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Characterization and Distribution of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* Isolated in Indonesia

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Previous studies have shown that *Vibrio alginolyticus* and *Vibrio parahaemolyticus* can be isolated from similar types of marine samples. In this report, the results of an examination of 567 *V. alginolyticus* and *V. parahaemolyticus* strains, isolated from seawater in Jakarta Bay and from more than 30 types of seafood from markets in Jakarta, Indonesia, are presented. Most isolates were from mackerel, shrimp, or squid. Numerical taxonomic analyses clustered 337 isolates and three *V. alginolyticus* reference strains at S ≥ 80%. These strains produced acid from sucrose, but only approximately 80% produced acetoin or grew in the presence of 10% NaCl. The frequency of occurrence of *V. alginolyticus* in seawater samples ranged from 0% (in February and March 1972) to 100% (in September and December 1972) and was highest in seafood samples from August to December 1972. A second cluster of 230 isolates and seven *V. parahaemolyticus* reference strains was observed at S ≥ 82%. These strains did not produce acetoin or acid from sucrose, and approximately 20% grew in the presence of 10% NaCl. *V. parahaemolyticus* was detected in seawater samples each month, with the highest frequency of occurrence (83.3%) in May 1972. Twenty-nine K antigen serotypes were demonstrated in *V. parahaemolyticus* isolates, and another 40% were untypable. The modal antibiotic resistance pattern for each species included five drugs. Only 12% of the *V. parahaemolyticus* strains were Kanagawa positive, and 10% elicited fluid accumulation in ligated rabbit ileal loops. All of the 7 *V. alginolyticus* strains and 94 (70%) of the *V. parahaemolyticus* strains tested killed mice when inoculated intraperitoneally. Both *V. alginolyticus* and *V. parahaemolyticus* were concluded to be indigenous to seawater and were frequently isolated from seafood in Jakarta, Indonesia. For this reason they should be of concern to clinicians and health authorities in this and other tropical areas.

In the last two decades, research on medical, environmental, and taxonomic aspects of *Vibrio* species has expanded greatly. Nine species have been associated with gastroenteritis, wound infections, or septicemia (4, 12).

Epidemiological studies have established *Vibrio parahaemolyticus* as a worldwide agent of gastroenteritis, and results of ecological studies demonstrate that it can be isolated from seafood, as well as estuarine, neritic, and brackish waters (12). Isolation from the environment in temperate regions has been shown to be related to water temperature, whereas isolation in tropical waters appears to be related to season (rainy or dry) and salinity (5, 11, 12, 21). Although the presence of *V. parahaemolyticus* is extensive in estuarine environments, virulent strains are essentially limited to those that are positive for the Kanagawa phenomenon (KP+) (12). KP+ strains are rarely isolated from environmental samples but constitute the majority of clinical strains. KP+ strains are infectious at lower doses than are KP− strains, and they elicit fluid accumulation in ligated rabbit ileal loops (RIL) more frequently (12). Attempts to link specific serotypes of *V. parahaemolyticus* with illness or with environmental specimens have been unsuccessful to date, but an association with copepods has been suggested (16). No serotype predominates from year to year in environmental or clinical strains (12).

Vibrio alginolyticus has been isolated more frequently in recent years from wounds exposed to seawater. Although *V. alginolyticus* can be isolated from the same types of samples as can *V. parahaemolyticus*, ecological and epidemiological studies have been somewhat limited and have included only isolates from temperate waters (12). *V. alginolyticus* has been shown to be lethal for mice injected intraperitoneally (i.p.), with death occurring in 24 to 48 h (24). Ecological interrelationships between *V. alginolyticus* and *V. parahaemolyticus* merit further investigation, as does the association of serotypes with virulence factors, illness, or both.

Taxonomic studies (2, 6, 18, 29, 34) of genetic, biochemical, and physiological data clearly distinguish *V. parahaemolyticus* from *V. alginolyticus*, with differential taxonomic characteristics shown for isolates from geographically diverse areas. Here we report the results of analyses of taxonomic, ecological, pathogenic, and serologic data for 567 *V. parahaemolyticus* and *V. alginolyticus* isolates from seawater samples collected in Jakarta Bay and from seafood samples taken from markets in Jakarta, Indonesia.

