Incidence of *Vibrio cholerae* non-O1 in seafood from the retail outlets and processing plants of Cochin area, India

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Abstract

The incidence of Vibrio cholerae *in different fresh fish and fish products from the retail outlets and processing plants of Cochin area, for a period of sixteen months were studied .The detection and isolation was done using TCBS agar as per USFDA method. The cultures isolated were purified and confirmed by biochemical test and polyvalent antisera. From the result it could be observed that all* Vibrio cholerae *cultures isolated were non –O1 (Non agglutinating) type. The incidence is 22.5 %. Comparing to fin fish (11.1 %) high incidence was recorded in shellfishes like clams (55 %), mussels (33.3 %), shrimps (35.7 %), and crabs (33 %). Antibiotic sensitivity of the cultures towards all the 8 antibiotics, notified by the EU were examined and it was found that all the isolates were sensitive to the antibiotics tested. Frequent isolation of* Vibrio cholerae non-O1 *could indicate that it is a part of the natural flora of the aquatic environments and not necessarily a contamination during handling.*

Keywords: *Vibrio cholerae, Antibiotic sensitivity, Vibrio cholerae non – O1*

1. Introduction

Vibrio cholerae as a species includes both pathogenic and non-pathogenic strains that vary in their virulence gene content. Nearly 200 *Vibrio cholerae* serogroups have been identified to date (Yamai *et al.*, 1997) but only two serogroups, serogroup O1and serogroup O139 are associated with epidemic cholera. *Vibrio cholerae* has since been detected in natural waters worldwide including areas where clinical cases of cholera did not exist (Hood *et al.*, 1983; Jesudasan *et al.*, 2000; Keysner *et al.*, 1987 and Yamai *et al.*, 1997) They not only survive in riverine, estuarine and coastal waters, but also live in association with crustacean copepods and aquatic plants either in the viable and culturable state or in the viable but non culturable state (VBNC). Since this human pathogen is primarily an inhabitant of the aquatic environment, water plays an important role in the transmission and epidemology of cholera.

Vibrio cholerae non-O1 is a non-agglutinating group of *Vibrio cholerae*, which occur as a part of autochthonous flora of marine environment. It is a Gram negative bacillus that require trace

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amounts of sodium chloride for growth .The clinical syndromes of non-O1 *Vibrio cholerae* infection are skin infection, acute gastroenteritis and septicemia. Other presentations include central nervous system syndrome such as cerebritis and meningitis but these are less common. *Vibrio cholerae* non –O1 have been isolated from seafoods (Karunasagar *et al.*, 1988; Mathew *et al.*, 1988; Iyer *et al.*, 1990). The incidence of Non-O1 *Vibrio cholerae* strains in sewage (77.3 %), seawater (40.4 %) and fresh water (33.3 %) were reported by Martin (1988). *Vibrio cholerae* non-O1 was isolated from seawater and oyster samples by (Rodrigue *et al.*, 1986). The aim of this study was to investigate the incidence of *Vibrio cholerae* non-O1 in seafood from the retail outlets and processing plants of Cochin area.

2. Materials and methods

A total of 89 samples were collected from different markets and processing plants of Cochin area during the period of July 2003 and October 2004. Both fresh and frozen samples were collected. The samples included fin fishes like pearl spot (*Etroplus* suratensis), seer fish (*Scomberomorus* species), *Thryssa species*, Indian mackerel (*Rastreliger kanagurta*), Oil sardine (*Sardinella longiceps*), Catla (*Catla catla*), Pomfrt (*Pampus argentius*), Tilapia (*Tilapia mossambica*) and rohu (*Labeo rohita*) shellfishes like black clam (*Villoritta cyprinoids*), Oyster (*Crassostrea madrasensis*), green mussel (*Perna viridis*), sea crab (*Portunus pelagicus*), and shrimps like white prawn (*Penaeus indicus*), and tiger prawn (*Penaeus monodon*). The samples were collected in sterile bags and transported to the laboratory under ice for bacteriological examination. The procedure for analysis was generally based on the method outlined by USFDA (1995).

2.1 Isolation and characterization of *vibrio cholerae*

Twenty-five gram tissue of the fish sample were cut aseptically and transferred into 225 ml alkaline peptone water (APW) in a sterile polythene bag and blended in a stomacher 400 (Seward) for one min at 230 rpm and incubated at 36±1 °C for 18 - 24 h of incubation. A loopful of the culture was streaked on pre-dried plates of Thiosulphate Citrate Bile Salt (T.C.B.S) Medium (Oxoid) after 6 h and 24 h. The plates were incubated at 36±1 °C for 18 - 24 h. Characteristic colonies were picked and sub cultured on Tryptone Soy Agar plates. They were characterized morphologically and biochemically. Cultures identified as *Vibrio cholerae* were confirmed by slide agglutination test with commercial anti *Vibrio cholerae* O1 (Difco) serum.

