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Incidence of Motile Aeromonads in Marine Environment, Fishes and Processed Fishery Products

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Abstract : Motile Aeromonds ubiquitous to the aquatic environment has been frequently associated with disease of fishes and other aquatic animals. Recent studies point to its role as a causative agent for foodborne gastroenteritis. This paper reports the incidence of motile aeromonds in the fishing ground, raw marine fish and processed fishery products. The density of motile aeromonad in the samples were analysed by direct plating and enrichment methods along with total plate count. Typical isolates were identified upto species level. In sea water of the fishing grounds off Cochin, the aeromonad count ranged between 4.0×10^2 to 2.1×10^3/ml in July-August. The aeromonad count in the skin with muscle of the fishes during that period were in the range of $9. \times 10^2$/g to 9.9×10^5/g corresponding to a TPC range of 6.7×10^4/g to 7.12×10^6/g. In frozen fishes the aeromonad count ranged between 9.0/g to 2.0×10^4/g and the TPC, in the range of 4.5×10^4/g to 9.7×10^5/g . Cured or salted fishes were found to be free from motile aeromonads. Random colonies picked from the plate were identified upto species level and the dominant species in water, raw fish and frozen fish were found to *A. caviae* which is considered as non-toxigenic. The incidence of *Aermonas hydrophila* was more in the frozen fish than fresh fish. The enterotoxigenicity of the isolates were assessed on the basis of biochemical tests.

Introduction

Aeromonads ubiquitous to the aquatic environment have been frequently associated with disease of fishes and other aquatic animals. However recent studies have identified the role of Aeromonads in human illness and this microorganism has been recognized as a potent cause of food borne gastroenteritis (Morgan and Wood, 1988). In addition to being an enteric pathogen, the bacteria is capable of producing wound infections as well.

Seafood products are considered to be the prime source of motile Aeromonads (Abeyta *et al.*, 1990; Araujo *et al.*, 1991; Neves *et al.*, 1990). But other food products such as meat, poultry dairy products, chicken, vegetable and confectionary are also reported to carry substantial numbers of motile Aeromonads (Callister and Agger, 1989; Majeed *et al.*, 1989; Palumbo *et al.*, 1985). They have also been isolated from chlorinated or non chlorinated water supplies (Burke *et al.*, 1984).

Though Aeromonad are identified as part of natural flora of tropical fishes, quantitative data on the occurrence of this pathogen in seafood and marine environment is generally lacking. Hence the purpose of this investigation is to assess the distribution and species-wise occurrence of motile Aeromonad in the coastal waters off Cochin, fishes and fishery products.

Materials and Methods

Sample

The study represented samples of water, fish and processed fishery products collected during the period July to September, 1994 and 1995. Water samples were collected from onboard fishing vessel of the Institute. Samples of water from 6 sites were collected in each trial, in wide-mouthed glass stoppered bottle from 1 m below the surface, transferred immediately to polythene bottles and brought to the laboratory. Fish samples were procured from the landing places or market. Frozen fish samples were purchased from local retail stores in regular consumer packs while cured fish samples were procured from fish markets. All the samples were analysed on the day of purchase itself.

Enumeration of motile Aeromonads

For estimation of the cell numbers of motile Aeromonads in sea water, the method proposed by FDA (Abeyta and Stelma, 1984) was used.Tryptic soy amplicillin broth was used for MPN determinations followed by streaking the content of the positive tubes on starch amplicillin agar (Palumbo *et al.*, 1985). Random isolates from starch ampicillin agar showing typical colony morphology were presumptively confirmed on *Aeromonas hydrophila* medium (AHM) of Kaper *et al.* (1979). Tentatively positive cultures were maintained for identification upto species level.From frozen and cured fishes also, the above procedure was adopted for estimation of motile Aeromonads.

In the case of fresh samples, 25 g portions of the muscle with skin and 10g. portions of the intestine were asceptically removed and homogenized with 225 and 90 ml of saline respectively in stomacher 400 for 2 min. Serial dilutions were surface plated on starch ampicillin agar and the rest of the procedure was the same as that adopted for water samples.

