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Application of pulsed-field gel electrophoresis to the investigation of a nosocomial outbreak of *Vibrio parahaemolyticus*

Po-Liang Lu, Shan-Chwen Chang, Hui-Jui Pan[1], Mei-Ling Chen[1], Kwen-Tay Luh[1]

Departments of Internal Medicine and [1]Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan, ROC

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The method of pulsed-field gel electrophoresis (PFGE) for *Vibrio parahaemolyticus* was first published in 1996. Since then, its application has been seldom reported in the literature. A food poisoning outbreak due to *V. parahaemolyticus* occurred in five wards of a hospital was investigated using this method. Twenty-five patients were involved and all of them had eaten food supplied by the hospital. Of the 15 cases whose stools were available for culture, only four cultures yielded *V. parahaemolyticus*. All four isolates were serotype K6 and were indistinguishable or closely related to each other based on PFGE patterns. Two isolates from food were recovered and they presented different characterizations from the patient isolates in both serotype and PFGE pattern. Successful typing by PFGE to identify the outbreak strain and differentiate *V. parahaemolyticus* strains between patient and food isolates in this study suggests the usefulness of PFGE for *V. parahaemolyticus*, the leading cause of food poisoning in Taiwan.

Key words: Pulsed-field gel electrophoresis (PFGE), *Vibrio parahaemolyticus*, outbreak, food poisoning

Vibrio parahaemolyticus, a halophilic gram-negative rod, is widely distributed in marine and estuarine environments and is the leading cause of food poisoning during the warm season in Taiwan and Japan [1,2]. Most of the reported cases of acute gastroenteritis due to *V. parahaemolyticus* were related to ingestion of raw or improperly cooked seafood [1,3,4]. In Taiwan, the source of pathogen in most *V. parahaemolyticus* outbreaks was also seafood, which was commonly supplied to elementary or junior high schools in lunch boxes or prepared by party caterers for social or religious activities [1]. Outbreak of food poisoning due to *V. parahaemolyticus* in a hospital setting, a place that should be of good sanitation and hygiene, has not before been reported in literature.

Serologic typing of O (somatic) and K (capsular) antigen is commonly used to differentiate isolates of *V. parahaemolyticus* and is helpful in epidemiological study and outbreak investigation. Besides serotyping, several subspecies typing methods for *V. parahaemolyticus* such as protein profiles by electrophoresis [5], arbitrarily primed polymerase chain reaction (APPCR) [6], plasmid analysis [7], and pulsed-field gel electrophoresis (PFGE) [8,9] have been developed. The procedure of PFGE for *V. parahaemolyticus* was first published in 1996 [8], and this method appeared useful in the investigation of an interstate outbreak in the United States of America [9]. In 1997, an outbreak of *V. parahaemolyticus* food poisoning occurred in a hospital in northern Taiwan and we were requested to help in the investigation of the outbreak. PFGE, in addition to serotyping, was employed in the investigation.

Corresponding author: Dr. Shan-Chwen Chang, Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei, Taiwan, ROC.

Materials and Methods

Outbreak investigation

In May 1997, nine patients in two adjacent wards in a hospital located in northern Taiwan were noticed to have diarrhea and abdominal pain of 2 days' duration. The infection control team of the hospital started surveillance to search for all patients with diarrhea, abdominal pain or vomiting in all wards during the period of outbreak. Clinical and epidemiological features of infected patients and their intake 3 days before they started to have gastrointestinal symptoms were reviewed by the medical personnel with the assistance of the infection control team. Stool from patients with symptoms was cultured during the investigation period. All the food supplied by the

hospital during the outbreak period was reserved in a separate refrigerator. The stool specimens and the preserved food were sent to the clinical microbiology laboratory of the hospital and the National Institute of Preventive Medicine for pathogen isolation and identification. Methods for cultivation and isolation of common bacterial gastroenteritis pathogens, *V. parahaemolyticus*, *Staphylococcus aureus*, pathogenic *Escherichia coli*, *Salmonella* spp., and *Bacillus cereus* from food and stool specimens were generally according to the previously described [1]. The bacterial isolates that grew from the specimens were identified according to previously described methods [2,10]. *V. parahaemolyticus* is identified as oxidase-positive, glucose-nonfermenting, gram-negative rods which could grow on 6% NaCl added agar medium and were arginine dehydrogenase-negative, lysine decarboxylase-, ornithine decarboxylase-, and urease-positive.

