APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2003, p. 3999–4005 Vol. 69, No. 7
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.7.3999–4005.2003

Molecular, Serological, and Virulence Characteristics of *Vibrio parahaemolyticus* Isolated from Environmental, Food, and Clinical Sources in North America and Asia

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Received 25 October 2002/Accepted 25 April 2003

Potential virulence attributes, serotypes, and ribotypes were determined for 178 pathogenic *Vibrio parahaemolyticus* isolates from clinical, environmental, and food sources on the Pacific, Atlantic, and Gulf Coasts of the United States and from clinical sources in Asia. The food and environmental isolates were generally from oysters, and they were defined as being pathogenic by using DNA probes to detect the presence of the thermostable direct hemolysin (*tdh*) gene. The clinical isolates from the United States were generally associated with oyster consumption, and most were obtained from outbreaks in Washington, Texas, and New York. Multiplex PCR was used to confirm the species identification and the presence of *tdh* and to test for the *tdh*-related hemolysin *trh*. Most of the environmental, food, and clinical isolates from the United States were positive for *tdh*, *trh*, and urease production. Outbreak-associated isolates from Texas, New York, and Asia were predominantly serotype O3:K6 and possessed only *tdh*. A total of 27 serotypes and 28 ribogroups were identified among the isolates, but the patterns of strain distribution differed between the serotypes and ribogroups. All but one of the O3:K6 isolates from Texas were in a different ribogroup from the O3:K6 isolates from New York or Asia. The O3:K6 serotype was not detected in any of the environmental and food isolates from the United States, and none of the food or environmental isolates belonged to any of the three ribogroups that contained all of the O3:K6 and related clinical isolates. The combination of serotyping and ribotyping showed that the Pacific Coast *V. parahaemolyticus* population appeared to be distinct from that of either the Atlantic Coast or Gulf Coast. The fact that certain serotypes and ribotypes contained both clinical and environmental isolates while many others contained only environmental isolates implies that certain serotypes or ribotypes are more relevant for human disease.

Vibrio parahaemolyticus is the leading cause of seafood-associated bacterial gastroenteritis in the United States (29) and is a major cause of food-borne illness in the world (21, 41). However, the relationship between strains isolated from estuarine environments, those isolated from seafood, and human clinical isolates is poorly understood. The presence of thermostable direct hemolysin (TDH) is a proven virulence factor (31), and TDH occurs in over 90% of clinical strains of *V. parahaemolyticus* isolated in both the United States (34; unpublished data from the Centers for Disease Control and Prevention) and internationally (30, 32, 41). Early work used a phenotypic assay known as the Kanagawa test to identify strains producing TDH (30), but this has been replaced by more efficient and definitive methods such as PCR (3, 37) and DNA probe assays (28, 41) that target the gene encoding TDH (*tdh*). Recently, these and other genotypic assays have been applied to environmental studies (8, 13) and seafood surveys (9, 17). While total *V. parahaemolyticus* levels in U.S. market oysters exceeding 10,000 g^{-1} have been observed frequently (9), usually less than 1% of the isolates from environmental and seafood samples possesses *tdh* (8, 9, 13, 38), except in a limited study in Grays Harbor, Washington (22). Few tdh^+ environmental or food isolates have been available for further characterization.

A proposed virulence factor, the TDH-related hemolysin (TRH), encoded by the gene *trh*, has been discovered in clinical stains of *V. parahaemolyticus* lacking *tdh* (19, 20). Most clinical isolates from the U.S. Pacific Coast have been reported to possess both *tdh* and *trh* (34). Environmental isolates possessing *trh* have been reported in Asia (41), only recently in the U.S. Gulf Coast, and not at all in the Atlantic Coast (9, 14, 38). *V. parahaemolyticus* possessing *trh* nearly always produces urease, which is atypical for other *V. parahaemolyticus* strains (34), and these genes may be part of a pathogenicity island (36). Neither urease nor *trh* has been shown to enhance the virulence of those *V. parahaemolyticus* strains that also possess *tdh*, but urease has been shown to increase acid tolerance in *Yersinia enterocolitica* (11) and *Helicobacter pylori* (39).

