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NOTES

Urea Hydrolysis Can Predict the Potential Pathogenicity of *Vibrio parahaemolyticus* Strains Isolated in the Pacific Northwest

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The ability of some strains of *Vibrio parahaemolyticus* to hydrolyze urea (uh$^+$) can be used as a marker to predict which strains isolated from molluscan shellfish harvested in the Pacific Northwest are potentially pathogenic. The thermostable direct hemolysin-producing (TDH$^+$) characteristic is a marker that is correlated with potential pathogenicity, and all of the TDH$^+$ strains that we have isolated have been found to be uh$^+$. Most of the uh$^+$ strains belong to somatic antigen groups O3, O4, and O5. TDH$^+$ strains are usually members of groups O4 and O5. The strains most often associated with human illness are members of the uh$^+$, O4 group. The test for urease production is a simple screening test that can be helpful in predicting which strains are potentially pathogenic.

We have previously described a biochemical trait of several strains of *Vibrio parahaemolyticus* isolated from an estuary in Washington State (6). The ability to hydrolyze urea (uh$^+$), found in approximately 58% of the *V. parahaemolyticus* isolates obtained from Willapa Bay, seemed to be geographically isolated in this estuary because only 6% or fewer of the strains obtained from two other large estuaries in the state were uh$^+$ (6). The potentially pathogenic Kanagawa hemolysin-producing strains (organisms which have the thermostable direct hemolysin-producing [TDH$^+$] characteristic, a trait closely associated with human gastroenteritis [1]) were also uh$^+$. Serogroup O4,K12 was a common *V. parahaemolyticus* serogroup among strains associated with human illnesses associated with the consumption of oysters (*Crassostrea gigas*) in Washington State (10). In addition, a majority of the patient isolates examined by workers at the California Department of Health Services were uh$^+$ O4,K12 (1). A few of the California patients reported that they had consumed oysters that had been harvested from Willapa Bay. A case of gastroenteritis associated with a uh$^+$ strain was also reported in the Pacific Northwest in 1982 (11). Illnesses involving strains having this phenotype have also been reported in other countries, including Bangladesh (4), Singapore (8), British Columbia, Canada (7), and Brazil (9). In Bangladesh, 4 of the 11 uh$^+$ patient isolates were members of serogroup O4 (4).

In Canada, the clinical isolates obtained from patients with locally acquired gastroenteritis were all uh$^+$ (7). However, none of the isolates was Kanagawa hemolysin positive as determined by the in vitro plate test. The results of both the study of Kelly and Stroh (7) and this study suggest that uh$^+$ strains are now the predominant biotype of *V. parahaemolyticus* associated with gastroenteritis in the Pacific Northwest.

V. parahaemolyticus strains obtained from water, sediment, and shellfish samples were enumerated and identified by workers in our laboratory by the most-probable-number (MPN) method, using the procedure described by Elliot et al. (2). Sucrose-negative isolates on thiosulfate-citrate-bile salts-sucrose agar were screened by a procedure in which the arginine, motility, oxidase, and sucrose fermentation tests were used. Up to three isolates per plate were categorized. Isolates identified presumptively as *V. parahaemolyticus* were grouped by the urease reaction, using either Christensen's urea agar supplemented with 2% NaCl or urea broth prepared in 2% NaCl (Difco Laboratories, Detroit, Mich.) before the test for beta-hemolysin (thermostable direct hemolysin) production on Wagatsuma agar (2). Colony blots obtained from tryptic soy–2% NaCl agar cultures of all sucrose-negative isolates were hybridized with a thermostable direct hemolysin gene probe, *tdh-3* (3).

