacteraemia in Laennec's cirrhosis caused by enteric organism. Ann. Intern. Med. 60:568-580, 1964.
5. Dadisman, T.A., Jr., Nelson, R., Molenda, J.R., & Garber, H.J: Vibrio parahaemolyticus gastroenteritis in Maryland. I. Clinical and epidemiological aspects. Am. J. Epidemiol. 96.414-426, 1973.
6. Fujino, T., Okuno, Y., Nakada, D., Aoyama, A., Fukai, K., Mukai, T., & Ueho, T: On the bacterial examination of Shirasu-food poisoning. Med. J. Osaka Univ. 4:299-304, 1953.
7. Hollis, D.G., Weaver, R.E., Baker, C.N., & Thornsberry, C: Halophilic Vibrio Species Isolated from Blood Cultures. J. Clin. Micro. Vol. 3, No. 4, pg. 425-431, April 1976.
8. Lam, S., Yu, M., Sng, E.H. & Doraisingham, S: First isolation of Vibrio parahaemolyticus in Singapore. Sing. Med. J. 15, No. 3 184-187, 1974.
9. Lam, S., Goh, K.T: Incidence of Vibrio parahaemolyticus Infections in Singapore. Ann. Acad. Med. Sing. 6, No. 4, 331-333, Oct. 1977.

10. Ohashi, M. & Shimada, T: Histopathological changes in the small intestines of suckling mice evoked by peronal challenge with a hemolysin elaborated by Vibrio parahaemolyticus. Proceedings of the 9th Joint Cholera Research Conference at Grand Canyon, Arizona, pg. 261-267, Oct. 1973.
11. Roland, F.P: Leg gangrene and endotoxin shock due to Vibrio parahaemolyticus - an infection acquired in New England Coastal Waters. New Eng. J. Med. 282:1306, 1970.
12. Twedt, R.M., Spaulding, P.L., Hall, H.E: Morphological, cultural, biochemical and serological comparison of Japanese strains of Vibrio parahaemolyticus with related cultures isolated in the United States. J. Bacteriol. 98:511-518, 1969.
13. Yamashiro, K.M., et al: Pasteurella pseudotuberculosis: Acute sepsis with survival. Arch. Intern. Med. 128:605-608, 1971.
14. Zide, N., Davis, J., & Ehrenkranz, J.N: Fulminating Vibrio Parahaemolyticus Septicaemia - A syndrome of erythema multiforme, hemolytic anaemia and hypotension. Arch. Intern. Med. 133:479-481, 1974.