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Ribotyping of *Vibrio parahaemolyticus* Isolates Obtained from Food Poisoning Outbreaks in Taiwan

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***Abstract*: *Vibrio parahaemolyticus* is a prevalent food-borne pathogen in Taiwan, Japan and other Asian countries. This work presents a novel ribotyping method for the molecular epidemiological examination of this pathogen. Genomic DNA was fragmented by *Hin*dIII digestion and hybridized with cDNA probe for *Escherichia coli* 16S and 23S RNA genes. A total of 121 isolates obtained from outbreaks during 1992 and 1994 in Taiwan were characterized by this ribotyping method. Four to seventeen restricted fragments were visualized in these isolates. After hierarchical cluster analysis, these isolates were grouped into thirty different ribotypes. In addition, A3, A7, E3 and F1 were the major ribotypes, consisting of 22.3, 13.2, 9.1, and 8.3% of the isolates, respectively. A, E, F, G and B were the major groups, consisting of 46.2, 14.0, 9.1, 6.7, and 6.7% of the isolates, respectively. The discriminatory ability of this ribotyping method, as determined by Simpson's index of diversity, was 0.93, which closely resembled that of a previously reported pulsed-field gel electrophoresis method.**

***Key words*: *Vibrio parahaemolyticus*, Ribotyping, Epidemiology**

Vibrio parahaemolyticus, a halophilic Gram-negative bacterium that causes acute gastroenteritis in humans, is a prevalent food-borne pathogen in Taiwan, Japan and other countries (1). A high incidence of this pathogen undoubtedly originates from the frequent consumption of marine foods in these countries. Clinical manifestations include diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills, with incubation periods ranging from 4 to 96 hr (1, 3, 5).

A unique clone of *V. parahaemolyticus* has recently been reported in India and Japan, and has likely spread in other Asian countries (6). The spreading of other clones will also increase due to the rapid increase of travelers in these countries. Therefore, methods must be developed to perform an epidemiological examination of this pathogen. *V. parahaemolyticus* can be differentiated by serotype and commercial serotyping antisera, which are available in Japan and other countries (Denka Seiken, Tokyo). In general, the serotyping method cannot differentiate all isolates originating from different regions or sources. Reliable molecular methods for the typing of strains would greatly facilitate epidemiological investigations. Our recent work developed two molecular methods, i.e. the pulsed-field gel electrophoresis (PFGE) (11) and the random amplified polymorphic DNA (RAPD) (Wong, H. C., Liu, C. C., Pan, T.-M., Wang, T.-K., and Shih, D. Y.-C. 1998. Molecular typing of *Vibrio parahaemolyticus* isolates obtained from food poisoning outbreaks in Taiwan by random amplified polymorphic DNA analysis, submitted for publication), for the subspecies typing of this pathogen. Ribosomal RNA gene restriction fragment polymorphism (ribotyping) is also a reliable method having been used in the typing of *V. cholerae* and other pathogens (2, 7). In light of the above developments, this work presents a novel ribotyping method of *V. parahaemolytics*. A total of 121 clinical isolates obtained from food poisoning outbreaks, mostly occurring during 1993–1995 in Taiwan, were characterized by this method.

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Abbreviations: KP+, Kanagawa phenomenon-positive; PFGE, pulsed-field gel electrophoresis; RAPD, random amplified polymorphic DNA; ribotyping, ribosomal RNA gene restriction fragment polymorphism; SDS, sodium dodecyl sulfate; TE, Tris-EDTA buffer; TSB-3% NaCl, tryptic soy broth-3% NaCl.

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