Total plate count

Total plate count of the water, fresh fish and frozen and cured fishes were carried out on tryptone glucose extract agar (Oxoid) by pour plating method and the plates were enumerated after 48h. at RT (29±1°C) and count per g. estimated.

Identification of the isolates

Bacterial cultures that were tentatively classified as Aeromonas were further identified upto species level based on biochemical tests (Popoff, 1976; Abeyta and Stelma, 1984)

Table 1. **Density of motile Aeromonad in the coastal waters off Cochin**

Station No.	Location	Temp. °C	Salinity %	Aeromonad MPN count g-1	TPC ml-1
1	12 nautical mile off Cochin	29	23.89	$2.1x10^{3}$*	$3.90x10^{5}$*
2	8 " "	29	23.05	$2.0x10^{3}$	$2.25x10^{5}$
3	2 " "	29	20.36	$4.0x10^{2}$	$1.60x10^{4}$
4	3 " "	29	19.45	$9.4x10^{2}$	$9.10x10^{4}$
5	2.5 " "	29	22.38	$4.4x10^{2}$	$1.50x10^{5}$
6	2 " "	29	24.82	$4.0x10^{2}$	$8.80x10^{4}$

*Average of 3 trials

Samples collected during July-September 1995

The salt and the moisture levels of the cured fish samples were determined by AOAC method (AOAC, 1975) and water activity by a_W meter (Durotherm, Germany).

Results

The density of motile Aeromonads in the marine environment is presented in Table 1. In the coastal waters off Cochin upto 10 nautical miles where most of the fishing activity is concentrated the Aeromonad count was found to vary between 4.0×10^2 ml. of the nearshore waters to 2.1×10^3 ml. of the farthest point of 12 nautical miles corresponding to a water temperature of 29°C and salinity of 23.89%. The results indicated that motile aeromonads formed 0.29 to 2.5% of the total bacterial population of sea water. Maximum value was observed in the case of water sample from the farthest site i.e. 12 nautical miles. In the near shore waters upto 3 nautical miles, there was not much variation in the Aeromonad count. A similar trend was noticed in the case of TPC as well.

The motile Aeromonad count of the skin with muscle portion of the fishes are presented in Table 2. The total Aeromonad count varied from the lowest value of 9.1×10^2/g of Mackerel to 9.1×10^5/g of the Croaker. About 50% of the samples showed an Aeromonad count of 10^4/g or below it. The total plate count also showed wide fluctuations from 6.7×10^4/g to 7.12×10^6/g. Thus the percentage of motile aeromonads in the skin with muscle of fishes ranged from 0.56 to 20% and in 80% of the fishes analysed, the percentage of motile Aeromonads was less than 10%.

The density of motile Aeromonads in the intestinal contents of the fishes are given in Table 3. The intestinal contents of the fishes were found to carry motile Aeromonads in the range of 10^4-10^5/g corresponding to a TPC of 10^6-10^7/g. This represented a percentage value of 0.4 to 8.3% of the TPC. Maximum value of 8.3% was oberved in the case of mackerel. Majority of fishes had an Aeromonad count of less than 5% in the case of intestine. Thus the results show that the Aeromound density in the skin surface and muscle of fishes are greater that of the surrounding water and also the intestinal contents.