Japanese researchers developed a *V. parahaemolyticus* serotyping system based on lipopolysaccharide (O) and capsular

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(K) antigens (21). *V. parahaemolyticus* isolates are diverse; at least 12 O groups and 65 K groups have been recognized, and antisera for all of these groups are commercially available (24). Despite this diversity, a single serotype (O4:K12) has had the highest prevalence among tested clinical *V. parahaemolyticus* isolates from the U.S. Pacific Coast for most years between 1979 and 1995 (34). A new clone of the O3:K6 serotype emerged in India in 1996 and quickly spread throughout Asia (35). While previous *V. parahaemolyticus* strains were usually associated with sporadic cases or small outbreaks, this clone caused larger outbreaks and appeared to have an unusually high attack rate (27). In 1998, O3:K6 *V. parahaemolyticus* caused the largest reported *V. parahaemolyticus* outbreak in U.S. history, an outbreak which was linked to consumption of raw oysters from Galveston Bay, Tex. (10); a smaller outbreak of O3:K6 *V. parahaemolyticus* associated with Long Island Sound oysters occurred later that year (5). Recently isolated O4:K68 and O1:K untypeable (O1:Kuk) *V. parahaemolyticus* from Asia have been reported to be closely related to the new O3:K6 clone according to molecular methods that are typically more discriminatory than serotyping, and therefore concern has arisen that these serotypes may potentially cause outbreaks and pandemic spread (27).

Molecular methods such as ribotyping (1, 18, 26), pulsed-field gel electrophoresis (1, 26, 41), enterobacterial repetitive intergenic consensus sequence amplification (26), restriction fragment length polymorphism (RFLP) analysis (26), and arbitrarily primed PCR (27, 35) are increasingly being applied for typing *V. parahaemolyticus*. Gendel et al. recently reported the existence of at least 12 different ribotype patterns among O3:K6 strains from the United States and Asia by using three restriction enzymes, and they found that most of the strains from the 1998 Galveston Bay outbreak were different than those from New York or Asia (18).

The primary objective of this study was to examine the relationship between *tdh*$^+$ *V. parahaemolyticus* from U.S. environmental and market oysters and clinical isolates from the United States and Asia. The relative ability of ribotyping and serotyping to differentiate among *V. parahaemolyticus* isolates and the agreement between these two methods were also examined.

MATERIALS AND METHODS

***V. parahaemolyticus* isolates.** The sources of the *V. parahaemolyticus* isolates are shown in Table 1. Oyster-associated clinical isolates were collected by the Washington State Department of Health from 1988 to 2001. Clinical isolates from the 1998 oyster-associated outbreaks were provided by the Texas Department of Health and the Health Departments of New York and Connecticut (18). Mitsuaki Nishibuchi (Kyoto, Japan) provided 21 clinical isolates collected between 1980 and 1998 in eight Asian countries (18).

Environmental and food *V. parahaemolyticus* isolates obtained in previous surveys and environmental studies were included in the present study only if they possessed a virulence marker, primarily *tdh*. Environmental isolates from oyster, clam, or sediment samples from the Pacific Coasts of Washington and Oregon were obtained between 1982 and 2001 (22, 23). An ecological study conducted by the authors at Hood Canal, Wash., in August 2001 yielded 31 isolates. On the Gulf Coast, environmental oyster isolates were collected in Alabama in 1999 and 2000 (14) and Louisiana in 1999 (8). Food isolates were collected from oyster-processing plants in Washington in 1990 and 1991 and in Oregon in 1997. Twelve isolates from Atlantic and Gulf Coast oysters were collected during a nationwide market survey of shellstock oysters (9). Standard culture methodology (16) utilizing alkaline peptone water enrichment following isolation on thiosulfate-citrate-bile salts-sucrose agar was used to obtain *V. parahaemolyticus* isolates. Some environmental isolates (obtained in Alabama and Hood Canal, Wash., from 1999 to 2001) were isolated using a protocol involving direct plating on T_1N_3 (1% tryptone, 3% NaCl, 2% agar) in conjunction with DNA probe colony hybridization for *tdh* (14, 33).

