

Isolation of an Enteropathogenic, Kanagawa-Positive Strain of *Vibrio parahaemolyticus* from Seafood Implicated in Acute Gastroenteritis

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A strain of *Vibrio parahaemolyticus*, serotype O4/K4, that was Kanagawa positive and reactive in the rabbit ileal loop test, was isolated in low numbers from raw oysters implicated in a case of acute gastroenteritis. To our knowledge, this is the first report of the isolation of a Kanagawa-positive strain from suspect food in the United States.

A quarter of a century has elapsed since Fujino et al. (8) did their pioneer study of food poisoning caused by *Vibrio parahaemolyticus*. In the interim, the organism has been recognized not only as the principal cause of foodborne outbreaks of gastroenteritis in Japan, but also as a health hazard in seafood consumed throughout the world (2). The source of *V. parahaemolyticus* isolates is very closely associated with their hemolytic ability (10, 12, 14). In this relationship, termed the Kanagawa phenomenon, most clinical isolates are hemolytic, whereas most environmental strains are not. A strain's hemolytic ability is directly related to its ability to produce dilatation in the rabbit ileal loop test (3, 15, 16). Epidemiologists, therefore, use the Kanagawa test as a measure of an isolate's enteropathogenicity.

Although large numbers of Kanagawa-positive *V. parahaemolyticus* are frequently detected in stool samples from victims of food poisoning outbreaks caused by this bacterium, documented isolation of this organism from incriminated food has been extremely rare. Of 4,426 patient stool isolates obtained during 606 food poisoning outbreaks in Tokyo from 1963 to 1973, over 88% were Kanagawa positive. In contrast, only 2.3% of the 814 strains isolated from incriminated foods were hemolytic (11).

In 1973, an outbreak of food poisoning caused by *V. parahaemolyticus* occurred aboard an international charter airlift from Bangkok to London. Kanagawa-positive strains of serotype O2/K3 were isolated from patients and from suspected raw and dressed crab meat in duplicate meals (13). However, there have been no reported isolations of Kanagawa-positive strains

from food incriminated in the 16 documented outbreaks of *V. parahaemolyticus* gastroenteritis in the United States (4-6). This report describes the first isolation and identification of an enteropathogenic Kanagawa-positive strain from the food implicated in an acute case of food poisoning in this country.

In April 1977, a woman residing in Long Island, N.Y., became acutely ill 3 h after consuming raw oysters and boiled scallops. Her symptoms included numbness of hands and fingers, dizziness, nausea, vomiting, diarrhea with rectal bleeding, chills, and a fever of 38.3°C (101°F). Because of the food type and the neurological symptoms, paralytic shellfish poisoning was initially suspected by Food and Drug Administration investigators. A bacterial agent was strongly suggested by the additional symptoms: the relatively long onset time (3 h), fever, gastrointestinal involvement, and the absence of respiratory symptoms. Furthermore, the consumed shellfish were harvested from an area of the Chesapeake Bay that was previously (1971) involved in gastroenteritis outbreaks caused by *V. parahaemolyticus* (4). Samples of raw, seasoned scallops and raw oysters from the same lot as those consumed by the victim were tested for paralytic shellfish poison according to Methods of Analysis (1) and for *V. parahaemolyticus* as well as traditional food-poisoning species according to the *Bacteriological Analytical Manual for Foods* (7).

Suspect food samples were negative for paralytic shellfish poison, coagulase-positive staphylococci, and pathogenic enterobacteria. No typical *V. parahaemolyticus* colonies were detected in thiosulfate-citrate-bile salts-sucrose agar plates from scallops. However, two of three replicate plates of the 10⁰ dilution and one of the 10⁻¹ dilution of the oyster meat gave typical

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dark-centered, smooth, green colonies. From these plates, four different isolates were chosen and tested for 34 morphological, physiological, and biochemical characteristics of *V. parahaemolyticus* (7, 9). Two isolates were *Vibrio* species; one was *V. anguillarum* (a fish pathogen) and the other, designated NY 477, was verified as Kanagawa-positive *V. parahaemolyticus* serotype O4/K4.

In addition to tests for the 17 identifying characteristics listed in the *Bacteriological Analytical Manual for Foods* (7), biochemical tests for 17 additional traits were performed, including the presence of catalase, urease, and ornithine decarboxylase; citrate utilization; nitrate and nitrite reduction; starch and esculin hydrolysis; and fermentation of arabinose, lactose, mannose, raffinose, rhamnose, xylose, adonitol, dulcitol, and salicin. In all tests, reactions of strain NY 477 were identical to those of the type strain (9) with the exception of delayed arabinose fermentation. In addition, strain NY 477 was reactive when tested by a rabbit ileal loop technique (16).

The enteropathogen was isolated from the 10⁶ level in one of two 50-g samples of raw oysters. Such a low level may reflect adverse conditions of sample exposure before analysis (namely, refrigeration storage for 1 day outside of the Food and Drug Administration facility and 2 days before the laboratory analysis for *Vibrio*). In addition, the oysters were harvested in April, when water temperatures are inimical to *V. parahaemolyticus* growth and when few food poisoning outbreaks have been documented.

Unfortunately, the hospital laboratory was not directed to look for *V. parahaemolyticus* in the patient's stools. Coagulase-positive staphylococci and other pathogenic bacteria were not detected. However, the patient's symptomology, typical of *V. parahaemolyticus* gastroenteritis, and the isolation of an enteropathogenic strain from the implicated food seems more than circumstantial to us. To our knowledge, this is the first report of the isolation of a Kanagawa-positive *V. parahaemolyticus* strain from food suspected of causing gastroenteritis in the United States.

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