MATERIALS AND METHODS

Sample collection. Seawater samples (200 ml each) were collected in neritic waters within 3 m of the shoreline of Jakarta Bay. Samples were membrane filtered (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.), and the filter disks were aseptically placed onto thiosulfate-citrate-bile-sucrose agar (BBL Microbiology Systems, Cockeysville, Md.) and incubated for 24 h at 37°C.

Seafood samples were collected from various markets (Fig. 1) in Jakarta, Indonesia. The gills, gut, and portions of the skin and muscle were used in the processing of fish and crab specimens, while whole bodies were used for shrimp,

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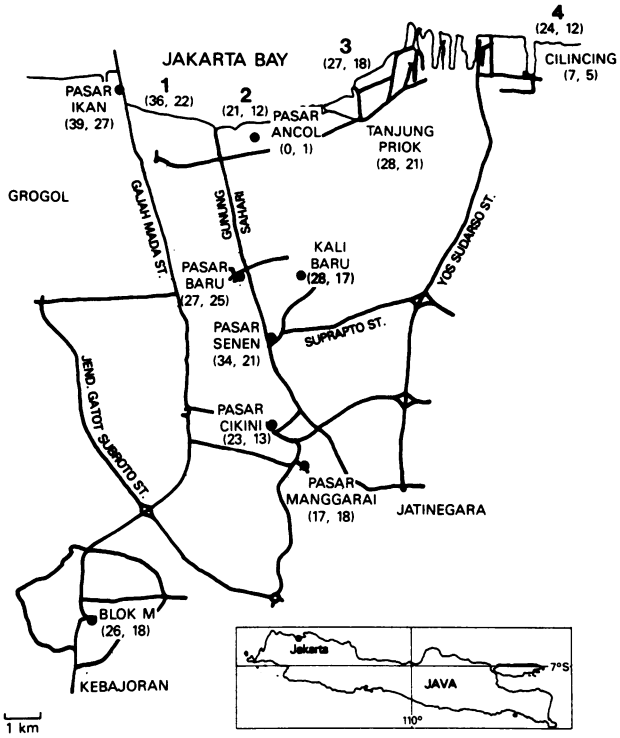

FIG. 1. Map of Jakarta, Indonesia, showing collection sites for seawater (boldface numbers) and seafood. Numbers in parentheses represent the numbers of *V. alginolyticus* and *V. parahaemolyticus*, respectively, isolated from samples collected at each site.

clam, and squid specimens. Samples were processed as recommended previously (31).

Biochemical tests. Presumptive *V. alginolyticus* and *V. parahaemolyticus* isolates were characterized by using standard methods (31). In addition, the following tests were performed (media used were from BBL unless otherwise noted): oxidase reaction, nitrate reduction, and esculin hydrolysis as described by the manufacturer (Difco Laboratories, Detroit, Mich.); lipase (22); cellulose utilization (27); and decarboxylation of L-lysine, L-ornithine, and L-arginine (all at 1%, wt/vol) in Moeller decarboxylase basal medium (Difco). Reactions were determined in TSI Agar (BBL), sulfide-indole-motility agar, litmus milk, and Simmons citrate agar (each amended with 2.5% NaCl [wt/vol]).

Antibiotic susceptibility. Tests of susceptibility to the following antibiotics were performed by using the method of Bauer et al. (1): ampicillin (10 μg), cephalin (30 μg), chloramphenicol (30 μg), colymicin (10 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), methicillin (5 μg), nafcillin (1 μg), nalidixic acid (30 μg), neomycin (30 μg), penicillin (10 U), polymyxin B (300 U), streptomycin (10 μg), and tetracycline (30 μg).

Serology. Serological characterization of the *V. parahaemolyticus* isolates was performed either by using commercially available sera (Toshiba Kagaku Co.) or through the courtesy of H. Zen-Yoji.

Kanagawa phenomenon. The ability to hemolyze erythrocytes was tested on Wagatsuma agar (31). Inoculated media were incubated at 37°C for 24 h and were examined visually for zones of hemolysis.