Table 2. **Aeromonad count in the skin and muscle of fishes of marine fishes**

Fish species	Aeromonad count g-1	TPC g-1	%
Croaker *(Johniops dussumieri)*	5.70×10^4	7.20×10^5	7.90
Indian Mackerel *(Rastrelliger kanagurta)*	9.10×10^2	1.62×10^5	0.56
Oil sardine *(Sardinella longiceps)*	4.00×10^3	6.70×10^4	5.90
Anchovies *(Thryssa malabarica)*	1.90×10^4	9.20×10^4	0.20
Mullet *(Liza parsia)*	1.39×10^5	3.60×10^6	3.86
Croaker *(Otolithes cuvieri)*	9.00×10^5	7.12×10^6	12.60
Black pomfret *(Parastromateus niger)*	9.40×10^4	1.01×10^6	9.30
Carangids *(Alepes djeddaba)*	2.39×10^4	5.31×10^6	0.75
Seer *(Scomberomorus commerson)*	1.10×10^4	1.20×10^5	9.16

Table 3. **Aeromonad count of the intestinal contents of marine fishes**

Fish species	Aeromonad count g-1	TPC g-1	%
Croaker *(Johniops dussumieri)*	$4.00x10^4$	$3.96x10^6$	1.00
Indian Mackerel *(Rastrelliger kanagurta)*	$1.70x10^5$	$2.04x10^6$	8.3
Oil sardine*(Sardinella Iongiceps)*	$1.93x10^4$	$1.28x10^6$	1.52
Thread fin bream *(Nemipterus japonicus)*	$5.30x10^4$	$1.16x10^7$	0.45
Mullet *(Liza parsia)*	$1.07x10^6$	$4.24x10^7$	2.52
Croaker *(Otolithes cuvieri)*	$4.55x10^6$	$7.21x10^7$	6.31
Carangids *(Alepes djeddaba)*	$8.38x10^4$	$9.38x10^6$	0.89
Seer*(Scomberomorus commerson)*	$1.63x10^6$	$8.58x10^7$	1.90
Black pomfret *(Parastromateus niger)*	$1.51x10^6$	$1.37x10^8$	1.10
Barracuda*(Sphyraena jello)*	Nil	$4.91x10^6$	-

The incidence of motile Aeromonads in frozen fishes are presented in Table 4. Out of 24 samples of frozen fish comprising pomfret, seer, mackerel, carangids and reef-cod, mackeral and reef-cod did not carry this pathogen. Samples which yielded high numbers of Aeromonads were seer and pomfret and constituted 10.5% and 11.02% of the total count.

The cured fishes from the retail markets were also found to be totally free from motile Aeromonads. The salt content of these samples ranged from 34.03 to 58.86% and salt content from 12.43 to 23.94%. The water activity of these samples were between 0.71 and 0.86. The Aeromonads are reported to grow in salt concentrations upto 6%. The combined effect of salt content and

Table 4. **Aeromonad count in the frozen fishes of retail market**

Fish species	Aeromonad count g-1	TPC g-1	%
Indian Mackerel *(Rasterelliger kanagurta)*	ND	$4.91x10^5$	-
Carangids *(Alepes djeddaba)*	9	$4.50x10^4$	0.02
White pomfert *(Pampus argenteus)*	$1.50x10^4$	$1.36x10^5$	11.02
Black pomfret *(Parastromateus niger)*	$2.00x10^4$	$9.70x10^5$	2.05
Reef cod *(Epinephelus malabaricus)*	ND	$7.56x10^4$	-
Seer *(Scomberomorus commerson)*	4	$1.48x10^5$	0.02

Table 5. **Speciation of motile aeromonads isolated from water, fish and fishery products**

	Percentage occurrence in			
	Sea water	Fresh fish muscle	Fresh fish intestine	Frozen fish
A. hydrophila	23	21	18	42
Atypical *(A. hydrophila)*	12	7	16	8
A. caviae	59	64	55	50
A sobriae	Nil	7	4	Nil
Unidentified	6	1	7	Nil
Total cultures studied	53	70	61	84

water activity may be the reason for the absence of Aeromonads in cured fishes.