Serotyping

Serotyping of the bacterial isolates was performed with commercial serotyping antisera (Denka Seiken Co., Tokyo, Japan), according to the manufacture's instructions, at the National Institute of Preventive Medicine, Taipei, Taiwan.

Genomic fingerprinting by PFGE

Genomic DNA was prepared by a modification of the procedure of Smith and Cantor [11]. *V. parahaemolyticus* cultures were grown for 48 h on tryptic soy agar plates (Difco Laboratories, Detroit, MI, USA) and 3% NaCl at 37 °C. The cells were harvested, washed and then resuspended in Pett IV buffer (1.0 M NaCl, 10 mM Tris-HCl [pH 7.6]). A portion of this suspension was mixed with an equal volume of 1.5% low-melting-temperature agarose, distributed into molds, and allowed to solidify at 4 °C for 30 min. The bacterial cells in the agarose plugs were lysed by treatment with 1 mL of lysis solution containing 1 μg/mL lysozyme and 20 μg/mL RNase for 3 h at 37 °C. They were then treated with ESP (0.5 M EDTA [pH 8.0], 1.0% *N*-lauroylsarcosine, 1 mg of proteinase K per mL) and incubated at 50 °C overnight. After lysis, the plug was washed five times (for 12 min each time) with TE buffer (10 mM Tris-HCl, 1 mM EDTA) and stored at 4 °C.

For restriction endonuclease digestion, one section of the plug (about 4 x 9 x 1.2 mm) was equilibrated with 100 μL of enzyme buffer supplied by New England Biolabs (Beverly, MA, USA) at 4 °C for 1 h. Ten U restriction enzyme *Sfi*I (New England Biolabs, Beverly, MA, USA) was added to the mixture. The mixture of restriction enzyme, enzyme buffer and plug was incubated at 4 °C for 16 h and was then incubated in TE buffer at 37 °C for 1 h. PFGE was performed as described previously [8,12]. Restriction fragments were separated by PFGE in 1% agarose gel (Bio-Rad, Hercules, CA, USA) in 0.5X TBE buffer (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA, pH 8.0) using the Bio-Rad CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, CA, USA). The initial pulse time of 3 sec was increased linearly to 80 sec over 24 h at 190 V and 14 °C. After electrophoresis, the gels were stained in a solution of ethidium bromide (0.5 μg/mL) for 30 min. Gels were then washed in distilled water for 60 min before being photographed. DNA bands were visualized with short wave UV light (254 nm) and photographed.

Band patterns were visually compared and classified as indistinguishable (clonal), closely related (clonal variants, three or less band differences), possibly related (four to six band differences), and unrelated according to previously described criteria [13].

Results

After investigation by the infection control team of the hospital, a total of 25 patients distributed in five wards had symptoms of gastroenteritis. The five wards were distributed in two separate buildings: two adjacent wards and one ward at different floor were located in one building and the other two wards at different floor were of another building. The onset time of gastroenteritis symptoms of the infected patients were clustered in a period of 48 h (Fig. 1). The patients' clinical symptoms included diarrhea (96%), abdominal cramps (60%), fever (24%), chills (12%), nausea (12%), vomiting (4%), and headache (4%). The amplitude of fever ranged from 37.8 °C to 38.5 °C. There was no mortality or sequela of illness among the patients.