2.2 Antibiotic sensitivity

Antibiotic sensitivity of all strains of *Vibrio cholerae* isolated from the samples was tested for 8 antibiotics notified by EU using disc diffusion method (Bauer-Kirby 1966). The antibiotic discs were

dispensed on seeded plates of Muller-Hinton agar and incubated at 36±1 °C for 24 h. Based on the diameter of the zone of inhibition the behavior of each strain towards individual antibiotic was interpreted using standard methods. The strains were classified as resistant and sensitive.

3. Results and discussion

A total of 89 samples including fresh and frozen fish and shellfish belonging to different species have been analyzed .The range of distribution of different organisms in fresh and frozen samples are given in Table 1, 2 and 3 respectively. Among the fresh samples the highest and the lowest total plate counts were obtained in oyster samples. According to the existing standards for fresh fish, a TPC up to $5x10^5$ cfu g^{-1} is considered acceptable. At this level, only 48 % of the sample could be considered acceptable.52 % of the samples exceed the limit of 5 lakhs g^{-1}. This could be due to unhygienic handling and exposure of the fish to ambient temperature. (Lakshmanan *et al.*, 1984) have reported that 66.7 % of fish collected from the landing centers of Cochin had plate counts more than 10^5 cfu g^{-1}, and 8.5 % had TPC counts more than $5.5x10^5$ cfu g^{-1}. Nambiar and Iyer (1990) reported that 69.9 % of the samples had total plate counts more than $1x10^5$ cfu g^{-1} of which 49.4 % had counts more than $5x10^5$ cfu g^{-1}. The high plate counts in the fish sample from retail market is an indication of the poor hygienic standards of the markets. About 93 % of the samples were contaminated with *E.coli* and Faecal streptococci. Generally samples with *E. coli* count more than 20 g^{-1} and Faecal streptococci count more than 1,000 g^{-1} are considered unacceptable. Nambiar and Iyer (1990) reported very high incidence of *E. coli* and Faecal streptococci in samples from the retail markets of Cochin. Similar results were obtained in the present study also. The presence of very high number of faecal indicator organisms in fish gives an indication of the level of contamination with faecal matter and the possibility of other entero pathogenic microorganisms being present. The pathogen *Salmonella* was not detected in any of the samples. (Iyer *et al.*, 1986) detected *Salmonella* in 4.4 % of the samples from retail markets of Bombay. Nambiar and Iyer (1990) reported the incidence of Salmonella in 5.8 % of the samples from retail markets of Cochin. Coagulase positive *Staphylococcus aureus* was detected in only 4 % of the samples. This showed a count comparable to that reported by Nambiar and Iyer, 1990. The total enterobacteriaceae count was in the range of 0 — $>10^6$ cfu g^{-1}. The presence of the halophylic pathogen *Vibrio parahaemolyticus* was detected in 7 % of the samples. The pathogenic *Vibrio cholerae* O1 was not detected in any of the samples. *Vibrio cholerae* non –O1 was detected in 25.4 % of the samples. Varma and his colleagues have studied the incidence of *Vibrio cholerae* non –

O1 in fish and fishery products collected from Kerala and Tamil Nadu during 1986 - 1988.The study showed a clear difference in the levels of the organism in different products, process and environments. Their study result shows that raw material collected from trawl net were not contaminated with *Vibrio Cholerae non -O1*.This clearly indicates that *Vibrio cholerae* non-O1 is not a natural flora of fish. It is clear that the estuarine water, which is a natural reservoir of *V. cholerae non-01*, used for washing the catch just before landing might be the contaminating agent. The mishandling of the catch also plays an important role.

Table 1. Bacteriological Quality of raw fish from retail markets of Cochin (n=59)

TPC	$1.6x10^4$ cfu g^{-1} — $>10^6$ cfu g^{-1}
E.coli	$0 - 140^+ g^{-1}$
Faecal coliform (MPN)	$2.5\text{-}140^+ g^{-1}$
Total coliform (MPN)	$2.5\text{-}140^+$ g^{-1}
Faecal streptococci	$2x10^2 - 8.8x10^4$ *cfu* g^{-1}
Staphylococcus aureus	$0\text{-}3.8x10^4$ *cfu* g^{-1}
Total Enterobacteriaceae count	$0 - >10^6$ cfu g^{-1}
Vibrio cholerae-O1	N.D
Vibrio parahaemolyticus	Present in 7 % of the samples
Vibrio cholerae non-O1	25.4 % of the samples

Table 2. Bacteriological quality of frozen fish samples from seafood processing plants (n= 15)

TPC	$5.6x10^4\text{-}3.2x10^6$ *cfu* g^{-1}
E.coli	$0\text{-}0.9^+$
Faecal coliforms	$0\text{-}45^+$
Total coliforms	$0\text{-}45^+$
Faecal streptococci	$0\text{-}2x10^4$ cfu g^{-1}
Staphylococcus aureus	*0-20 cfu* g^{-1}
Salmonella	Not detected
Vibrio cholerae	Not detected
Vibrio parahaemolyticus	Not detected
Vibrio cholerae non-O1	Not detected
Total Enterobacteriaceae count	$0 - 10^2$ cfu g^{-1}