Since the original report of illness caused by a uh$^+$ strain in Washington State (10), the follow-up incidence study performed with samples from Willapa Bay (6), and an additional study performed with samples from Grays Harbor (5), we have analyzed 137 samples from oyster-growing areas within the state. These samples have included samples of oysters (*C. gigas*), sediment samples, and overlying marine water samples collected aseptically by B. Cleland (Washington State Department of Health Shellfish Section) at various times of the year. Two growing areas in Puget Sound, Skookum Inlet and Gallagher Cove, were sampled monthly from March to December 1991. The Elk River bed in Grays Harbor was sampled monthly from July 1992 to September 1993, except for the months of January and April 1993. The water temperature ranged from 6 to 21°C, and the salinity ranged from 16 to 31 ppt. Samples were transported on ice and were analyzed within 24 h of collection. Representative isolates were then grouped with somatic antisera (Denka Seiken Co., Ltd., Tokyo, Japan) by performing slide agglutination tests (2).

During the summer and early fall of 1990, workers in the state of Washington reported 30 confirmed cases of *V. parahaemolyticus* gastroenteritis after consumption of oysters. Several patient isolates obtained from J. Lewis, Washington State Public Health Laboratories, Seattle, were examined by workers in our laboratory for the Kanagawa reaction, for the presence

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TABLE 1. Phenotypes and genotypes of *V. parahaemolyticus* strains obtained from patients and related environmental samples

Strain(s)	Source	Urea hydrolysis[a]	Kanagawa reaction[a]	*tdh* Gene[b]	Serotype[c]
851	Patient (Washington)	+	+	+	O4,K12
853	Patient (Washington)	+	+	+	O6,K18
855	Patient (Washington)	+	+	+	O4,K63
857	Patient (Washington)	+	+	+	O4,K12
8511	Patient (Washington)	+	+	+	O4,K12
8513	Patient (Washington)	+	−	−	O8
8515	Patient (Washington)	+	+	+	O1,K56
8517	Patient (Washington)	+	+	+	O4,K12
8521	Patient (Washington)	+	+	+	O12,K12
8523	Patient (Washington)	+	+	+	O4,K12
902811	Patient (Idaho)	−	−	−	O6
F11-3A	Clam[d]	+	+	+	O4,K12
F12-1A	Oyster[d]	−	−	−	O4
S904[e]	Sediment	+	−	−	O4,K42
S911[f]	Sediment	+	+	+	O4,K12
S914[f]	Sediment	+	−	−	O8
S905[e]	Oyster	+	+	+	O5,K52
S915[f]	Oyster	+	−	+	O5,K52
S909,S910[f]	Oyster	+	+	+	O4,K12
S912,S913[f]	Oyster	+	+	+	O4,K12
S906[e]	Oyster	+	+	+	O4
S907[e]	Oyster	+	−	+	O4,K49
S908[e]	Oyster	+	+	+	O4,K63

[a] Methodology from Elliot et al. (2).
[b] Methodology from Hill et al. (3).
[c] Serotypes were determined by Y. Takeda, Kyoto University, Kyoto, Japan.
[d] Shellfish related to one case of illness in Washington.
[e] Strains isolated from Grays Harbor, Wash., as described by Kaysner et al. (5).
[f] Strains isolated from Gallagher Cove, Wash., in 1991.

of the *tdh* gene, and for urease production (Table 1). Two shellfish isolates that were related to at least one of the cases were also received from state of Washington workers, but unfortunately were not related to any patient isolate that we examined. A single patient isolate was obtained from workers at the Idaho State Health Department. Data obtained from oyster and sediment isolates from two growing areas are shown in Table 1. Serotyping was performed by Y. Takeda, Kyoto University, Kyoto, Japan.

Serogroup O4 was the most common patient isolate serogroup, and the strains were also uh^+ (Table 1). Strains with this biotype were also isolated from a clam sample implicated in one case of illness. Two growing area oyster isolates did not express hemolysin on Wagatsuma agar, but were found to hybridize with the *tdh-3* gene probe (3), a characteristic that we have encountered with a only small number of strains obtained from environmental samples.