Identification and virulence characterization. Isolates were identified as *V. parahaemolyticus* by using API-20E diagnostic strips (bioMerieux, Durham, N.C.) as previously recommended (16). In addition to API-20E testing, urease production was also determined using Christensen's urea agar or urea broth (BBL, Becton Dickinson and Co., Cockeysville, Md.) (16). Unless stated otherwise, the source of other bacteriological media used in this study was Difco Laboratories (Sparks, Md.). Both DNA probes (28) and a PCR method (3) were used to test for the presence of *tdh,* while the presences of *trh* and the thermolabile direct hemolysin (*tlh*), which is species specific, were determined by multiplex PCR (3).

Serotyping. O (lipopolysaccharide) and K (capsular) serotypes were determined using specific antisera (Denka; Seiken Corp., Tokyo, Japan) as previously described (16).

Ribotyping. Ribotyping was carried out using the Qualicon (Wilmington, Del.) Riboprinter microbial characterization system according to the manufacturer's instructions. Cultures were grown overnight on brain heart infusion agar plates, and cell picks were transferred directly to the lysis buffer supplied. Cells were inactivated by incubation at 90°C for 10 min and transferred to the Riboprinter. The Riboprinter carried out cell lysis, DNA digestion, gel electrophoresis, DNA transfer to a hybridization membrane, and Southern hybridization using a chemiluminescent ribosomal probe (4). The resulting riboprint patterns were recorded and analyzed using the software supplied with the Riboprinter system. The riboprint pattern for each isolate was compared with the patterns produced for all other isolates using the same restriction enzyme. These isolate-to-isolate comparisons were used to define ribogroups, each consisting of those patterns that were $\geq$0.92 similar. The sample number of the first pattern in each group became the label used to identify that group. The analysis software derived a single average pattern for each ribogroup, as well as information on the similarity between each pattern within the group and the group average pattern. Riboprint patterns were produced for all 178 isolates of *V. parahaemolyticus* by using the restriction enzyme *Eco*RI. A dendrogram showing the similarity relationships between the ribogroup patterns was prepared using UPGMA (unweighted pair group method with arithmetic mean) for the cluster analysis based on the similarity values between the ribogroup patterns obtained using the Riboprinter system software.

RESULTS

The virulence attributes, serotypes, ribogroups, and sources of the 178 *V. parahaemolyticus* isolates tested are shown in Table 1. PCR analysis for *tlh* confirmed the identities of all *V. parahaemolyticus* isolates. All isolates that possessed *trh* also produced urease. Using *Eco*RI, we found that these isolates were diverse, with 27 different serotypes and 28 ribogroup patterns. Approximately 40% of the serotypes and ribogroups contained a single isolate; most of the ribogroups with more than two isolates contained multiple serotypes and vice versa. While diverse, there were apparent trends in the characteristics of these isolates within and between certain source groupings.

Outside of the Pacific Coast, 39 of 43 clinical isolates were serotype O3:K6 and all but one O3:K6 isolate possessed *tdh* but not *trh* and did not produce urease (isolate AQ4037, isolated in 1985 from a traveler returning to Japan from the Maldives, produced urease and possessed *trh* but not *tdh*). Three ribogroups (190-6, 193-1, and 252-5) were observed among the O3:K6 isolates. The largest ribogroup, 190-6, contained 31 isolates, all of which were clinical isolates, with most being serotype O3:K6, along with one isolate each of O4:K68 and O1:Kuk. Except for a single isolate from Texas, the isolates in ribogroup 190-6 were from Asia or the Long Island Sound oyster-associated outbreak. Ribogroup 193-1 consisted exclusively of O3:K6 isolates associated with the Galveston Bay outbreak.

