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NOTES

Occurrence of Urease-Positive *Vibrio parahaemolyticus* in Kanagawa, Japan, with Specific Reference to Presence of Thermostable Direct Hemolysin (TDH) and the TDH-Related-Hemolysin Genes

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A total of 132 strains of *V. parahaemolyticus* isolated from patients and from the suspected causal food items of past food poisoning cases occurring in Kanagawa Prefecture, Japan, were examined for the ability to hydrolyze urea, with specific reference to the presence of the thermostable direct hemolysin gene (*tdh*) and the gene for thermostable direct hemolysin-related hemolysin (*trh*). Ten strains belonging to five different O-antigen serotypes were positive for urea hydrolysis (UH^+), and four of these strains did not carry *tdh*. A total of 106 strains carried *tdh*, but less than 6% of them were UH^+, whereas all *trh*-carrying strains were UH^+. The evidence suggests that urea hydrolysis is not a reliable marker for identifying *tdh*-carrying *V. parahaemolyticus* strains in Japan (the Pacific Northeast) but may be a marker for *trh*-carrying strains.

Vibrio parahaemolyticus is one of the major gastroenteritis-causing bacteria, frequently associated with consumption of raw or improperly cooked seafood (1, 2). Past epidemiological studies revealed that almost all clinical isolates induced the Kanagawa phenomenon, a zone of beta-type hemolysis around the colony on a special blood agar (Wagatsuma agar [3]). The phenomenon is caused by Kanagawa hemolysin or the thermostable direct hemolysin (TDH) produced by the isolates, and thus TDH has been conventionally considered a major virulence factor of the bacterium (6, 12). Honda et al. (4), however, reported that a recent outbreak of gastroenteritis in the Republic of Maldives was caused by a type of Kanagawa phenomenon-negative *V. parahaemolyticus*. The isolates did not carry the *tdh* gene, but it was subsequently found that one isolate produced a TDH-related hemolysin. The TDH-related hemolysin was immunologically similar to TDH, but the two hemolysins had significantly different physiochemical characteristics and lytic activities for various erthrocytes (5). Recently, Kelly and Stroh (8) reported that clinical isolates obtained from patients with locally acquired gastroenteritis in Canada all hydrolyzed urea, but none of the isolates were Kanagawa hemolysin positive, as determined by the in vitro plate test. The evidence suggests that the urea-hydrolyzing (UH^+) strains are the predominant biotype of *V. parahaemolyticus* associated with gastroenteritis in the Pacific Northwest. Stimulated by these studies, Kaysner et al. (7) reported a correlation between the TDH-producing characteristic and the ability to hydrolyze urea by *V. parahaemolyticus* strains isolated from patients and the environments in the Pacific Northwest and found that all TDH gene (*tdh*)-carrying strains isolated from patients and the

TABLE 1. Phenotypes and genotypes of *V. parahaemolyticus* strains isolated from patients and from suspected causal food items in clinical cases in Kanagawa, Japan, between 1980 and 1994[a]

O-antigen serotype	No. of isolates examined	No. of strains that are:							
		UH^+ *tdh*$^+$ *trh*$^+$	UH^+ *tdh*$^+$ *trh*$^-$	UH^+ *tdh*$^-$ *trh*$^+$	UH^+ *tdh*$^-$ *trh*$^-$	UH^- *tdh*$^+$ *trh*$^+$	UH^- *tdh*$^+$ *trh*$^-$	UH^- *tdh*$^-$ *trh*$^+$	UH^- *tdh*$^-$ *trh*$^-$
O:1	15	0	3	0	0	0	7	0	5
O:2	13	0	0	0	0	0	10	0	3
O:3	15	0	0	2	0	0	12	0	1
O:4	70	0	0	0	1	0	59	0	10
O:5	6	0	0	0	1	0	5	0	0
O:6	3	3	0	0	0	0	0	0	0
O:8	8	0	0	0	0	0	6	0	2
O:10	2	0	0	0	0	0	1	0	1
Total	132	3	3	2	2	0	100	0	22

[a] UH^+ or UH^-, able or unable to hydrolyze urea, respectively; *tdh*$^+$ or *tdh*$^-$, carrying or not carrying the *tdh* gene; *trh*$^+$ or *trh*$^-$, carrying or not carrying the *trh* gene, respectively.