All the affected patients had eaten food supplied by the hospital before their gastroenteritis symptoms occurred. These infected patients could not clearly point out which meal consisted of seafood or undercooked foods. After reviewing various kinds of food that patients had eaten during and before the outbreak period according to patients' memory, we could not identify which foods were the exact source of infection. Fifteen stool samples from 15 patients were collected for culture. Four isolates of *V. parahaemolyticus* grew from four patients' stool specimens. Three stool isolates were recovered from patients in the same ward and the other isolate was from a patient in another ward. From the food specimens, two isolates of *V. parahaemolyticus* were recovered from two different foods.

All four isolates from the patients were belonged

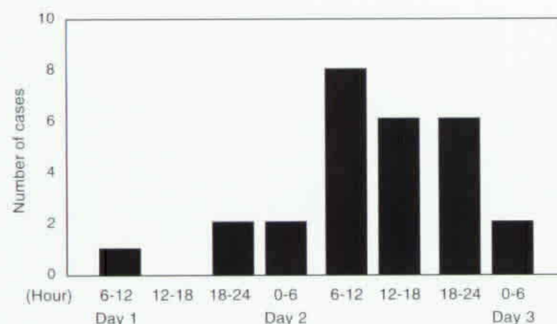

Fig. 1. Onset time of gastroenteritis symptoms of the 25 patients in the outbreak.

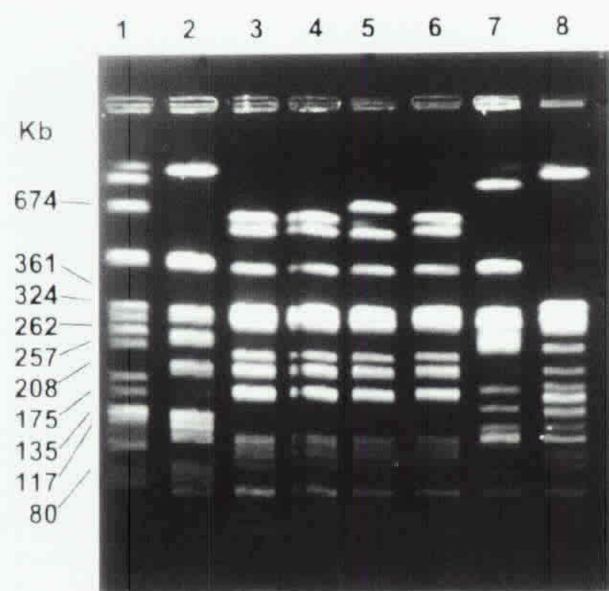

Fig. 2. Profiles produced by PFGE of *Sfi*I macrorestriction fragments of *V. parahaemolyticus* isolates. Molecular size markers are indicated with reference to the *Sma*I digested *Staphylococcus aureus* NCTC 8325 DNA ladder. Lanes 1 and 2 refer to the two food isolates, lanes 3-6 refer to four patient isolates, and lanes 7 and 8 refer to two *V. parahaemolyticus* isolates unrelated to this outbreak.

to serotype K6, whereas the two isolates from food were of untypeable serotype. The results of PFGE (Fig. 2) revealed that two patient isolates were of the same clone (lanes 3 and 6), while the other two patient isolates (lanes 4 and 5) were closely related to the clonal isolates. The two isolates cultured from food (lanes 1 and 2) were unrelated to each other and were different from the patient isolates. Two other clinical *V. parahaemolyticus* isolates not recovered in this outbreak (lanes 7 and 8) also had different PFGE pattern compared to the patient isolates and food isolates.

Discussion

A PFGE typing method for *V. parahaemolyticus* was first published in 1996 and was seldom used [8,9]. In this study, the relationship of *V. parahaemolyticus* isolates from four patients in two wards was successfully established by PFGE typing. Two isolates were genetically indistinguishable by PFGE, and the other two isolates had less than three fragment differences in PFGE patterns; these two isolates were closely related to the outbreak strain [13]. The PFGE patterns of two food isolates and two outbreak-unrelated isolates were different from the patient isolates and were different from each other, suggesting that PFGE had adequate discriminatory ability to identify the clonality of *V. parahaemolyticus* isolates.

