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SURVEY ON THE OCCURRENCE OF VIBRIO PARAHAEMOLYTICUS AND VIBRIO ALGINOLYTICUS IN FISH AND SHELLFISH FROM THE KARACHI COASTAL WATERS

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Abstract. The first reported isolations of *V. parahaemolyticus* and related organism *V. alginolyticus* from fish and shrimps harvested from Karachi coastal waters are described. Sixty per cent of the samples examined yielded *V. parahaemolyticus*. In 62 of the 65 positive samples. *V. parahaemolyticus* could be isolated from direct plating on TCBS. Morphological and biochemical characteristics of the isolates generally confirm to those described for this organism in the literature. Forty two of the 80 isolates showed a positive Kanagawa phenomenon. Todate, there have been no reports of *V. parahaemolyticus* food poisoning out breaks due to the consumption of fish or shrimps from this region. *V. alginolyticus* was isolated from 107 of the 108 samples examined.

Vibrio parahaemolyticus is well established as a major cause of food poisoning in Japan[21,22,28] and has been repeatedly isolated from Japanese sea food and environmental samples [7,17,27] In recent years it has been incriminated with increasing frequency as a cause of food poisoning in Australia [4] and the United States[6]. The organism has also been isolated from infected injuries[16] and in case of fatal fulminating septicemia.[29] It has also been reported that *V. parahaemolyticus* causes disease in shrimp,[25] blue crabs[11] and possibly oysters.[13]

Sakazaki *et al.*[22] described three biotypes of *V. parahaemolyticus.* These organisms were found to differ in their ability to grow at various salt concentrations, ferment various sugars and produce acetyl methyl carbinol. Biotype I *V. parahaemolyticus* and to a lesser extent Biotype II *V. alginolyticus* are considered possible food-borne pathogens, whereas Biotype III. *V. anguillarum* is non pathogenic.[2] These biotypes were later given a separate species identification.[19]

Pakistan has a coast line of about 550 miles (880 k.m) and the present fishing is confined to about 12 miles (19.2 k.m.) off the coast. This coast is an important source of all types of fish and shellfish consumed locally and commercially. Pakistans total marine fish catch is reported to have reached over 0.2 million meteric tons annually and shrimp is its thriving industry. With an annual export of Rupees 300 - 400 million, it is a valuable earner of foreign exchange for the country.

No published information is available on the occurrence of *V. parahaemolyticus* and *V. alginolyticus* in fish and shellfish resource exported and offered for sale in local retail markets. Since a significant number of persons may be at risk, a limited survey was carried out to estimate the distribution of these organisms in samples of fish and shellfish from local markets and from the Karachi Harbour where approximately 60% of the total catch is landed and auctioned. Common edible variety of fish which are widely consumed locally, shrimps which are mainly exported and other marine samples such as crab, skate etc. were selected for the survey.

This paper reports the results of a study on the occurrence of *V. parahaemolyticus* and *V. alginolyticus* in fishery products sold in the local market.

Material and Methods

During the period June 1975 - March 1976, each week samples of fish and shellfish were taken either from the local retail markets or fish harbour. The samples were packed in clean plastic bags immediately after sampling and transported to the laboratory where they were processed within 4 hr of collection. The type of fish and shellfish examined in this survey, their source and their numbers are summarized in Table 1.

Strain

For comparison the Japanese strain of antigenic formula O_3K_{43} was procured through the courtesy of Dr. R.G.A. Sutton, Commonwealth Department of Health, Sydney, Australia.

Isolation Media for V. parahaemolyticus.

1. Glucose salt teepol broth (GSTB).[18] (2). Salt peptone broth (SPB).[12] (3). Salt nutrient broth (SNB) contained 3% NaCl in 2.8% nutrient broth (Oxoid) pH 8.0. (4). Salt nutrient penicilling broth (SNPB)

TABLE 1. REVIEW OF LOCATION AND TYPES OF FISH AND SHELLFISH SAMPLES EXAMINED FOR *V.PARAHAEMOLYTICUS* AND *V. ALGINOLYTICUS*

Locality	English name	Scientific name	Number of samples examined
Market	Cat fish	*Arius* sp.	1
	Mackeral	*Cybium* sp.	3
	Pomfret	*Stromateus* sp.	4
	Shrimps	*Penaeus indicus, P. merguensis*	11
	Total		19
Harbour	Big eyed Jumper	*Lactaruis* sp.	5
	Crab	*Geryon.* sp.	1
	Crookers or Drums	*Pseudosceiaena* sp.	7
	Eel	*Muraenea* sp.	3
	Indian Shad	*Hilsa* sp.	5
	Jew fish	*Sciaean* sp.	6
	Mackerel	*Cybium* sp.	2
	Mullet	*Mugil* sp.	2
	Pomfret	*Stromateus* sp.	10
	Red snapper	*Serranus* sp.	6
	Sardine	*Clupea* sp.	8
	Shrimps	*Penaeus indicus, P. merguensis*	22
	Silver bar fish	*Chirocentrus* sp.	1
	Skate	*Rhynchobatus* sp.	1
	Sole (Ray)	*Trygon* sp.	1
	Spade fish	*Scatophagus* sp.	4
	Thread fin	*Polynumus* sp.	5
	Total		89
	Grand Total	19 +/ 89 =	108

same as no.3 but contained an additional 20.1.U. of penicillin/ml.