Ligated RIL. The RIL test was performed as described previously (28). New Zealand White rabbits of either sex and weighing approximately 1.0 kg were anesthetized by intravenous injection of 45 mg of sodium pentobarbitol per kg of body weight. The ileum was externalized and ligated, and 2.0 ml of whole culture in brain heart infusion broth was injected into each loop. Test loops were separated by control intervals which were injected with sterile brain heart infusion broth. The ileum was returned, and the incision was closed. After 18 to 20 h, the rabbits were sacrificed, and the loops were examined for fluid accumulation. Response was measured as the ratio of milliliter of fluid accumulation to centimeter of loop length. Most RIL tests were repeated for each isolate.

Mouse i.p. inoculations. For each strain, 0.5 ml of whole culture was inoculated i.p. into two 18- to 21-day-old white mice, NIH/NMRI CU strain. The mice were kept in holding cages at room temperature and were examined hourly for 10 h. They were subsequently examined daily for 10 days.

Reference strains. All reference strains used in this study were isolated from temperate areas (Table 1). All but two *V. parahaemolyticus* strains were from environmental sources.

G+C determinations. Moles percent guanine plus cytosine (G+C) was determined by the method of Mandel et al. (20). Preparation of DNA samples was performed as previously described (10) with the following modifications. The cells were suspended and lysed in Tris saline buffer, pH 8, containing 0.05 M Tris (Bethesda Research Laboratories, Inc., Rockville, Md.) and 0.2 M NaCl. The Tris saline buffer was supplemented with sodium EDTA (final concentration, 0.02 M) at the time of lysis, and the cell suspension was frozen at −70°C until required for further preparation. The DNA was precipitated and purified (10), and after 1 h of incubation in the presence of RNase, pronase (Sigma Chemical Co., St. Louis, Mo.) was added at a concentration of 100 μg/ml. The incubation was continued for 1 h at 35°C. The purity of the DNA was examined by measuring A_{230}, A_{260}, and A_{280} in a Gilford 2600 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio), which also was used to melt the DNA at a temperature increasing by 1°C/min to determine the moles percent G+C value. *V. cholerae* (ATCC 14035) and *V. parahaemolyticus* (ATCC 17802) were included as controls for moles percent G+C calculations.

Computer analysis. Computer analysis was performed by using programs available at the Microbial Systematics Section, National Institute of Dental Research, Bethesda, Md. Biochemical and physiological data were submitted to the

TABLE 1. Reference strains[a]

Species	Strain	Geographical location	Source
V. alginolyticus	B1	Florida	Pompano nose erosion
	16670	Japan	Fish
	17749	Japan	Fish
V. parahaemolyticus	8657	Maryland	Gastroenteritis
	NH4	New Hampshire	Estuary
	BCL	Washington	Clams
	CRAB	Maryland	Blue crab
	NIC	Texas	Shrimp
	4AA	Maryland	Blue crab
	SAK4	Japan	Gastroenteritis

[a] All strains were from the collection of one of the authors (R.R.C).