Table 2 and 3 gives the bacteriological quality of the frozen products. Out of 30 samples, 15 were collected from the retail outlets and rest from processing plants. The total plate count was above the limit in 26.6 % of the samples. Coagulase positive *Staphylococcus*

aureus was detected in one sample. The indicatior organisms *E. coli* was present in 33.3 % of the samples and faecal streptococci in 46.6 %. Together they were present in 26 % sample.5 out of the 15 samples were totally free from the prescence of *E. coli*, Faecal streptococci and coliform bacteria. The faecal streptococci count was in the range 0-$2x10^4$ cfu g^{-1}. coliform level was above the limit in four samples. The total enterobacteriaceae count was in the range 0 — 10^2 cfu g^{-1}. The pathogens like *Vibrio cholerae* O1, non-O1, *Vibrio parahaemolyticus* and *Salmonella* were not at all detected in any of the samples.

Table 3. Bacteriological quality of seafood from the retail cold storages n=15

TPC	$6.4x10^4$ cfu g^{-1}-$1.1x10^7$ cfu g^{-1}
E.coli	0 — 140+
Total coliforms	4.5-140+
Faecal coliforms	4.5-140 +
Faecal streptococci	0-$6x10^2$ cfu g^{-1}
Staphylococcus aureus	0-$4x10^3$ cfu g^{-1}
Salmonella	13.33 % of the samples
Vibrio cholerae non -o1	16.6 % of the samples
Vibrio parahaemolyticus	10 of the samples
Vibrio cholerae O1	Not detected
Total Enterobacteriaceae Count	0 - 5.5 x10 ^{3} cfu g^{-1}

The frozen samples collected from the retail outlet were found to be poor in quality. The total plate count was found above $5x10^5$ cfu g^{-1} in 46.6 % of the samples. The coliform level was above the maximum admissible level in all the samples. The presence of *E. coli* and faecal streptococci was confirmed in 86 % of the samples. Coagulase positive Staphylococcus was detected in 40 % of the samples. Total enterobacteriaceae count was in the range of 0 - 5.5 x10 ^{3} cfu g^{-1}. The enteric pathogen salmonella was present in two shrimp samples. The percentage incidence of *Vibrio parahaemolyticus* and *Vibrio cholerae* non-O1 is 20 % and 33.3 % respectively. Iyer *et al.*, in 1989 reported the incidence of *Vibrio cholerae* non-O1 from fish contact surfaces, workers palm and utensils for fish handling and processing. They also reported the maximum percentage of *Vibrio cholerae* from swabs collected from the market .The reasons attributed for the low quality of fish in the retail cold storages are improper washing, cleaning and cross contamination during handling.

Table 4. Incidence of *Vibrio cholerae* non -O1 in different raw and frozen fish samples from the retail markets and processing plants

a) Frozen products			
Species	**No of samples**	**No of samples +ve for *V.cholerae* non-O1**	**% incidence**
Shrimp	19	4	21 %
Fish	9	1	11 %
Crab	1	—	-
Mussel	1	—	-
b) Fresh fish and shellfish			
Shrimp	14	5	35.71 %
Fish	18	2	11.1 %
Crab	3	1	33 %
Mussel	6	2	33.3 %
Oyster	6	—	-
Clams	9	5	55.55 %
Cephalopods	3	-	-

The antibiotic sensitivity test were carried out using the following antibiotics Chloramphenicol, Tetracyclin, Oxytetracyclin., Furazolidone, Nalidixic acid, Oxolinic acid, Sulphamethazole and Neomycin. The organism showed sensitivity towards all the 8 antibiotics used. From this it is interpreted that the organism has not acquired any drug resistant properties. Table 5 gives the details of antibiotics used, concentration per disc and the average zone diameter in millimeters.

Table 5. Antibiotic sensitivity

Antibiotic used	ig/disc	Avg. zone diameter (mm)
Chloramphenicol (C)	30	25
Tetracyclin (TE)	30	14.5
Oxytetracyclin (OT)	30	11.2
Furazolidone (FR)	15	17.8
Nalidixic acid (N A)	30	24.4
Oxolinic acid (OA)	2	22
Sulphamethazole (SXT)	25	18
Neomycin (N)	30	17.4

4. Conclusion

The result indicates that *Vibrio cholerae* non-O1 is frequently isolated from both fresh and frozen samples. The presence of *Vibrio*

cholerae non-O1' in frozen foods indicates its low temperature survival. The High incidences of *Vibrio cholerae* non-O1 from shellfish confirm the needs to improve the quality of shellfishes. Being the autochthonous flora of the aquatic environment the remedial measure to eliminate *Vibrio cholerae* non-O1 from seafood are hygienic handling and proper cooking practices.

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