TABLE 1. Sources, virulence attributes, and serotypes of *V. parahaemolyticus* isolates

Origin	Location (no. of isolates)	Date(s) of isolation	No. of isolates			Serotype(s) (no. of isolates)	Ribogroup(s) (no. of isolates)
			tdh$^+$	*trh*$^+$	Producing urease		
Clinical	Washington (26)	1988–2001	26	26	26	O4:K12 (17)	146-1 (11), 159-5 (2), 147-4 (1), 159-6 (1), 293-3 (1)
						O1:K56 (3)	147-4 (2), 146-1 (1)
						O6:K18 (2)	146-2 (2)
						O4:K63 (2)	147-4 (2)
						O3:K36 (1)	293-1 (1)
						O12:K12 (1)	146-1 (1)
	Texas (10)	1998	10	0	0	O3:K6 (10)	193-1 (9), 190-6 (1)
	New York (10)	1998	9	0	0	O3:K6 (9)	190-6 (9)
						O4:K55 (1)	162-3 (1)
	Connecticut (2)	1998	2	0	0	O3:K6 (2)	190-6 (2)
	India (8)	1996, 1997	8	0	0	O3:K6 (8)	190-6 (7), 252-5 (1)
	Bangladesh (4)	1980, 1998	4	0	0	O3:K6 (2)	190-6 (2)
						O1:Kuk (1)	190-6 (1)
						O4:K68 (1)	190-6 (1)
	Japan (3)	1998	2	1	1	O3:K6 (2)	190-6 (2)
						O4:K12 (1)	147-1 (1)
	Thailand (2)	1996	2	0	0	O3:K6 (2)	190-6 (1)
	Korea (1)	1998	1	0	0	O3:K6 (1)	190-6 (1)
	Laos (1)	1997	1	0	0	O3:K6 (1)	190-6 (1)
	Maldives (1)	1985	0	1	1	O3:K6 (1)	190-6 (1)
	Taiwan (1)	1997	1	0	0	O3:K6 (1)	190-6 (1)
Environmental	Washington (28)	1982–2001	28	27	27	O1:K56 (19)	147-4 (16), 293-7 (3)
						O1:Kuk (3)	293-7 (2), 358-1 (1)
						O4:K12 (3)	146-1 (3)
						O4:Kuk (2)	146-1 (2)
						O5:Kuk (1)	161-3 (1)
	Washington (9)[a]	1982, 2001	9	9	9	O1:K56 (4)	147-4 (3), 293-7 (1)
						O1:Kuk (4)	293-7 (4)
						O4:K12 (1)	146-1 (1)
	Oregon (1)	1997	1	1	1	O4:K12 (1)	146-1 (1)
	California (1)	1995	1	1	1	O4:K8 (1)	161-3 (1)
	Alabama (49)	1999–2000	49	48	48	O11:Kuk (16)	250-6 (16)
						O4:K9 (12)	163-1 (12)
						O1:K30 (6)	203-7 (6)
						O1:K56 (3)	203-7 (3)
						O1:Kuk (3)	276-3 (2), 203-7 (1)
						O1:K54 (2)	161-3 (2)
						O1:K55 (2)	312-7 (2)
						O1:K22 (1)	162-3 (1)
						O4:K8 (1)	146-1 (1)
						O8:K70 (1)	310-3 (10)
						O11:K40 (1)	202-6 (1)
						O11:K61 (1)	275-1 (1)
	Louisiana (5)	1999	5	5	5	O3:Kuk (3)	219-7 (3)
						O4:Kuk (2)	251-1 (2)
	New York (1)	1977	1	0	0	O4:K8 (1)	146-8 (1)
Food	Washington (4)	1990–1997	4	4	4	O4:K63 (2)	147-4 (2)
						O4:K12 (1)	146-1 (1)
						O4:K49 (1)	161-3 (1)
	Louisiana (4)	1998, 1999	4	2	2	O4:K9 (2)	161-3 (2)
						O3:K49 (1)	219-6 (1)
						O11:Kuk (1)	219-8 (1)
	Connecticut (3)	1998	3	0	0	O5:K17 (2)	219-4 (2)
						O3:K34 (1)	219-7 (1)
	Maryland (2)	1998	2	2	2	O4:K63 (2)	219-6 (2)
	Massachusetts (1)	1998	1	0	0	O4:K9 (1)	161-3 (1)
	Virginia (2)	1998	2	2	2	O5:K17 (2)	219-4 (1)

[a] These nine isolates are from sediment samples. The remainder of the environmental and food isolates are from shellfish.