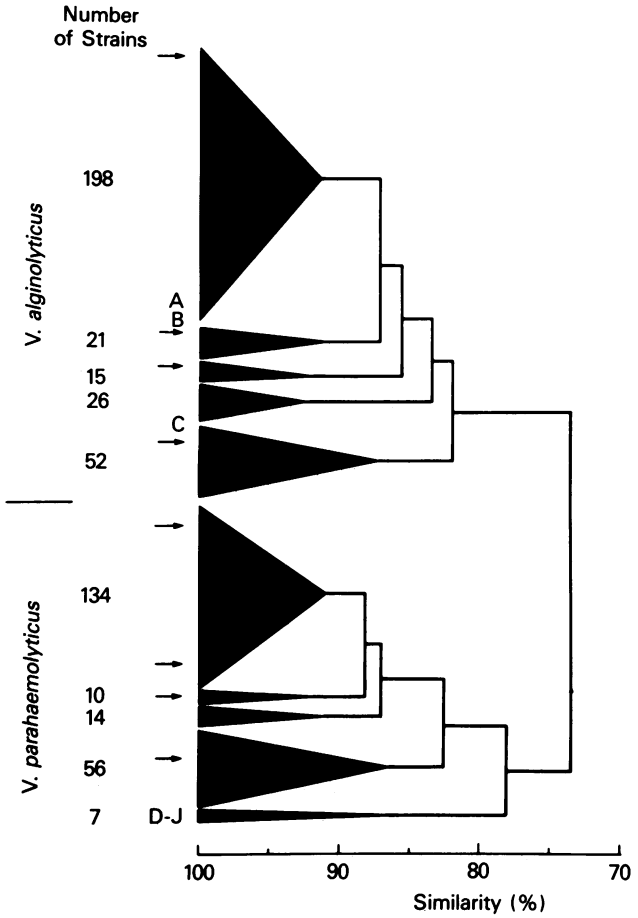

FIG. 2. Simplified dendrogram showing the clustering of the isolates which was produced by using the simple matching coefficient. Uniformly positive and negative features were deleted to increase the sensitivity of the analysis. Arrows indicate strains for which DNA base composition data were obtained. Letters indicate the following reference strains: B1 (A), 16670 (B), 17749 (C), 8657 (D), NH4 (E), BCL (F), CRAB (G), NIC (H), 4AA (I), and SAK4 (J).

program TAXON (32) for cluster analysis, using the simple matching coefficient (S) and unweighted average linkage.

RESULTS

Cluster analysis placed 577 strains (98%) into two major groups that clustered at S ≥ 75%. Group 1 included 337 isolates and three *V. alginolyticus* reference strains that clustered at S ≥ 80% (Fig. 2). Selected feature frequencies of this group were examined (Table 2). *V. alginolyticus* produced acetoin, acid from sucrose, and an acid slant in TSI Agar, and grew in the presence of 10% NaCl. All strains hydrolyzed esculin, and many produced hydrogen sulfide (66%), hemolysin (79%), and acid from arabinose (56%) (Table 2). Base ratios of the DNA of strains selected from this group ranged from 42 to 44 mol% G+C.

Group 2 from the cluster analysis included 230 isolates and seven *V. parahaemolyticus* reference strains. They clustered at S ≥ 82%, with the reference strains forming a subgroup at S ≥ 86% and joining the main cluster at S ≥ 78%. The features of this group were typical of *V. parahaemolyticus*: an alkaline slant was produced on TSI Agar, acetoin was not produced (<8%), no acid was produced from sucrose

TABLE 2. Frequency of tested features of *V. parahaemolyticus* and *V. alginolyticus*

Feature[a]	% of strains	
	V. alginolyticus (*n* = 340)	*V. parahaemolyticus* (*n* = 237)
Motility	98.5	98.3
TSI Agar reaction		
K/A[b]	1.5	100.0
A/A	97.9	0.0
Gas	2.7	0.4
H_2S[c]	65.9	59.1
Growth in % NaCl (wt/vol):		
0	2.6	4.2
7	98.8	94.1
10	78.5	19.8
Reduction of nitrate	93.8	99.3
Production of:		
Gelatinase	99.7	100.0
Lipase	85.2	66.1
Urease	2.4	9.4
Indole	96.5	99.2
Phenylpyruvate	0.0	0.9
Acetoin	84.0	7.7
Litmus milk		
Acid	2.2	2.2
Coagulation	65.2	42.2
Alkaline	31.5	55.6
Peptonization	68.1	74.1
Reduction	99.1	100.0
Methyl red	78.3	97.5
Hemolysis		
Alpha	68.8	67.5
Beta	10.0	8.4
Acid from:		
Arabinose	56.3	79.5
Xylose	3.0	1.0
Galactose	82.4	97.9
Glucose	100.0	99.6
Mannose	100.0	99.5
Cellobiose	15.0	4.8
Lactose	2.7	2.1
Maltose	97.6	95.8
Melibiose	0.0	1.4
Sucrose	97.6	5.9
Raffinose	5.9	0.0
Dextrin	76.5	93.8
Mannitol	98.5	92.4
Utilization of citrate	99.4	98.3
Decarboxylation of:		
Arginine	1.6	0.0
Lysine	97.0	98.7
Ornithine	41.7	69.0
Hydrolysis of starch	99.1	97.0

[a] All strains grew in the presence of 3% NaCl, produced catalase, oxidase, and acid from fructose and trehalose, and hydrolyzed esculin. None of the strains utilized cellulose or produced acid from rhamnose, salicin, melezitose, adonitol, dulcitol, or sorbitol.

[b] K/A, Alkaline slant/acid butt; A/A, acid slant/acid butt.