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TABLE 2. Genotypes and serotypes of urea-hydrolyzing *V. parahaemolyticus* strains and their biochemical characteristics as determined by API 20E

Strain	Gene		Serotype	Presence or absence of given biochemical characteristic[a]																				
	tdh	*trh*		ONPG	ADH	LDC	OCD	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX
K9922	+	+	O6:K18	−	−	+	+	−	−	+	−	−	−	+	+	+	−	−	−	−	−	−	+	+
K9927	+	+	O6:K18	−	−	+	+	−	−	+	−	+	−	+	+	+	−	−	−	−	−	−	+	+
K9928	+	+	O6:K18	−	−	+	+	−	−	+	−	+	−	+	+	+	−	−	−	−	−	−	−	+
K9984	−	+	O3:K6	−	−	+	−	−	−	+	−	+	−	+	+	+	−	−	+	−	−	−	−	+
K9985	−	+	O3:K6	−	−	+	+	−	−	−	−	+	−	+	+	+	−	−	+	−	−	−	+	+
K10076	−	−	O5:UT[b]	−	−	+	+	−	−	+	−	+	−	+	+	+	−	−	−	−	−	−	−	+
K10215	+	−	O1:K1	−	−	+	−	−	−	+	−	+	−	+	+	+	−	−	−	−	−	−	+	+
K10223	+	−	O1:K1	−	−	+	+	−	−	−	−	−	−	+	+	+	−	−	−	−	−	+	+	+
K10260	+	−	O1:K20	−	−	+	+	−	−	+	−	+	−	+	+	+	−	−	−	−	−	−	−	+
K10294	−	−	O4:K53	−	−	+	+	−	−	+	−	+	−	+	+	+	−	−	−	−	−	−	+	+

[a] ONPG, β-galactosidase; ADH, arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; CIT, citrate utilization; H_2S, H_2S production; URE, urease; TDA, tryptophan deaminase; IND, indole production; VP, acetoin production; GEL, gelatinase; GLU, glucose hydrolysis; MAN, mannitol hydrolysis; INO, inositol hydrolysis; SOR, sorbitol hydrolysis; RHA, rhamnose hydrolysis; SAC, sucrose hydrolysis; MEL, melibiose hydrolysis; AMY, amygdalin hydrolysis; ARA, arabinose hydrolysis; OX, oxidase.

[b] UT, untypeable.

environments are UH^+. They concluded that the urea hydrolysis test is a simple and useful screening test for potentially pathogenic *V. parahaemolyticus* bacteria, although they did not report detection of the TDH-related-hemolysin gene (*tdh*) or whether strains were evaluated for the presence of this gene. We thus determined the occurrence of UH^+ strains among clinical strains of *V. parahaemolyticus* isolated over a 15-year period in Kanagawa Prefecture, Japan, in relation to the presence of *tdh* and *trh* genes.

The bacteriological screening procedure used in the present study was essentially that described by Kaysner et al. (7). A total of 132 strains, which were presumptively identified as *V. parahaemolyticus* by a differential plate medium, thiosulfate citrate bile salts sucrose agar (Eiken Chemical Co., Ltd., Tokyo, Japan), were confirmed as such by further biochemical tests as described by Elliot et al. (3). Serotyping (O and K antigens) with a commercially available rabbit antiserum (Denka Seiken Co., Ltd., Tokyo, Japan) was also performed on these strains. All strains were obtained from food poisoning cases occurring in Kanagawa during the period 1980 to 1994, including 113 strains isolated from feces of patients and 19 strains isolated from suspected causal food items. Urea hydrolysis by the strains was determined with Christensen's urea agar (Difco Laboratories, Detroit, Mich.) supplemented with NaCl (2% final concentration) as described by Kaysner et al. (7). Monitoring of reactions in the agar at 37°C was continued for a total of 7 days after inoculation.