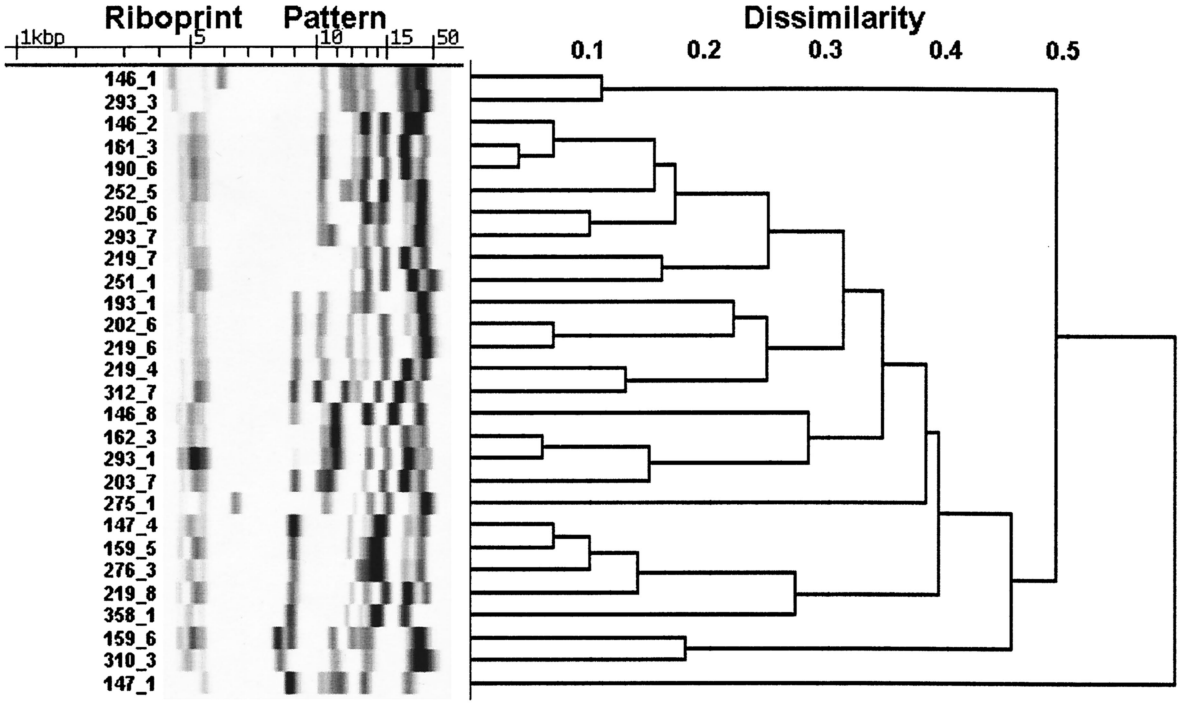

FIG. 1. Dendrogram showing the relatedness of ribogroup patterns for *V. parahaemolyticus* isolates.

The Pacific Coast clinical isolates clearly differ from the other clinical isolates in virulence attributes, serotypes, and ribogroups. The O3:K6 serotype was not observed among the 26 Pacific Coast clinical isolates, which were all positive for all of the tested virulence attributes. The O4:K12 serotype was the most prevalent (17 of 26 isolates) among the Pacific Coast clinical isolates but was found only once among the other clinical isolates, in an isolate from Japan. The O4:K12 serotype contained six ribogroups, the most observed in this study. Ribogroup 146-1 contained 12 of 17 clinical O4:K12 isolates but also included single isolates from two other serotypes (O1:K56 and O12:K12). Multiple isolates of serotypes O1:K56 (three isolates) and O6:K18 (two isolates) were also found among the Pacific Coast clinical isolates. Ribogroup 147-4 contained only Pacific Coast isolates, including clinical isolates with three serotypes (O1:K56, O4:K12, and O4:K63).