[c] Negative after 24 h at 37°C; positive reactions recorded after 7 days at room temperature.

(<6%), and most strains (94%) were able to grow in the presence of 3 to 7% NaCl and unable (80%) to grow in the presence of 10% NaCl (Table 2). The strains hydrolyzed esculin, and many produced hydrogen sulfide in TSI Agar (59%), hemolysin (76%), acid from arabinose (80%), and ornithine decarboxylase (69%). Less than 10% produced urease or acid from cellobiose. DNA base ratios ranged from 44 to 45 mol% G+C.

Distribution in the environment. Overall, 32% of the *V. alginolyticus* strains and 28% of the *V. parahaemolyticus* strains were isolated from seawater; however, *V. alginolyticus* was isolated more frequently from each of the sampling sites than was *V. parahaemolyticus* (Fig. 1). Most *V. alginolyticus* and *V. parahaemolyticus* strains were isolated from seawater throughout the year, when the water temperature ranged between 29 and 32°C. The proportion of each species isolated at each temperature was very similar.

Isolates from samples representing 32 species of fish and shellfish composed approximately 68 and 72% of the *V. alginolyticus* and *V. parahaemolyticus* strains, respectively (Table 3). Distribution of the two bacterial species among types of seafood was approximately the same. Most of the seafood strains were obtained from mackerel, shrimp, or squid.

The frequency of *V. alginolyticus* and *V. parahaemolyticus* strains in seawater and seafood samples was confirmed for each month (Table 4). The occurrence of *V. alginolyticus* in seawater samples increased from 0%, in February and March 1972, to 100%, in September and December 1972. In contrast, *V. parahaemolyticus* was present in seawater every month, with the highest occurrence (83.3%) in May 1972. The presence of *V. parahaemolyticus* was confirmed in at least 56% of the samples collected in May through October 1972, contrasting with an occurrence in fewer than 50% of the samples for February through April 1972. Another decrease occurred after October 1972 (Table 4).

The occurrence of *V. alginolyticus* in seafood peaked at approximately the same time of year (August to December 1972) that its occurrence was highest in seawater (July to December 1972) (Table 4). The frequency of occurrence of *V. parahaemolyticus* in seafood peaked earlier in the year (April to July 1972) than did that of *V. alginolyticus*.

Serology. A total of 29 K antigen serotypes of *V. parahaemolyticus* were detected. However, 42% of the strains isolated from seawater and 37% of those isolated from seafood were untypable by using available antisera. The modal serotype K28 was found in 19 and 13% of the seawater and seafood isolates, respectively. The modal serotype for each month of the study for each type of sample was determined (Table 4). Except for strains isolated in December 1972, the most common serotype in seawater did not coincide with that from seafood.

TABLE 3. Sources of *V. alginolyticus* and *V. parahaemolyticus* isolates

Specimen type	No. (%) of strains	
	V. alginolyticus (*n* = 229)	*V. parahaemolyticus* (*n* = 166)
Mackerel (*Rastrelliger* sp.)	44 (19.0)	33 (20.0)
Shrimp (*Penaeus* sp.)	30 (13.1)	26 (16.0)
Squid (*Loligo* sp.)	24 (10.0)	18 (10.9)
Pomfret (*Stromateus* sp.)	17 (7.4)	8 (4.9)
Trevally (*Caranx* sp.)	17 (7.4)	10 (6.1)
Milk fish (*Chanos* sp.)	16 (7.0)	11 (6.7)
Crab	13 (5.7)	12 (7.8)
Fusilier (*Caesio* sp.)	10 (4.4)	9 (5.5)

Resistance to antibiotics. The method of Bauer et al. (1) for testing for resistance to 16 antibiotics resulted in 114 different resistance patterns for *V. alginolyticus* strains. The majority of the patterns (57%) were represented by single isolates; the modal pattern (25 strains, 8.2%) of *V. alginolyticus* (*n* = 306) included resistance to ampicillin, lincomycin, methicillin, nafcillin, and penicillin. The two next-most-common patterns occurred with equal frequency (15 strains, 4.9%) and included streptomycin or streptomycin and kanamycin in addition to the antibiotics in the modal pattern.