Colony blots of all strains, obtained from heart infusion (Difco) supplemented with 2% NaCl and 2% agar, were hybridized with commercially available enzyme (alkaline phosphatase)-labeled *tdh* and *trh* probes, VP-TDH and VP-TRH (Toyobo Co. Ltd., Osaka, Japan), for detection of *tdh* and *trh*, respectively, by following procedures described by Yamamoto et al. (13). The DNA sequences of the probes used were 5′-CCCCGGTTCTGAXGAGATATTGTT-3′ for *tdh* and 5′-TCCAGGTTCGGAXGAGCTACTATT-3′ for *trh*, where X denotes deoxyuridine with a side arm labeled with the enzyme. The T^d values for *tdh* and *trh* probes are 71.1 and 69.2°C, respectively.

The *V. parahaemolyticus* strains were classified into 8 of the 12 serogroups recognized. Serogroup O4 was predominant (53%) (Table 1). Urea hydrolysis was observed in 10 strains (7.5%) belonging to five serotypes. Since a commercially available identification kit, API 20E (API System, Montalieu Vercieu, France), has been claimed to be a useful tool for identification of potentially pathogenic members of the *Vibrionaceae* (11), an additional biochemical characterization of the UH^+ strains by API 20E was made. The diluent used for the kit contained 2% NaCl as recommended by MacDonell et al. (9). The results are summarized in Table 2, together with additional data of K-antigen serotyping. The phenotypic profiles of all UH^+ strains were identical to that expected of *V. parahaemolyticus*, except that K9984 and K10215 gave atypical results of negative ornithine decarboxylase activity and K9922 and K10223 gave atypical results in not producing indole. These results with the API 20E system corresponded to those obtained from the tests described by Elliot et al. (3). Interestingly, K9985 and K10223, which were positive for urea hydrolysis in Christensen's urea agar, gave negative results by the API 20E method. The evidence suggests that urea hydrolysis tested by the API 20E system is of limited use for characterization of *V. parahaemolyticus*.

Genotypically, 106 strains (80% of the strains examined) carried *tdh*, of which 3 carried *trh* concomitantly, whereas 26 strains (20%) did not carry *tdh* but strains 2 carried *trh* (Table 1). It should be noted that more than 80% of the O4 strains were found to carry *tdh*. Contrary to the recent report presented by Kaysner et al. (7), the presence of *tdh* did not correspond to a strain's ability to hydrolyze urea in most of our isolates; of the 106 *tdh*-carrying strains, 100 strains were found to be negative for urea hydrolysis, and only 6 strains were UH^+. By contrast, we found that the presence of *trh* corresponded to the ability to hydrolyze urea; all *trh*-carrying strains are UH^+. The use of the UH^+ biotype as a marker for identifying potentially pathogenic strains of *V. parahaemolyticus*, as proposed by Kaysner et al. (7), does not seem to be practical, at least in our laboratory's screening system. The differences between our results and those of Kaysner et al. (7) may be ascribed to a difference in choice of *tdh* gene probes used, or it may simply reflect a geographical difference in phenotypic or genotypic characteristics between the strains found in the Pacific Northeast and those found in the Pacific Northwest. Alternatively, we suggest that the UH^+ biotype can be used as an indicator for the possible presence of the *trh* gene in *V. parahaemolyticus*, although urea hydrolysis does not mean that the *trh* gene is present. A limited sample size of *trh*-carrying strains (only five strains) found in the present study, however, made it difficult for us to draw an overall conclusion on this matter.

Further work is in progress to evaluate a possible link between the ability of *V. parahaemolyticus* to hydrolyze urea and the presence of the *trh* gene.

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