While almost all (91 of 94) of the environmental *V. parahaemolyticus* isolates possessed all of the virulence attributes, they were serologically and genetically heterogeneous, with 16 serotypes and 16 ribogroups. Serotype O1:K56 accounted for 23 of the 39 Pacific Coast environmental *V. parahaemolyticus* isolates, all of which were from Washington. Nineteen of these isolates were in ribogroup 147-4, matching two of three Pacific Coast clinical O1:K56 isolates, and four were in ribogroup 293-7. The second most prevalent serotype among Pacific Coast environmental *V. parahaemolyticus* isolates was O1:Kuk, and six of the seven isolates were in ribotype 293-7. Serotype O4:K12 was also prevalent among Pacific Coast environmental *V. parahaemolyticus* isolates, and all five isolates were in ribogroup 146-1, matching the majority of O4:K12 clinical isolates.

Serotypes O11:Kuk (16 isolates) and O4:K9 (12 isolates), in ribogroups 250-6 and 161-3, respectively, were most prevalent among Gulf Coast environmental *V. parahaemolyticus* isolates. Several isolates each of serotypes O1:K56 and O1:Kuk were noted among Gulf Coast environmental *V. parahaemolyticus* isolates, but their ribotypes were different from those of Pacific Coast environmental isolates or any of the clinical isolates with corresponding serotypes. A single O4:K8 isolate was found among the environmental isolates from each coast, i.e., Atlantic, Gulf, and Pacific, but each of these isolates had a different ribogroup.

Each of the four Pacific Coast food isolates possessed all of the virulence attributes examined, while 6 of the 12 food isolates from the Atlantic and Gulf Coasts lacked *trh* and urease production. The O4:K63 and O4:K12 food isolates from the Pacific Coast were in ribogroups that matched those of the Pacific Coast clinical isolates with the corresponding serotypes, but not the O4:K63 food isolates from Maryland. The O4:K9 food isolates from Louisiana and Massachusetts were in the same ribogroup (161-3) as the O4:K9 environmental isolates from Alabama. The O5:K17 isolates from Connecticut and Virginia were all in ribogroup 219-4. However, the O11:Kuk food isolate from Louisiana was in a ribogroup different from that of the 16 O11:Kuk environmental isolates from Alabama. None of the environmental or food isolates were O3:K6, nor were they in any of the three ribogroups that contained all of the pandemic O3:K6 clone. With regard to both serotype and ribogroup, none of the environmental or food isolates from the Gulf or Atlantic Coasts matched any of the clinical isolates or any of the Pacific Coast isolates.

A dendrogram illustrating the similarity relationships between the ribogroup patterns is shown in Fig. 1. The 28 patterns were divided into six clusters (Table 2). In general, there were no apparent patterns of distribution by either serotype or

TABLE 2. Distribution of serotypes and isolate sources among the ribogroup clusters

Cluster[a]	No. of isolates	Ribogroup(s)	Clinical isolates		Environmental and food isolates		Serotype(s)
			% of total	Region(s) (no. of isolates)[b]	% of total	Region(s) (no. of isolates)[b]	
1	24	146-1, 293-3	62	PC (15)	38	PC (8), GC (1)	O4:K12, O1:K56, O4:Kuk, O4:K8, O12:K12
2a	105	146-2, 161-3, 190-6, 252-5, 250-6, 293-7, 219-7, 251-1, 193-1, 202-6, 219-6, 219-4, 312-7	41	Asn (20), AC (11), PC (2), GC (10)	59	GC (41), PC (13), AC (8)	O1:K54, O1:K55, O1:K56, O1:Kuk, O3:K6, O3:K34, O3:K49, O3:Kuk, O4:K8, O4:K9, O4:K49, O4:K63, O4:K68, O4:Kuk, O5:K17, O5:Kuk, O6:K18, O11:K40, O11:Kuk
2b	14	146-8, 162-3, 293-1, 203-7	14	AC (1), PC (1)	86	GC (11), AC (1)	O1:K22, O1:K30, O1:K56, O1:Kuk, O3:K36, O4:K8, O4:K55
3	1	275-1			100	GC (1)	O11:K61
4	32	147-4, 159-5, 276-3, 219-8, 358-1	22	PC (7)	78	PC (22), GC (3)	O1:K56, O1:Kuk, O4:K12, O4:K63, O11:Kuk
5	2	159-6, 310-3	50	PC (1)	50	GC (1)	O4:K12, O8:K70
6	1	147-1	100	Asn (1)			O4:K12