Since *V. parahaemolyticus* is a thermotrophic organism, it may be assumed that on chilled fish and shellfish relatively low numbers of Vibrio will occur. Therefore, search for these organisms in chilled fish and shellfish should be made by a suitable enrichment technique. The selectivity of various enrichment broths for this purpose was measured in accordance with the following procedure: Enrichment broths (90 ml) were inoculated with approximately 40 viable cells of *V. parahaemolyticus* and incubated for 12 - 16 hr at 37°. After shaking thoroughly, one loopful of each enriched broth was streaked on TCBS agar. To assess the reliability of the methods, enrichment broths containing normal fish flora and artificially inoculated cells of *V. parahaemolyticus* were examined in parallel. For this purpose, 10 g of fish homogenate containing normal fish flora in 90 ml enrichment broth were inoculated with approximately 40 viable cells of *V. parahaemolyticus.*

Isolation of V. parahaemolyticus

V. parahaemolyticus present on fish and shrimps occur mainly on the surface, because these bacteria originate primarily from the marine environment. It was, therefore, considered advisible to sample fish and shrimp by a non-destructive technique. The entire body surfaces of shrimps and comparatively smaller fish were streaked intensively. In fish particularly, the gills were included. Depending on the size, half of the fish containing mouth and gills were also used. Samples were transferred into clean plastic bags, which were not sterilized, but has negligible bacterial counts. 90 ml of SNPB was introduced aseptically. The sample was washed for 2 - 3 minutes, messaging over the entire surface and gills of the fish by external hand manipulation, forcing the rinse solution into gills and with vigorous shaking. The wash solution was transferred aseptically into sterile flask, and incubated for 12 - 16 hr at 73°. After shaking, a loopful of the enriched broth was streaked on thiosulphate citrate bile salts

sucrose (TCBS) agar and incubated for another 18 - 20 hr at 37°. In addition, a loopful of the surface washings was streaked directly on to TCBS and incubated for 18 - 20 hr at 37°. Blue green sucrose negative and yellow sucrose positive colonies on TCBS medium were considered to be presumptive *V. parahaemolyticus* and *V. alginolyticus* respectively.

All suspected isolates were screened using triple sugar iron agar, motility media and cytochrome oxidase production. If suspected isolates were cytochrome oxidase positive, motile and showed an acid butt/alkaline slant (V. *parahaemolyticus)* or an acid butt/acid slant (*V. alginolyticus*), negative gas and H_2S on triple sugar iron agar, biochemical testing was continued.

Counts of V. parahaemolyticus

Total counts of *V. parahaemolyticus* were obtained as follows: Rinse solution was obtained as described above. Serial decimal dilutions (10^{-1} –10^{-4}) were made in 3% salt solution containing 1% peptone, pH 7.5 and 0.1 ml of each dilution in duplicate were plated onto TCBS using the spread plate technique, incubated for 18-20 hr. at 37°. Typical blue green (non sucrose fermenting) colonies were considered positive for *V. parahaemolyticus.* When the blue green colonies were finally identified biochemically as *V. parahaemolyticus,* the enumeration was made.

Biochemical Tests

All suspected colonies were transferred into salt peptone broth and subjected to biochemical tests described for this organism in the literature.

Kanagawa Reaction

All biochemically positive isolates were tested for the Kanagawa reaction, using the medium of Wagatsuma and the method of Sakazaki. [22]

Results

All enrichment broths described above gave good recovery with pure culture of *V. parahaemolyticus.* However, when the strain in the same concentration was added to broths containing normal fish flora, SNPB showed comparatively better recovery of *V. parahaemolyticus* than the other three broths and therefore selected for use in the survey. *Vibrio parahaemolyticus* could be detected in 62 samples by direct plating on TCBS and in 65 of the 108 samples examined after enrichment showing that only at few occasions this organism could not be detected without enrichment.

A total of 108 samples were examined for *V. parahaemolyticus* and *V. alginolyticus.* The results are summarized in Table 2. Sixty five out of 108 (60%) samples yielded *V. parahaemolyticus* and 107 out of 108 (99%) yielded *V. alginolyticus.* Positive samples were found in both the areas, but the incidence in harbour samples was relatively lower than market samples. The counts of *V. parahaemolyticus* from the samples is illustrated in Table 3. Higher counts were common in both fish and shrimps. In shrimps it ranged from zero (not detected by the method used to a maximum of 8.5×10^6/g. Three of the 5 samples examined had count over 3.5×10^6/g. Both the fish samples showed a count over 2×10^5/g. No attempt was made to obtain accurate viable count of *V. alginolyticus,* but the count of sucrose fermenting yellow colonies on TCBS medium was usually 5-10 times greater than the corresponding count of *V. parahaemolyticus.*

Forty two of the 80 isolates (50%) produced β haemolytic zones with 24 hr on Wagatsumas agar containing freshly drawn human blood.

