Prevalence of Pathogenic Bacteria in Finfish and Shellfish Obtained from Domestic Markets of West Bengal, India

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Abstract: The objective of this study was to determine the microbiological quality and prevalence of pathogenic bacteria viz. *Vibrio cholerae, E. coli, Salmonella* spp. in fish and shellfish from domestic markets of West Bengal, India. Fresh *Penaeus monodon, Macrobrachium rozenbergii, Labeo rohita, Catla catla, Cirhinnus mrigala* were examined for total bacterial and faecal coliform loads and presence of *Vibrio cholerae, E. coli, Salmonella* spp by culture methods. While the total plate counts of bacteria were within the acceptable or marginally acceptable limits for most samples, fishes were contaminated with faecal coliforms indicating poor sanitary hygienic condition. The specific pathogens like *Vibrio cholerae* and *Salmonella* spp were also detected in fish and shellfish samples. Antibiotic sensitivity tests showed that the pathogens were resistant to antibiotics raising serious public health concerns.

Keywords: Microbiological Quality, Fish and Shellfish, *Vibrio cholerae*, *E. coli, Salmonella* spp, Antibiotic Sensitivity, Bacterial Load

1. Introduction

Fish is a widely preferred food item around the world and is cheap source of protein in developing countries. However, microbiological quality of fish and shellfish has been a matter of great public health and economic concern both for domestic and International markets. The major problems have been the presence of pathogenic microorganisms: for instance during 1990-98 about 7.2 % of fish and shellfish imported to US were contaminated with *Salmonella* which leads to detention and rejection of the consignment [1]. It is observed that aquaculture products are more prone to various biological hazards i.e. contamination with pathogenic microorganisms than those from marine resources [2]. Like several other Southeast Asian and African countries the main quality problems faced by Indian seafood exports were microbiological. During June 1995 to December 1997, 31 fish consignments exported to European Union (EU) nations were found to be of poor hygienic quality. In addition, during the same period, many consignments exported to USA and Japan was also rejected for the same reason [3]. The microbiological problem includes high bacterial load and presence of pathogens like *Salmonella* spp., *E.coli*, and *Vibrio cholerae* [3]. Outbreak of diseases from the consumption of contaminated shellfish is one of the major concerns in seafood industry and public health agencies around the world [4]. Seafood usually harbors infective agents like pathogenic bacteria, virus which are present in the aquatic environment either naturally or coming through human operations [5]. In live finfish, the pathogenic bacteria may be associated with the gill portion, skin and gut [6]. But Shellfish concentrate microorganisms in their tissues from the surrounding waters during the filter feeding process [7]. The members of the genus *Vibrio* are native to aquatic environment but pose a serious health hazard in human due to consumption of raw or inadequately cooked seafood [8,9]. *E.coli* and *Salmonella* are also introduced in the aquatic environment through the domestic sewage of the adjacent township [10]. Most of the outbreaks of illnesses occur in countries where seafood is eaten raw or is inadequately cooked; for instance, in Japan where seafood is eaten raw which results in food borne human illness of about 70% [11]. Shellfish is one of the sources for *Vibrio* transmission. *Vibrio cholerae* is an autochthonous, which is frequently related

with phyto-and zoo-plankton [12]. The association of *Vibrio cholerae* with plankton, notably copepods, provides further proof for the environmental origin of cholera, as well as an explanation for the sporadic and erratic occurrence of cholera epidemics [13]. Aquatic environments are the major reservoirs of *Salmonella* and fishery products have been recognized as a major carrier of food-borne pathogens in human health [14,15]. Occurrence of *Salmonella* in fishery products exported from India and Mexico are also reported by several researchers [16].

The motivations of the study are to assess prevalence of pathogenic bacteria and the microbiological quality of finfish and shellfish available in the domestic retail markets for human consumption. We had examined the overall microbiological quality with emphasis on food borne pathogens in shellfishes and Indian major carps which contributes more than 60% of fish consumption in West Bengal.

2. Materials and Methods

2.1. Collection of Samples

Fresh fin fishes, viz., Indian major carps (IMC) (*Labeo rohia*, *Catla catla* and *Cirrhinus mrigala*), shrimp (*Penaeus monodon*) and prawn (*Macrobrachium rosenbergii*) were randomly procured from different districts of West Bengal like South 24 pargans, North 24 parganas, Nadia, Howrah and Kolkata. The total no of samples were 150 including shrimps, freshwater prawns and Indian major carps i.e., 30 nos of sample pool (10 no prawns +10 n shrimps and 10no I.M.C) were collected from five districts mentioned above. These include fresh fishes cultured in the state as well as, carps transported under ice cover from Southern states of India. The study was conducted during April 2009 to September 2009. All samples were collected in fresh condition from retail and wholesale markets of the five districts and placed in sterile polythene bags, brought to laboratory under ice –cover and processed within 1 to 2 h.