[a] At 0.375 dissimilarity units
[b] PC, U.S. and Canadian Pacific Coasts; GC, U.S. Gulf Coast; AC, U.S. Atlantic Coast; Asn, Asian countries.

region of origin among the ribogroup clusters. For example, the six O4:K12 ribogroup patterns occurred in four clusters. While the three O3:K6 ribogroup patterns fell into a single cluster (2a), this cluster contained nearly half of the ribogroups and most of the isolates. Within cluster 2a, the Galveston Bay O3:K6 isolates (ribogroup 193-1) were much more distant from ribogroup 190-6, which contained most of the Asian and all of the New York and Connecticut O3:K6 isolates, than was ribogroup 252-5, which contained a single clinical isolate from India.

DISCUSSION

These data show that *tdh*$^+$ *V. parahaemolyticus* isolates from clinical food and environmental sources are both serologically (27 types) and genetically (28 ribogroups) diverse with many small groups and a few dominant ones. Many of the serotypes consisted of multiple ribogroups and vice versa. Using these two typing systems in combination greatly enhanced the ability to link or distinguish strains from various sources. Neither the O3:K6 serotype nor any of the ribogroups associated with the pandemic clone was found among any of the environmental or food isolates examined, supporting previous results (13) suggesting that it has not become established in the United States (5, 10).

Surprisingly, given this diversity, over 90% of *tdh*$^+$ food and environmental isolates examined from U.S. coastal areas were *trh*$^+$ and produced urease. All isolates possessing either of these traits had both of them and may be part of a pathogenicity island (36). This is the first report of urease-producing and *trh*$^+$ *V. parahaemolyticus* isolates from the U.S. Atlantic Coast, extending the known occurrence of *V. parahaemolyticus* with these traits to all three major coasts of the United States, a phenomenon that was recently reported only on the Pacific Coast (14). It is possible that the prevalence of urease-producing and *trh*$^+$ *V. parahaemolyticus* is increasing on the Gulf and Atlantic Coasts, since this was not reported until recently. However, previous environmental studies of *V. parahaemolyticus* from these regions found relatively few *tdh*$^+$ *V. parahaemolyticus* isolates, and none of these isolates were tested for *trh* (2, 9, 12, 15). Urease production has been observed in some environmental isolates from the Chesapeake Bay, but the other virulence factors were not examined (7). While *trh* was not described until 1988 (20), reexamination of clinical isolates from the Pacific Coast dating back to at least 1979 found that most were *trh*$^+$ and urease producing (34). Since the mid 1980s, the majority of the clinical and *tdh*$^+$ environmental *V. parahaemolyticus* isolates from the U.S. and Canadian Pacific Coasts have been reported to produce urease (13, 23, 25, 34) and also, when tested, to usually possess *trh* (13, 34). It does not appear that urease-producing and *trh*$^+$ *V. parahaemolyticus* from the Pacific Coast were introduced to the Atlantic and Gulf Coasts, as many of the Pacific Coast strains have maintained a stable serotype and ribogroup pattern for 1 to 2 decades and all of the Pacific Coast strains were distinguishable from Gulf and Atlantic Coast strains in terms of both serotype and ribogroup.

While these data indicate that urease-producing and *trh*$^+$ *V. parahaemolyticus* are widespread in U.S. coastal environments (>95% of *tdh*$^+$ isolates), their prevalence appears to be considerably lower in market samples of Gulf and Atlantic Coast oysters (approximately 50% of *tdh*$^+$ isolates). This difference may be attributed to different harvest locations, as most environmental isolates were from the Gulf Coast and most food isolates were from the Atlantic Coast. Another possibility is that strains possessing *trh* and urease may not grow or survive as well during the handling and storage of oysters as do *tdh*$^+$ strains lacking these traits.

The sharp contrast in the prevalence of *trh* and urease production between the clinical *V. parahaemolyticus* isolates from the Pacific Coast (26 of 26 isolates) and those from the Gulf and Atlantic Coasts (0 of 22) is due largely to the selection of

isolates. The Atlantic and Gulf Coast isolates were almost exclusively from the only two U.S. outbreaks caused by the O3:K6 serotype, whereas Pacific Coast isolates represented a mix of sporadic and outbreak isolates. Approximately 60% of recent human clinical strains from the United States, including many from the Gulf and Atlantic Coasts, possess *trh* and produce urease (14). In contrast, only 4% of the 371 food-borne *V. parahaemolyticus* isolates obtained in Taiwan between 1992 and 1995 were urease positive (41).