Based on resistant, intermediate, and susceptible zone diameters established for enteric bacteria (1), *V. alginolyticus* strains were susceptible to chloramphenicol, resistant to ampicillin, lincomycin, methicillin, nafcillin, and penicillin, and had intermediate reactions to neomycin (Table 5).

A total of 92 different antibiotic resistance patterns were observed in *V. parahaemolyticus* (*n* = 199). Most patterns (53%) were represented by only a single isolate. The modal pattern (nine strains, 4.5%) consisted of resistance to colymicin, lincomycin, nafcillin, penicillin, and polymyxin B. The next-most-common pattern (seven strains, 3.5%) included ampicillin resistance as well as resistance to the antibiotics included in the modal pattern. *V. parahaemolyticus* was susceptible to chloramphenicol and resistant to lincomycin, nafcillin, and penicillin (Table 5).

Pathogenicity. Pathogenicity was tested by KP, RIL, and i.p. inoculation of mice (Table 6). Few *V. parahaemolyticus* isolates (12%) were KP+ or elicited fluid accumulation (10%) in ligated RIL. Most strains (70%) were lethal for mice injected i.p. Only limited testing of *V. alginolyticus* strains was performed. One of the five strains tested was KP+, one of the eight strains tested elicited fluid accumulation, and all of the seven strains tested were lethal for mice.

DISCUSSION

Identification of *V. alginolyticus* from tropical waters and seafood was achieved by using established taxonomic characteristics of *V. alginolyticus* from temperate climates. Three *V. alginolyticus* reference strains occupied nearly central positions in the *V. alginolyticus* cluster (Fig. 2).

Growth in the presence of 10% NaCl, acetoin production, and fermentation of arabinose are important features which distinguish *V. alginolyticus* from *V. parahaemolyticus*. Incubation periods of at least 2 days for growth in the presence of increased NaCl concentratons have been used by other investigators (33, 34), and negative results have been reported for growth in the presence of 10% NaCl of *V. alginolyticus* (14, 19). Production of acetoin is influenced by both incubation time and temperature (33). Arabinose-positive *V. alginolyticus* strains from diverse geographical regions have been reported (9, 14, 30), although this characteristic is generally considered to be negative for *V. alginolyticus* (12, 23, 26, 34). Discrepancies between our fermentation results and those reported by others may be due to differences in the basal media, sterilization conditions, and indicators used. Thus, negative results for these tests should not be used to rule out *V. alginolyticus*.

Biochemical characteristics of *V. parahaemolyticus* were typical of this species, but about 6% of the strains produced acid from sucrose (Table 2). Other investigators have also

TABLE 4. Samples in which the presence of *V. alginolyticus* or *V. parahaemolyticus* was confirmed for each month

Mo	Sample type[a]	No. collected	No. (%) positive for:		Modal serotype[b]
			V. alginolyticus	*V. parahaemolyticus*	
February 1972	Sw	6	0 (0)	2 (33.3)	K17,K28
	Sf	16	2 (12.5)	7 (43.8)	UT
March 1972	Sw	2	0 (0)	1 (50.0)	NT
	Sf	15	7 (46.6)	9 (60.0)	UT
April 1972	Sw	2	1 (50.0)	1 (50.0)	K12
	Sf	20	13 (65.0)	18 (90.0)	UT
May 1972	Sw	6	4 (66.7)	5 (83.3)	UT (K5,K24,K28)
	Sf	24	15 (62.5)	18 (75.0)	UT
June 1972	Sw	3	1 (33.3)	2 (66.7)	UT (K17)
	Sf	25	15 (60.0)	23 (92.0)	UT
July 1972	Sw	10	8 (80.0)	6 (60.0)	UT
	Sf	9	5 (55.6)	9 (100.0)	K3
August 1972	Sw	7	5 (71.0)	5 (71.0)	UT
	Sf	11	11 (100.0)	8 (73.0)	UT
September 1972	Sw	16	16 (100.0)	9 (56.2)	UT
	Sf	25	24 (96.0)	15 (60.0)	UT
October 1972	Sw	21	18 (85.7)	13 (61.9)	UT
	Sf	33	33 (100.0)	23 (69.7)	UT
November 1972	Sw	18	7 (38.9)	6 (33.3)	UT (K28)
	Sf	19	14 (73.6)	13 (68.4)	UT
December 1972	Sw	11	11 (100.0)	4 (36.4)	K17
	Sf	12	12 (100.0)	4 (33.3)	K17,K28
January 1973	Sw	14	9 (64.3)	7 (50.0)	K5,K28
	Sf	24	23 (95.8)	18 (75.0)	UT