V. parahaemolyticus was routinely isolated from water, sediment, and oysters during the warmer months, July through September, in our seasonal studies. During the colder months, *V. parahaemolyticus* was not found in water and was only occasionally found in oysters, but it was consistently found in sediment. Overall, *V. parahaemolyticus* was isolated from approximately 65% of the 137 samples analyzed. The highest levels of *V. parahaemolyticus*, 240 to 4,600 MPN/g, were found in the sediment during the summer in samples obtained from the two estuarine areas. The levels of the potentially pathogenic tdh^+ strains were consistently lower (15 to 24 MPN/g) than the levels of the total *V. parahaemolyticus* population. However, only tdh^+ strains were isolated from a few samples.

Of 836 *V. parahaemolyticus* isolates recovered from samples analyzed since 1990, 298 (35.6%) were uh^+. A total of 57.2% of the isolates obtained from Grays Harbor were uh^+, whereas only 28.5% of the Puget Sound isolates were uh^+. These percentages of uh^+ strains are greater than the value originally reported for these two estuaries (6%) (6); however, the results may have been influenced by the more intensive recent sampling or may have been due to the spread or increased numbers of the uh^+ biotype. Serogroup O3 was the most common serogroup among strains isolated from Puget Sound, while serogroup O5 was more common among the isolates obtained from Grays Harbor. The tdh^+, O4 group appeared to be equally dispersed in both areas. Only 7 of the 11 recognized somatic groups were detected in our samples (Table 2). Of 38 oyster samples, 21 (65.6%) contained *V. parahaemolyticus*, and uh^+ strains were found in 16 (76.2%) of those samples. Only six oyster samples (28.6%) contained both uh^+ and tdh^+ strains. A uh^+, tdh^+, O4 strain was found at a concentration of 4 MPN/g in one oyster sample collected from Grays Harbor.

TABLE 2. Somatic antigen groups, urea hydrolysis reactions, and *tdh* hybridization reactions of representative *V. parahaemolyticus* strains isolated from samples of Washington State oysters and growing area water and sediment since 1990[a]

O antigen	No. of isolates examined	No. of isolates that are:			
		uh^- tdh^-	uh^- tdh^+	uh^+ tdh^-	uh^+ tdh^+
O:1	3	1	0	0	2
O:3	42	19	0	21	2
O:4	14	2	0	2	10
O:5	25	0	0	8	17
O:6	12	10	0	2	0
O:8	13	11	0	2	0
O:11	9	8	0	1	0
Total	118	51	0	36	31

[a] A total of 137 samples were analyzed, and 65% were positive. The *V. parahaemolyticus* isolates studied were randomly selected from 836 isolates and represented all of the sample types and locations. At least one isolate per sample was included in the serogrouping analysis.

All of the tdh^+ isolates obtained from shellfish-growing areas were also uh^+; however, not all of the uh^+ strains produced thermostable direct hemolysin (Table 2). A total of 81 (69.2%) of representative *V. parahaemolyticus* isolates obtained from all sample types and locations were members of serogroup O3, O4, or O5. These three serogroups contained 89.6% of the uh^+ isolates and 93.5% of the tdh^+ isolates. Serogroups O4 and O5 contained 87% of the potentially pathogenic strains (tdh^+), and serogroups O1, O6, O8, and O11 contained less than 10% of the tdh^+ isolates. Two uh^-, tdh^-, O4 strains were isolated from water samples. Members of serogroup O1 appear to be rare in these estuaries.

We concluded that the uh^+ biotype is a useful marker for identifying potentially pathogenic strains of *V. parahaemolyticus* isolated from molluscan shellfish grown in the Pacific Northwest. We found that all tdh^+ strains are also uh^+. Likewise, there is a high probability that a uh^+, tdh^+ strain belongs to either serogroup O4 or serogroup O5. The procedure used to detect the production of urease is a simple biochemical test that can be performed during screening procedures used to group isolates before further testing for pathogenicity markers.

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