Epidemiologic evidence showed that the ill patients had similar symptoms of gastroenteritis during a short period, and all the patients had exposure to food supplied by a hospital. Microbiologic evidence of patients' *V. parahaemolyticus* isolates from two wards were the same serotype (K6) and were clonally or closely related according to PFGE patterns. Thus, the outbreak of food poisoning due to *V. parahaemolyticus* was confirmed. The meals supplied by the hospital were suspected to be the incriminating foods, based on the epidemiologic evidence.

The inconsistency of serotypes and PFGE typing results between the patient isolates and the food isolates does not exclude causality of hospital foods for the outbreak. According to previous food poisoning investigations, it is not unusual that the same causative serotypes are not found in the food specimens as in the patient isolates [14-17]. Presence of other nonpathogenic *V. parahaemolyticus* strains in the incriminated foods is proposed to be the reason for failure to find the same pathogenic strains as in patients' stool. Mixed infection of *V. parahaemolyticus* isolates of different serotypes in an outbreak [3,8] suggests that there might more than one strain in the contaminated food. In one outbreak, there were 12 different serotypes of *V. parahaemolyticus* identified among the numerous colonies subcultured from the foods examined [15]. Tomoyasu demonstrated that the patient's strain of *V. parahaemolyticus*, which could not be found in the food by using conventional culture methods, might be found using an immunomagnetic enrichment method [14].

V. parahaemolyticus serotype K6 in Taiwan is not reported in the literature before 1997 [3,8,18] but became the predominant serotype in early 1997 [19]. Emergence of a serotype K6 clone of *V. parahaemolyticus* in Calcutta, India, and in Southeast Asia since 1996 is reported [6]. However, the cause and

significance of the predilection for a single clone of serotype K6 to dominate among *V. parahaemolyticus* infections is not clear yet. Further comparison of isolates of K6 serotype in Taiwan with the unique clone in India and Southeast Asia might provide epidemiological information of *V. parahaemolyticus* in nearby areas.

This nosocomial food poisoning outbreak occurred in May, one of the warm months in Taiwan. It is reported that colony counts of *V. parahaemolyticus* in the contaminated seafood are higher in the warm season (from April to October) in Taiwan [18], and most of the *V. parahaemolyticus* outbreaks in Taiwan occur in the warm season [18,20]. Although seafood was not identified to be the food source responsible for the outbreak in this study, the lack of carrier state, no known mammalian reservoir of *V. parahaemolyticus* [21] and no use of seawater in the hospital kitchen, made contaminated food to be the only reasonable source of infection. Furthermore, cross contamination between seafood and other kinds of food may occur during the process of food handling and cooking.

The clinical symptoms of diarrhea without bloody stool, abdominal cramps, nausea, and vomiting in most patients, and fever and chills in some patients in this outbreak are compatible with the manifestations of gastroenteritis due to *V. parahaemolyticus* [4,19,21]. Although *V. parahaemolyticus* gastroenteritis is usually self-limiting [21], such an infection might harm vulnerable patients in a hospital setting. The occurrence of a nosocomial food poisoning outbreak highlights that supervision of food sanitation practices may require strengthening, especially in the warm season.

In summary, we present the investigation results of a nosocomial outbreak due to *V. parahaemolyticus* infection with emphasis on the successful application of PFGE for isolates from patients of different wards. In this outbreak, the outbreak strain was confirmed by PFGE in addition to serotyping. The food isolates were different from the patient isolates in serotyping and the difference was also demonstrated by PFGE typing method.