[a] Sw, Seawater; Sf, seafood.
[b] UT, Untypable; NT, not tested. Serotypes in parentheses are those which occurred at the same frequency as did the untypable strains.

reported that *V. parahaemolyticus* produced acid from sucrose (6, 14, 18). Thus, it must be concluded that some strains of *V. parahaemolyticus* are indeed sucrose positive and that these strains should not be overlooked in clinical screening for causative agents of enteritis.

The inability of *V. parahaemolyticus* to grow in the presence of 10% NaCl is useful but in itself is not sufficient for distinguishing *V. parahaemolyticus* from *V. alginolyticus*. Other studies have reported that *V. parahaemolyticus* is capable of growth in the presence of 10% NaCl (6, 8, 14, 26). Zen-Yoji et al. (34) reported that all *V. parahaemolyticus* strains tested grew in the presence of 9% NaCl but that only one strain grew in the presence of 11% NaCl. Although care has been recommended in the selection of the basal medium and in the autoclaving of salt tolerance media (33), the variability of *V. parahaemolyticus* growth in the presence of 10% NaCl suggests that 10% NaCl is too close to the concentration limit for this species.

Production of hydrogen sulfide by *V. parahaemolyticus* has been reported to be dependent upon the medium and method used (18) and is generally negative in TSI Agar (18, 24). Sulfur reduction has been reported by Schandevyl et al. (26) for almost 50% of tropical *V. parahaemolyticus* isolates after 2 days when NaCl-supplemented Kligler iron agar was used.

V. alginolyticus is known to occur within the same habitat as does *V. parahaemolyticus* and is often isolated from the same types of samples (12). Both species can survive in conditions prevalent at seafood markets in Jakarta. Stephen et al. (30) isolated *V. alginolyticus* from 9 of 12 types of seafood from the Arabian Sea, with most isolates originating from freshly caught sardines, mackerel, and crabs.

A consistently higher frequency of occurrence for *V. alginolyticus* than for *V. parahaemolyticus* was reported for seawater and seafood samples collected in Norway, the Netherlands, and Japan (8, 14, 25). The relative abundance of *V. alginolyticus* and *V. parahaemolyticus* in tropical seawater and seafood sources is less well studied, but a recent report showed that a greater percentage of freshly caught sea fish samples yielded sucrose-positive colonies than sucrose-negative colonies on thiosulfate-citrate-bile-sucrose agar (26). Sakazaki et al. (25) proposed a *V. alginolyticus* index for secondary surface contamination of seafood and utensils by *V. parahaemolyticus*. However, modification of this proposal may be necessary in tropical areas, where the relative abundance of *V. alginolyticus* and *V. parahaemolyticus* does not provide a distinct difference.

Gjerde and Boe (8) reported that the incidence of *V. alginolyticus* increased in mussel, fish, water, and sediment samples from the Norwegian coast during the summer months (June to September) when water temperature was warmest (10 to 16°C). In the results reported here, seasonal

TABLE 5. Resistance of *V. alginolyticus* and *V. parahaemolyticus* to selected antibiotics