Speciation of the Aeromonads islated from water, fish and fishery products were attempted (Table 5). The isolates that fermented glucose with gas, produced acid from arabinose and salicin and were positive for V.P. and asculin reactions were considered as *A. hydrophila* and isolates that were V.P. and asculin positive but differed in other tests were grouped as atypical. *A. hydrophila* (Majeed *et al.*, 1989). A dominance of *A. caviae* was noted in sea water and fresh fish. But frozen fishes carried equal numbers of *A. caviae* and *A. hydrophila* provided the atypical strains of *A. hydrophila* are also grouped with *A. hydrophila*. Thus a conspicuous abundance of *A. hydrophila* was noted in the frozen fishes when compared to fresh fish muscle or intestine and sea water. *A. sobriae* was isolated from fresh fish muscle in very low numbers.

The enterotoxigenicity of the isolates were not determined although efforts were made to correlate it with certain biochemical traits (Table 6). Correlation have been reported between toxin production and lysine decarboxylase reaction (Kaper *et al.*, 1981) and toxin production with arabinose fermentation (Abeyta *et al.*, 1986). In the present study, a greater percentage of strains from frozen fish showed haemolysis and lysine decarboxylation than that from fresh fish or water. However arabinose fermentation which correlate inversely with toxin production was not apparent in the case of these isolates. Even then

Table 6. **Correlation to enterotoxigenicity of *A. hydrophila* isolates on the basis of biochemical tests**

	Percentage of the isolates from		
	Sea water	Fresh fish	Frozen fish
Voges Prauskaur	100	100	100
Arabinose fermentation	70	50	80
Lysine decarboxylation	60	50	90
Haemolysis	70	60	80

these *Aeromonas hydrophila* isolates indicate a possibility of being toxigenic.

Discussion

In the study of Araujo *et al.* (1991) the oceanic waters in the north western Spain were found to harbour Aermonad in the range of $4.0x10^2$/ml to 1.78x 10^4/ml. Kaper *et al.* (1981) studying the Aeromonad count of estuarine waters found a range of 10.3 to $5x10^3$/ml and also noted a positive correlation with TPC. Pathak *et al.* (1988) have reported that Aeromonas count of river fish was greater than riven water. The results of the present investigation is in agreement with these findings.

In the marine environment and Ocean fresh fish, a dominance of *A. caviae* over other Aeromonas species have been reported by several workers. Araujo *et al.* (1991) reported that out of 883 cultures isolated from salt water, 55% were *A. caviae*, 34% *A. hydrophila* and 6% *A. sobriae*. Neves *et al.* (1990) studying fresh and marine waters reported that in sea water environment *A. caviae* predominated, the levels being 60% *A. caviae*, 1% *A. hydrophila*, 14% *A. veronii* and 1% *A. sobriae*. According to Suchubert (1975) and Araujo *et al.* (1991) *A. caviae* predominated in sewage and water with high degree of pollution while in less polluted waters of fresh or marine origin *A. caviae* and *A. hydrophila* are almost equally distributed. It is not surprising that coastal waters off Cochin showed a dominance of *A. caviae* and hence chances of a pollution.

According to the results of this study, frozen fishes were found to yield greater numbers of *A. hydrophila*. In this case, the Aeromonas content of frozen seafood products shows a resemblance to other frozen products such as lamb meat. Chicken, confectionary (Callister and Agger, 1987; Kirov *et al.*, 1989; Majeed *et al.*, 1989; Palumbo *et al.*, 1985) wherein a dominance of *A. hydrophila* was noted. It is to be assumed that *A. caviae* is confined to aquatic habitat only. Psychotrophic nature of *A. hydrophila* coupled with other growth characteristics could be the reason for the abundance of *A. hydrophila* in frozen seafoods. *A. hydrophila* and *A. sobriae* are reported to be enterotoxigenic by animal assay while *A. caviae* is found to be non-toxigenic (Arujo *et al.*, 1991; Majeed *et al.*, 1989).

Thus the study shows that Aeromonads like vibrio species is an inhabitant of the coastal waters and the skin surface or intestine of the fishes are good reservoirs of this bacteria. *A. caviae* which is relatively non-pathogenic is present in water and fishes. But frozen fishes poses a risk to the human health considering greater number of *A. hydrophila* isolated from these samples.

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