2.2. Preparation of Samples

Further, sections of muscles with skin / exoskeleton from shrimp, prawn and fish were cut and homogenized in PBS [Phosphate Buffered Saline and pH is adjusted to 7.3-7.4]to 10 % (w/v) suspension.

2.3. Enumeration of Bacteria

The homogenized tissue samples was serially diluted in

PBS and plated on to tryptic soy agar (DIFCO, Becton Dickson and Company, Sparks Md., USA) for total plate count(TPC) of bacteria and pour plated on to mFC agar (DIFCO) for enumeration of faecal coliforms. The plates were incubated at 37^0C for 24 h and at 44.5^0C for 24h for TPC and coliforms respectively.

2.4. Enrichment of Culture, Isolation and Identification of Bacteria

The homogenized tissue samples were enriched in Alkaline peptone water (APW, DIFCO), EC broth [E.coli broth] and Tetrathionate broth at 37^0C for 6-8, 18h and 24h for isolation of *Vibrio cholerae*, *E.coli* and *Salmonella* respectively. The enriched culture media were plated on to Thiosulfate Citrate Bile Salt Sucrose Agar (TCBS Difco), Eosin Methylene Blue agar (EMB agar, DIFCO) and Xylose Lysine Deoxycholate Agar (XLDA, DIFCO)respectively. Following incubation at 37^0C for 24h, the presumptive *Vibrio cholerae, E.coli* and *Salmonella* were isolated in pure culture and identified by cultural and biochemical characters [17].

2.5. Antibiotic Sensitivity Test

The sensitivity of bacterial isolates to six broad spectrum antibiotics viz chloramphenicol (30mcg), ciprofloxacin (5mcg), gentamicin (10mcg), ampicillin (10mcg), norfloxacin (10mcg), erythromicin (15mcg) (Himedia Laboratories, Mumbai, India) were examined by agar disc diffusion method [18] on Mueller Hinton Agar (DIFCO).

2.6. Statistical Analysis

Statistical analysis: Mean and Standard deviation (SD) were calculated using Excel2007 (Microsoft Corp. Seattle, W.A).

3. Results

In the present study, the faecal coliform count for shrimp, prawn and IMC were 1.48×10^4 cfu/g, 1.65×10^4 cfu/g and 6.6×10^3 cfu/g respectively. Similarly the bacterial load of shrimp, prawn and fish are 2.5×10^5 cfu/g, 1.8×10^5 cfu/g and 1.20×10^4 cfu/g. Pathogenic members viz. *Salmonella, Vibrio* and *E. coli* were present more frequently in shrimp and prawn than finfishes (Table-1).The antimicrobial sensitivity test of pathogenic bacteria i.e. *Vibrio, Salmonella* and *E. coli* detected from shellfish and finfish are given in (Table 2).

Table1. Presence of pathogenic bacterial species in finfish and shellfish.

Sample	No of sample	% of samples positive [a]			Faecal Coliform count [b](Mean ± S.D)	Total [b]Plate Count(Mean± S.D)
		Salmonella	Vibrio	E.coli		
P. monodon	50	40	90	100	$1.48\pm2.3 \times 10^4$	$2.5\pm3.4 \times 10^5$
M. rosenbergii	50	30	100	90	$1.65\pm2.6 \times 10^4$	$1.8\pm2.6. \times 10^5$
IMC	50	10	10	50	$6.6\pm8.5 \times 1^3$	$1.20\pm1.6 \times 10^4$

a Percentage of samples positive = number of samples positive / total number of sample examined.
b Faecal coliform counts and total plate counts are expressed as CFU/g.

Table2. Antibiotic sensitivity test of bacteria isolated from samples.

Test organismAntibiotics Discs	% of *V. cholera* positive strains			% of *Salmonella* positive strains			% of *E.coli* positive strains		
	R	I	S	R	I	S	R	I	S
Gentamicin (10 mcg))	0	25.4	75.6	100	0	0	0	20	80
Erythromycin (15 mcg)	100	0	0	100	0	0	72	28	0
Norfloxacin (10 mcg)	0	0	100	100	0	0	0	12	88
Chlorampehenicol (30mcg)	0	2.3	98.7	0	1	99	0	0	100
Ampicillin (10 mcg)	100	0	0	8	20	72	6	94	0
Ciprofloxacin (5 mcg)	0	7.5	93.5	26	0	74	0	0	100

R= Resistant, I= Intermediate and S= Sensitivity.

4. Discussion

Microbiological quality of fish and shell fish determines its shelf life, quality of the product and human safety. A higher side bacterial load was detected in fish and shellfish in present study. As per ICMSF (1986) guidelines, the bacterial loads in the fresh finfishes were acceptable or marginally acceptable for most of the samples, whereas according to ICMSF 1986 [19] acceptable limit for fish and fishery products is 5.0 x10^5 cfu/g. The TPC of finfish and shellfish observed in the present study were found to be in conformity with the result as described by Manna [6]. The bacterial load in *P. monodon* and *M. rosenbergii* were also higher than in finfishes. This was possibly due to polluted water of bheries, the heavy presence of faecal coliform in shrimp, prawn and fin fishes also indicated poor hygienic and sanitary qualities which pose a major constraint for Indian seafood industry.

