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Microbiological Examination of Fish Collected from Markets of Kolkata

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ABSTRACT

This study aimed to evaluate the microbiological quality of *Labeo rohita*, *Mugil cephalus*, *Macrobrachium rosenbergii* and ice samples from New Garia, Jadavpur and Mukundapur fish market of Kolkata, West Bengal. A total of 27 fish samples (n=9) were collected from those markets (3 fish samples of every species from each market). The presence of Escherichia coli was found highest followed by Vibrio spp., Aeromonas spp. and Salmonella spp. in collected sample. The total viable bacterial counts in muscle of *M. rosenbergii*, *Mugil cephalus* and *Labeo rohita* were found highest 7.75±0.38 log cfu/gm, 6.90±0.41 log cfu/gm and 6.64±0.17 log cfu/gm, respectively from New Garia fish markets. The qualities of fish from other markets were also found not satisfactory. The Most Probable Number (MPN) of ice samples from those markets were recorded very high with a maximum 275 MPN/100 ml of ice. All the fish sold in those aforesaid markets had the potential to be contaminated with foodborne bacteria. Efficient control measures are highly needed to avert the contamination during handling of fish in those markets.

KEYWORDS

Fish market, Microbiological quality

Introduction

Humans have been consuming fish since 1.95 million years ago. With the rise in human population the consumption of fish and fishery product has also been rising upto 122% from 1990-2018 (FAO 2019). Fishes are known for their exceptional health benefits, particularly against cardiovascular diseases (Maehre *et al*., 2014) and for infant brain development (Boucher *et al*., 2011) due to their long-chain polyunsaturated fatty acids (PUFAs) content.

The development of seafood industry over the years was observed to be accompanied by increase in risk of contamination of seafood by food borne pathogens. The contamination of pathogenic bacteria in food is become a serious issue as it may cause many food borne diseases in humans. Salmonellosis, one of the food borne diseases can spread diarrheal disease. Consumption of these contaminated fish may cause infection or intoxication to the consumers. Bacterial pathogens associated with fish market can be categorized into three general groups. The bacteria abundant in aquatic environment (e.g., *Vibrio* spp., *Psedomonas* spp. and *Aeromonas hydrophila*), the enteric bacteria present due to faecal contamination (*E. coli*) and bacteria introduced during handling and marketing (*Salmonella* spp., *Shigella* spp.). Good hygiene practices before, during, and after fish handling can reduce the chances of contracting pathogenic microorganisms, which otherwise causes illness as bacteria are a common cause of food borne illness (Sahu *et. al*., 2012). Thus, it is particularly important to avoid contamination of food with pathogenic bacteria. Some pathogenic bacteria in fish are naturally present and some are present during harvesting and storage as a result of mishandling. This study is mainly aimed to investigate the bacterial load and presence of pathogenic bacteria in three commercially important species including fish and prawn collected from the wet market in Kolkata. Two objectives were taken for this study. First one was to enumerate the bacterial load in rohu (*Labeo rohita),* mullet (*Mugil cephalus*), *Macrobrachium rosenbergii* (Galda) and ice samples used for their preservation from selected markets. Second was to confirm the presence of *E. coli*, *Salmonella* spp., *Vibrio* spp. and *Aeromonas* spp. from those collected fish and prawn samples.

Materials and methods

Sampling

The fish and ice sample were collected from three different fish market; New Garia, Jadavpur and Mukundapur fish market of Kolkata, West Bengal. Three different species was collected namely rohu (*Labeo rohita),* mullet (*Mugil cephalus*), *Macrobrachium rosenbergii* (Galda). A total of 27 fish samples, including Rohu, Mugil and Galda (n=9 of each fish species and 3 numbers of each species from each market) were collected randomly from those three markets. Fish samples were collected in the morning, kept in plastic insulated boxes with ice and then transferred to the laboratory. The fork length and wet weight of each fish were recorded as mentioned below (Table 1).

Table 1: The average weight and length of fish samples (n=9)

Name of fish species	Weight (gm)	Length (cm)
Labeo rohita	520 ± 44.5	24 ± 2.5
Mugil cephalus	110 ± 9.5	12 ± 1.3
Macrobrachium rosenbergii	90 ± 5.2	16 ± 2.2

Values presented as Mean ± SD for fish species collected from markets.

Bacteriological chemical and media

Normal saline (0.85%) was prepared for serial dilution. Brain Heart Infusion (BHI) agar (HiMedia, India) was prepared for the total viable bacterial count. Different enrichment media like Peptone water, Lactose broth, Alkaline peptone water and Tetrathionate broth were used for pre-enrichment of different bacteria.