Discussion

It is evident from the results that *V. parahaemolyticus* can be isolated with related ease from the fish and shrimps of Karachi coastal waters, probably because of the relatively high water temperature which ranges between 25° and 30° throughout the year[15] around the Karachi coast. This confirms the findings of other workers [2,3,9,22] who have shown that *V. parahaemolyticus* is most abundant in inshore and estuarine areas where ambient temperatures rise seasonally (25° to 30°) permitting growth of the organism and decrease sharply when water temperatures drop below 15°.

This is particularly significant in view of the capability of *V. parahaemolyticus* to produce gastro. - enteritis and other human diseases. Of greater significance is the relatively high count (Table 3), the number of samples containing *V. parahaemolyticus* found in this study (Table 2) and the fact that in 92% of the positive samples the organism could be detected without any enrichment. Since fish and shrimps are consumed only after cooking in this country, food poisoning due to *V. parahaemolyticus* is unlikely.

High potential exists for cross-contamination of uncontaminated fish and shellfish collected at the same

TABLE 2. LOCATION AND NUMBER OF SAMPLES POSITIVE FOR *V. PARAHAEMOLYTICUS* AND *V. ALGINOLYTICUS*

Location	Material	No. of samples examined	No. of samples positive for *V. parahaemolyticus.*	+Ve %	*V. alginolyticus.*	+Ve %
A.Fish Harbour						
	Shrimps	22	13	59	21	95
	Fish	67	39	58	67	100
Total		89	52	58.5	88	99
B.Retail Market.						
	Shrimps	11	9	82	11	100
	Fish	8	4	50	8	100
Total		19	13	68.4	19	100
Grand total: (A+B)		108	65	60	107	99.5

TABLE 3. COUNTS OF *V.PARAHAEMOLYTICUS* IN FISH AND SHRIMPS.

Type	Sample tested	Low	Mean* count	High
Shrimps	5	Zero	3.8×10^6	8.5×10^6
Fish	2	2×10^5	3.5×10^5	5×10^5

time. The potential hazard can be multiplied under careless handling and summer temperature conditions if fish and shrimps are not properly iced during transportation and storage at retail markets, with the result that consumer may be commonly getting *V. parahaemolyticus* in larger numbers than presently realized.

In this study, *V. alginolyticus* was encountered in 107 of 108 (99%) samples examined. Considering the close association and phenotypic similarity of *V. parahaemolyticus* to *V. alginolyticus*[9] it is not surprising, because *V. alginolyticus* has been reported to respond more sharply to high ambient water temperatures then *V.parahaemolyticus*[2] and has more frequently been isolated from coastal sea water and seafish in warm months of the year.[20]

In the present study, isolates with the basic biochemical characteristics of *V. parahaemolyticus* resembling to the reference strain O_3K_{43} were considered. These isolates were not serologically typed, since many other marine vibrios are agglutinated with *V. parahaemolyticus* antisera and it is realized[22] that the agglutination test by itself is not conclusive for the recognition of this organism. Complete morphological, physiological and biochemical characterizations are required for the identification *of V. parahaemolyticus.* All the isolates were subjected to the Kanagawa haemolysin test on Wagatsumas agar which is considered to be the most important criteria for the confirmation of pathogenicity of this organism.

It has been reported that *V. parahaemolyticus* strains isolated from marine sources differ from strains isolated from persons suffering from *V. parahaemolyticus* gastro-enteritis, is being unable to haemolyze human blood in the so-called Kanagawa phenomenon[14]. An association of Kanagawa positive strains of *V. parahaemolyticus* with pathogenecity has been observed by Kato *et al.*[10]

In the present study 42 out of 80 (52.5%) isolates of *V. parahaemolyticus* were Kanagawa positive. These results are not in agreement with the findings of other workers[14,23] who have found that haemolytic reactions may be used to differentiate between isolates from the marine samples and from human sources. In addition, the percentage of Kanagawa positive isolates from marine sources was significantly higher than 1% reported by Sakazaki *et al.*[23]

Pakistan exports a considerable quantity of the frozen shrimps. *V. parahaemolyticus* has been shown to be highly sensitive to low temperature storage[1,5,8,24,26] and it seems unlikely that *V. Parahaemolyticus* will survive during freezing and frozen storage. In addition shrimps are always eaten after cooking and

frying, this will further eliminate any organisms that may survive freezing and other processing. These circumstances make it unlikely that 'Frozen Shrimps' can play any role in gastroenteritis or other human diseases due to *V. parahaemolyticus.*

It is evident from this limited study that *V. parahaemolyticus and V. alginolyticus* are commonly present in the waters around the Karachi coast, and that Kanagawa positive strains of *V. parahaemolyticus* are not very uncommon. No epidemological evidence is available to show that fish and shrimps from these waters have caused food poisoning due to this organism. Because of the lack of knowledge about the prevalance of *V. parahaemolyticus* foodborne infection in Pakistan, there is a need to collect information to assess its relative importance as foodborne disease.

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