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References

1. Pan TM, Wang TK, Lee CC, Chien SW, Horng CB. Food-borne disease outbreaks due to bacteria in Taiwan, 1986 to 1995. J Clin Microbiol 1997;35:1260-2.
2. Janda JM, Powers C, Bryant RG, Abbott SL. Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. Clin Microbiol Rev 1988;1:245-67.
3. Hsu ST, Liu CH, Wang TK, Liu TP. Food poisoning due to *Vibrio parahaemolyticus*. Chinese J Microbiol 1977;10:87-9.
4. Rodrick GE, Hood MA, Blake NJ. Human *Vibrio* gastroenteritis. Med Clin North Am 1982;66:665-73.
5. Abbott SL, Powers C, Kaysner CA, Takeda Y, Ishibashi M, Joseph SW, Janda JM. Emergence of a restricted bioserovar of *Vibrio parahaemolyticus* as the predominant cause of *Vibrio*-associated gastroenteritis on the West coast of the United States and Mexico. J Clin Microbiol 1989;27:2891-3.
6. Okuda J, Ishibashi M, Hayakawa E, Nishino T, Takeda Y, Mukhopadhyay AK, Garg S, Bhattacharya SK, Nair GB, Nishibuchi M. Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of strains from the same clonal group from southeast Asian travelers arriving in Japan. J Clin Microbiol 1997;35:3150-5.
7. Nolan CM, Ballard J, Kaysner CA, Lilja JL, Williams Jr LP, Tenover FC. *Vibrio parahaemolyticus* gastroenteritis: an outbreak associated with raw oysters in the pacific northwest. Diagn Microbiol Infect Dis 1984;2:119-28.
8. Wong HC, Lu KT, Pan TM, Lee CL, Shih DYC. Subspecies typing of *Vibrio parahaemolyticus* by pulsed-field gel electrophoresis. J Clin Microbiol 1996;34:1535-9.
9. Center for Disease Control. Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island Sound - Connecticut, New Jersey, and New York, 1998. JAMA 1999;281:603-4.
10. Wong HC, Ting SH, Shieh WR. Incidence of toxigenic *Vibrio* in foods available in Taiwan. J Appl Bacteriol 1992;73:197-202.
11. Smith CL, Cantor CR. Purification, specific fragmentation, and separation of large DNA molecules. Methods Enzymol 1987; 155:449-67.
12. Schoonmaker D, Heimberger T, Birkhead G. Comparison of ribotyping and restriction enzyme analysis using pulse-field gel electrophoresis for distinguishing *Legionella pneumophila* isolates obtained during a nosocomial outbreak. J Clin Microbiol 1992;30:1491-8.
13. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulse-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
14. Tomoyasu T. Development of the immunomagnetic enrichment method selective for *Vibrio parahaemolyticus* serotype K and its application to food poisoning study. App Environ Microbiol 1992;58:2679-82.
15. Peffers AS, Bailey J, Barroe GI, Hobbs BC. *Vibrio parahaemolyticus* gastroenteritis and international air travel. Lancet 1973;i:143-5.
16. Baker WH, Weaver RE, Morris GK, Martin WT. Epidemiology of *Vibrio parahaemolyticus* infection in humans. In: Schlessinger D, ed. Microbiology. Washington, D.C.: American Society for Microbiology, 1974:257-62.
17. Dadisman Jr TA, Nelson R, Molenda JR, Garber HJ. *Vibrio parahaemolyticus* gastroenteritis in Maryland. I. clinical and epidemiologic aspects. Am J Epidemiol 1973;96:414-26.
18. Fang SW, Huang WW, Chen LH. Contamination of seafood by *Vibrio parahaemolyticus* in Taiwan. Chinese J Microbiol

Immunol 1987;20:140-7.
19. Pan TM. *Vibrio parahaemolyticus* and food poisoning. Epidemiol Bull (Taiwan) 1997;13:245-50.
20. Pan TM, Chiou CS, Hsu SY, Huang HC, Wang TK, Chiu SI, Yea HL, Lee CL. Food-borne disease outbreaks in Taiwan, 1994. J Formos Med Assoc 1996;95:417-20.
21. Carpenter CCJ. Other pathogenic Vibrios. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 4th ed. New York: Churchill Livingstone, 1995:1945-6.