Four selective media were used *i.e*., Rimler Shotts (RS) agar (HiMedia, India) for detection of *Aeromonas* spp., Thiosulfate-Citrate-Bile Salt-Sucrose (TCBS) agar (HiMedia, India) for detection of *Vibrio* spp., Eosin Methylene Blue (EMB) agar (HiMedia, India) for detection of *Escherichia coli* and Salmonella Shigella Agar (SSA) (HiMedia, India) was used for detection of *Salmonella* and *Shigella* species. A 2% of agar powder was used for all the media for solidification. Only EMB media was sterilized by autoclave at 121^{o}C and 15 psi for 15 min, whereas other media were steam sterilized. (Ronald *et al*., 2010)

Sample pretreatment

Collected samples were then brought to lab and the skin sample was excised from the 3 cm^2 of the skin surface in the central area of the fish using a sterile scalpel. Total 10 gm of muscle was cut and minced (Gao *et al*., 2022). The samples were diluted with NSS (Normal saline, 0.85% of NaCl) up to 100 ml. Before starting work each glassware

were sterilized in an autoclave at 121°C, 15 psi and 15 min, and the dissecting apparatus like scalpel, scissors, pestle mortar and knives were also disinfected with alcohol.

Serial dilution and spread plating for Total viable bacterial count

The diluted sample were transferred to the test tube with the help of micro pipette of 1 ml in 9 ml NSS tube, mixed properly using vortex mixer for 10^{-1} dilution. Now from the 10^{-1} dilution upto 10^{-8} dilution was prepared in triplicate. With the help of 100 µl pipette the 10^{-5} to 10^{-8} dilution were transferred to BHI agar plate (20 ml media/plate) in triplicate for spread plate technique and incubated at 37°C for 24 h (Ronald *et al*., 2010). The colony counts in between 30 to 300 were considered (Table 2).

Most Probable Number

For confirmation of *E. coli* in ice water, 5 tube methods were used. Ten (10) ml, 1 ml and 0.1 ml ice samples were transferred to the Lactose broth in a test tube with Durham's tube which was incubated for 24 h at 37°C. Tubes with gas production were taken as positive and results were taken as per standard 5 tube table (Ronald *et al*., 2010).

Bacterial analyses

The bacterial analysis for presence of pathogenic bacteria in the collected fish and prawn samples were performed according to the modified method proposed by Roberts and Greenwood (2008). Bacteria were isolated from the edible portion like skin and flesh of fish.

Detection of *Aermonas* spp.

Briefly, 5 gm of fish sample was homogenized in 45 ml 0.1% (w/v) peptone water and incubated at 30°C for 18–24 h. Then it was streaked on Rimler Shotts (RS) agar and incubated at 30°C for 18–24 h. The typical yellow colonies appeared on the agar were chosen and confirmed by the biochemical tests (Citrate, Gas production, Oxidation/Fermentation, Oxidase, Catalase, Motility, Methyl Red, Voges Proskear's) (Gao *et al*., 2022).

Detection of *Escherichia coli*

Ten gm of muscle and skin sample were mixed with NSS (0.85%) to make the volume up to 100 ml and then 1 ml of those 10^{-1} diluents was inoculated into 10 ml of Lactose broth with Durham tube at 35°C for 24–48 h. After incubation, tubes with acid (Colour changes) and gas (Durham tube floating) formation were considered a positive result. A loopful of inoculums from every positive tube was transferred to Eosin

Methylene Blue (EMB) agar and biochemical tests (Indole, Methyl Red, Voges Proskear's and Citrate test) were used for confirmation of typical *E. coli* colonies (Gao *et al*., 2022).

Detection of *Vibrio* spp.

Ten gm of muscle and skin sample were mixed with Alkaline peptone water (1 gm peptone and 0.05 gm NaCl in 100 ml) to make the volume up to 100 ml. Such 10^{-1} homogenate of fish samples was incubated at 37°C for 20 h. Then it was streaked in TCBS agar at 37°C for 18-24 h. According to the colour and size of the bacteria on the medium, the suspected bacteria are screened out and further biochemical tests (Urease, Catalase, Voges Proskear's, Methyl Red, Indole and Triple Suger Iron test) were carried out to confirm *Vibrio* spp (Gao *et al*., 2022).

Detection of *Salmonella* spp.

Ten gm of muscle and skin sample were mixed with 90 ml of Lactose broth. The 10^{-1} homogenate of fish samples in Lactose broth was incubated at 37°C for 18-24 h and then it was further pre-enriched in Tetrathionate broth and incubated at 37°C for 18-24 h. Then it was streaked on the Salmonella Shigella Agar (SSA) agar and incubated at 37°C for 18-24 h. A typical Salmonella colony with black centre was identified (Gao *et al*., 2022).

Isolation of Bacteria culture

Based on the dominance, pigmentation and definite colony morphology, colony characterization was observed in selective media. The representative colony was picked aseptically from the plate after 24 h and the purified colony was maintained in Nutrient Agar (NA) slants for further biochemical test.

Biochemical tests

After observing the colony shape, size, colour, appearance, elevation, margins, and optical density were described by looking at the plate. The phenotypic characteristics of the bacterial strain were determined by the conventional biochemical tests like Gram staining, Oxidase, Oxidation and Fermentation (O/F), Acid and Gas production, Catalase, Motility, Indole, Methyl Red, VP, Citrate test, Triple Sugar Iron, Urease test etc.