The prevalence of *tdh*$^+$ *V. parahaemolyticus* in clinical isolates from the Pacific Coast described here (all 26 isolates associated with Washington oysters from 1988 to 2001) is higher than that previously reported in British Columbia (0 of 5 isolates from locally acquired diarrhea cases between 1984 and 1987 [25]) or California (35 of 43 fecal isolates collected from 1979 to 1995 [34]). In contrast to the clinical isolates, the environmental and food isolates were generally screened for *tdh* prior to further characterization, which precluded the determination of the frequency of *trh* or urease production in isolates not possessing *tdh*.

The predominant serotypes among the clinical isolates from the Pacific Coast (O4:K12, O1:K56, and O4:K63) were also prevalent among the environmental and food isolates from this region. While multiple ribogroups were observed among these serotypes, the most prevalent ribogroups were generally the same for each source. This suggests that the risk of illness probably correlates well with the density of *tdh*$^+$ *V. parahaemolyticus* found in oysters. In previous surveys, these three serotypes were also prevalent in clinical isolates from California (34) but were not observed among 18 *tdh*$^+$ *V. parahaemolyticus* environmental isolates from Willapa Bay, Wash. (23). Similarly, Wagatsuma reported close agreement between the K serotypes found in Kanagawa-positive strains isolated from environmental sources and those in outbreak-associated isolates in Japan (40). The evaluation of Gulf and Atlantic Coast clinical *V. parahaemolyticus* isolates associated with sporadic illnesses may show a similar relationship with food and environmental isolates from these regions, and we suggest such an evaluation for further study.

Combined consideration of both serotype and ribogroup also revealed some important relationships among isolates from other regions. All but one of the O3:K6 isolates from Texas were in ribogroup 193-1, which contained none of the O3:K6 isolates from either New York or Asia (ribogroup 190-6), suggesting that the two 1998 outbreaks in the United States were from different sources. The O1:Kuk and O4:K68 isolates from Asia were also included in ribogroup 190-6, and these serotypes have been reported to be closely related to the new O3:K6 clone (1, 6, 27). None of the O1:Kuk isolates from the Gulf or Pacific Coast were in ribogroup 190-6. However, two old O3:K6 isolates were in ribogroup 190-6 but had previously been shown to differ from the new O3:K6 clone (1, 6, 27).

The similarity relationships between the ribogroup patterns did not appear to reflect underlying serological, geographic, or genetic relationships. For example, O4:K12 isolates from the Pacific Coast were scattered into three distantly related clusters. There is general agreement in the scientific community that DNA-RFLP data are reasonably good indicators of genetic relatedness, although genetic relatedness may not be quantifiable from RFLP data.

This study demonstrates the predominance of *trh* and urease, along with *tdh*, in pathogenic *V. parahaemolyticus* throughout U.S. coastal areas. Serotyping and ribotyping showed similar distributions of pathogenic *V. parahaemolyticus* isolated from clinical, environmental, and food sources on the Pacific Coast. No relationship was observed between the pathogenic *V. parahaemolyticus* population of the Pacific Coast and those of either the Atlantic or Gulf Coast when both serotyping and ribotyping were considered, suggesting that most *V. parahaemolyticus* strains are regional. The fact that certain serotypes and ribotypes contained both clinical and environmental isolates while many others contained only environmental isolates implies that certain serotypes or ribotypes are more relevant for human disease.

ACKNOWLEDGMENTS

The Texas Department of Health, the State of New York Department of Health, the Connecticut Department of Health, the Washington State Department of Health, and Mitsuaki Nishibuchi of Kyoto University, Kyoto, Japan, kindly provided *V. parahaemolyticus* clinical strains.

This work was partially supported by Cooperative Agreement FD-000431 between the U.S. Food and Drug Administration and the National Center for Food Safety and Technology.

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