Salmonella is one of the most common food borne pathogens throughout the globe. Like meat, the incidence of Salmonellosis has also been linked to the consumption of fish and fishery products [20,21].The USFDA/EU had declared *Salmonella* as " Zero tolerant" in seafood and their standards stipulated that the bacteria should be nil in 25 gm of test sample. The present results showed that about 40% of shrimp/ prawn and 10% of fishes contaminated with *Salmonella* raising serious public health concern that might originate from these raw items.

E. coli contamination of tropical seafood is quite common [22]. In India, *E. coli* has been isolated from beach seawater through the year. Estuaries and coastal waters are the major sources of seafood in India which are contaminated by partially treated or untreated sewage water. Inadequately cleaned and disinfected boat decks and fish containers are known to contaminate the catch with *E. coli* [10]. *E. coli* contamination can also occur from ice, unclean workers and handling after catching of fish and shellfishes. *E. coli* act as an indicator organism of faecal contamination of water and seafood [23]. The present result showed that almost all samples of fish and shellfish were contaminated with *E. coli* indicating poor hygienic and sanitary quality of fish and shellfish.

V. cholerae is endemic in Southeast Asian countries with occasional cholera outbreaks [24]. Out breaks of cholera was reported owing to consumption of seafood including oysters, crabs and shrimps by Oliver and Kaper [9].*V. cholerae* which is found associated with shellfish and crustaceans is native to aquatic environment [8,25-27]. *V. cholerae* O139 serogroups are causing outbreaks of cholera in India and Bangladesh [28]. In the present paper *V. cholerae* was positive in shrimp (100%) and prawns (90%) where as in finfish only (10%). *V. cholerae* was noticed in most of the shellfish samples due to chitinase acitivity of *V. cholerae* which may increase the affinity to crustacean and molluscs like shrimp, prawn, crab, oyster etc. The presence of *V. cholerae* in finfish was possibly due to sewage contamination in the ponds.

In the study *Vibrio cholera* contamination was detected in the all samples of shellfishes. This might be because of the fact that Vibrios are natural inhabitants in marine environment and for sewage contamination in coastal aquaculture farms. Most of the prawns and shrimps, collected in retail and wholesale markets for the study were originated from coastal aquaculture farms. Similarly *E. coli and Salmonella* contamination were detected in finishes and shellfishes during the study because of either poor unhygienic and sanitary condition of the retail and wholesale markets of West Bengal and cross contamination for human activity or sample originated from ponds contaminated with domestic sewage.

Here, all the *V. cholera* isolates were sensitive to norfloxacin. In addition, over 90% strains were sensitive to chloramphenicol, ciprofloxacin and over75 % were susceptible to gentamicin. These drugs are commonly used in bacterial diarrhoeal cases and were effective in vitro against *V. cholera*. Similar results were reported by Das and Khan [29,30] and as per their findings all isolated strains of *V. parahaemolyticus* obtained from shrimp showed resistance to erythromycin, ampicillin, penicillin and kanamycin. All *Salmonella* isolates were sensitive to chloramphenicol, ciprofloxacin and Ampicillin and resistant to gentamicin, erythromycin and norfloxacin. Similar results were reported by Duffy [31]. All *E. coli* isolates were sensitive to gentamicin, norfloxacin, ciprofloxacin and chloramphenicol and resistant to ampicillin which correlates with the findings of Kumar [22]. Resistance to most of the antibiotics indicated that most strains have developed the resistance, might be due to frequent use of antibiotics in diarrhoea and other gastro- intestinal problem, common in this part of India. The presence of antibiotic residues originating from hospital and city wastes which is released in the estuaries may increase the chance of antibiotic resistance development. Whether occurrence of antibiotic resistance among fish and shellfish bacterial isolates is a reflection of that occurring in human and animal health, and whether this also influences

development of resistance among aquatic microbial population needs critical evaluation. Similarly, there also seems to have been an impact on the environmental bacterial flora surrounding fish farms where antibacterial drugs are being used profusely [32,33].

5. Conclusion

The microbiological quality of fish and shellfish was within acceptable or marginally acceptable limits; however, fishes were contaminated with faecal coliforms indicating hygiene and sanitary conditions. The specific pathogens like *Vibrio, E. coli and Salmonella* were also detected in fish and shellfish samples. However, microbiological quality aspects of fish and shell fish in the domestic retail markets of Bengal have not been studied thoroughly so far but the present study has given an indication of hygienic standard of finfishes and shellfishes of domestic markets of Bengal which are available for human consumption and in this regard extension work needs to be done to draw a significant conclusion.

Acknowledgments

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