Antibiotic (dose)[a]	Zone size (mm)	Response[b]	% of strains	
			V. alginolyticus (n = 306)	*V. parahaemolyticus* (n = 199)
Amp (10 μg)	<11	R	97.4	67.3
	12–13	I	2.3	16.1
	>14	S	0.3	16.6
Cep (30 μg)	<14	R	43.5	27.6
	15–17	I	51.0	41.2
	>18	S	5.6	31.2
Cam (30 μg)	<12	R	0.3	0.0
	13–17	I	3.3	0.5
	>18	S	96.4	99.5
Col (10 μg)	<8	R	47.9	78.4
	9–10	I	39.0	17.6
	>11	S	13.1	4.0
Ery (15 μg)	<13	R	4.6	0.5
	14–17	I	71.9	51.8
	>18	S	23.5	47.7
Gen (10 μg)	<12	R	5.9	2.0
	13–14	I	19.0	11.6
	>15	S	75.1	86.4
Kan (30 μg)	<13	R	41.6	16.6
	14–17	I	58.0	72.9
	>18	S	0.3	10.6
Lin (2 μg)	<9	R	99.7	99.5
	10–14	I	0.3	0.0
	>15	S	0.0	0.5
Met (5 μg)	<9	R	94.3	52.9
	10–13	I	5.7	43.2
	>14	S	0.0	3.9
Naf (1 μg)	<10	R	100.0	99.5
	11–12	I	0.0	0.0
	>13	S	0.0	0.5
Nal (30 μg)	<13	R	1.3	3.0
	14–18	I	34.0	26.6
	>19	S	64.7	70.4
Neo (30 μg)	<12	R	6.5	1.5
	13–16	I	90.5	74.7
	>17	S	2.9	23.7
Pen (10 U)	<11	R	99.7	92.0
	12–13	I	0.3	7.0
	>14	S	0.0	1.0
Pmx (300 U)	<10	R	28.3	59.7
	11–14	I	48.6	22.1
	>15	S	23.1	18.2
Str (10 μg)	<11	R	68.6	64.3
	12–14	I	31.4	33.2
	>15	S	0.0	2.5
Tet (30 μg)	<14	R	11.4	5.7
	15–18	I	80.3	54.3
	>19	S	8.3	40.0

[a] Amp, Ampicillin; Cep, cephalin; Cam, chloramphenicol; Col, colymicin; Ery, erythromycin; Gen, gentamicin; Kan, kanamycin; Lin, lincomycin; Met, methicillin; Naf, nafcillin; Nal, nalidixic acid; Neo, neomycin; Pen, penicillin; Pmx, polymyxin B; Str, streptomycin; Tet, tetracycline.

[b] R, Resistant; I, intermediate; S, susceptible.

TABLE 6. Pathogenicity of *V. parahaemolyticus*

Test	No. of strains tested	No. of strains positive
KP	42	5
RIL	30	3[a]
Mouse inoculation (i.p.)	132	94[b]

[a] Six other strains were positive at least once, but when retested, results varied.

[b] A total of 26 other strains caused death in one of the two mice inoculated.

fluctuation in the occurrence of *V. alginolyticus* did not appear to be dependent upon warmer water temperatures during the dry season (May to September) in tropical areas.

The incidence of *V. parahaemolyticus* increased during the dry season (Table 4), as has been reported in other studies from tropical areas (5, 21). Seasonal fluctuation was not, however, as distinct as that reported in temperate climates (15, 17). *V. parahaemolyticus* has been isolated from a wide variety of tropical seafood, and its incidence in market, estuarine, and lagoon seafood specimens has been reported to be higher than its incidence in fresh, pelagic specimens (5, 21).

Results of K antigen serotyping were inconclusive because a large number of strains (about 40%) were untypable. These results are in agreement with those of previous studies (7, 12) on environmental isolates. The preponderance of untypable environmental strains does not permit correlation of serotype with geographical distribution, virulence, or specific environmental parameters.

V. alginolyticus has been reported to be resistant to ampicillin, methicillin, lincomycin, penicillin, and carbenicillin and to be susceptible to tetracycline, chloramphenicol, gentamicin, kanamycin, streptomycin, and neomycin (3, 12, 13). Very few of these isolates were susceptible to tetracycline, kanamycin, streptomycin, or neomycin. *V. parahaemolyticus* has been reported to be resistant to the same antibiotics as *V. alginolyticus* (6, 12, 18, 24). Most *V. parahaemolyticus* strains had an intermediate reaction to tetracycline, an antibiotic important in the treatment of gastroenteritis. Both the wide variety of resistance patterns found among *V. alginolyticus* and *V. parahaemolyticus* isolates in this study and their intermediate and resistant reactions to several antibiotics create a high potential for refractory infections caused by *V. parahaemolyticus* and *V. alginolyticus*.

The ubiquity of *V. alginolyticus* and *V. parahaemolyticus* in Jakarta Bay and in seafood collected in Jakarta markets which was observed in this study indicates a public health problem and also provides additional data supporting the conclusion that these organisms are autochthonous to bays, estuaries, and coastal regions of the world, both temperate and tropical.

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