Results and Discussion

Total viable bacterial Count

Bacterial numbers were calculated as the average of each set of triplicates and expressed as cfu/gm. The present study indicated the poor hygienic and

microbiological quality of fin fish and prawn samples of domestic markets of Kolkata. Significantly higher (P<0.05) value of total viable plate counts (TVC) in muscle samples were found in *Macrobrachium rosenbergii* followed by *Mugil cephalus* and *Labeo rohita* collected from all three markets (Table 2). According to International Commission for Microbiological Specifications for Foods (ICMSF, 1986) the raw fish meat having total viable counts less than 5.0 x 10^5 cfu/gm is acceptable, between 5.0 x 10^5 cfu/gm to 5.0 x 10^6 cfu/gm is marginally acceptable and more than 5.0 x 10^6 cfu/gm should be rejected. In this study all the *Macrobrachium rosenbergii* were found to be rejected and the *Mugil cephalus* and *Labeo rohita* were found to be marginally accepted.

Table 2: Total viable bacterial Count of the muscle of fish and prawn sample (n=3)

	Species	CFU/g	Log cfu/g
New Garia fish market	*Macrobrachium rosenbergii*	5.6×10^7	7.75 ± 0.38^c
	Mugil cephalus	8.1×10^6	6.90 ± 0.41^b
	Labeo rohita	4.4×10^6	6.64 ± 0.17^a
Jadavpur fish market	*Macrobrachium rosenbergii*	2.1×10^7	7.32 ± 0.44^b
	Mugil cephalus	3.4×10^6	6.53 ± 0.28^a
	Labeo rohita	3.2×10^6	6.50 ± 0.45^a
Mukundapur fish market	*Macrobrachium rosenbergii*	1.5×10^7	7.17 ± 0.52^c
	Mugil cephalus	3.6×10^6	6.55 ± 0.18^b
	Labeo rohita	2.5×10^6	6.34 ± 0.17^a

Values (Mean ± SD) denoting different alphabetic superscript differ significantly (P<0.05) among the fish species for each market.

Most Probable Number count of ice

The MPN value of ice samples also found very high in New Garia fish market compared to other two markets (Table 3). According to the World Health Organisation (WHO, 1997), the ice used for food

preservation should be of same quality and safety as of drinking water. The Indian Standard (IS 10500) recommends that the ice should be devoid of total coliforms, fecal coliforms and *E. coli* and should fulfil the drinking water standards i.e. MPN <1/ 100 ml water.

Table 3: 5-Tube Most Probable Number (MPN) Table for total coliforms of Ice samples

	Number of Positive tubes			MPN/100 ml
	10 ml	1 ml	0.1 ml	
New Garia fish market	5	4	3	275
	5	3	3	175
	5	3	3	175
Jadavpur fish market	5	3	1	110
	5	4	1	170
	5	3	2	140
Mukundapur fish market	5	2	3	120
	5	3	2	140
	5	1	1	45

The findings of this study indicate that the raw fish of those markets have high total viable count. In local markets, different kinds of fish are kept together and the sellers do not maintain proper hygiene which creates the possibilities to come in contact with several pathogens and the sellers' uses ice, mostly prepared from contaminated water, to preserve fish after being caught which may be a potential source of contamination. Therefore, hygenic handling, proper storage condition and maintaining a strict regulation between fishing and marketing claim could be considered as the appropriate practices of hygiene for ensuring the microbiological quality of fish as well the consumer safety. It is worth to note that the pathogens existing in the contaminated foods may harbour virulence genes which might be responsible for disease outbreaks (Noor *et al*., 2013).

Presence of *E. coli*

The existence of coliform bacteria, including *E. coli* in most of the samples examined indicated poor hygienic and sanitary condition (Table 5). This finding supports with the study of Dutta *et al.* (2016). Quality of fish depends on the quality of

waters from where the fishes are captured and the sanitary conditions of the landing centers. Proper sanitation facilities at the retail markets play an important role in the overall quality of the fish. However, *E. coli* does not thrive in the salt environment for long period of time and so this organism cannot be expected in the species chosen for study include fish from both freshwater and brackish water. Hence, conclusion should include considering both the environment and not brackish only (Martinez *et al*., 1992).

Presence of *Vibrio* spp.

Vibrios are indigenous bacterial pathogens for seafood. The higher incidence of *Vibrio* spp. in prawn sample could be correlated with the ability of the bacterium to utilize chitin, abundantly available in the crustaceans. Moreover, shrimps are rich in free amino acid content, which serves as an excellent growth medium for the proliferation of this bacterium (Chakraborty *et al*., 2008). However, salt preference of bacterium was also reflected due to its association in brackish water fin fish *i.e.*, *Mugil cephalus* in the study.

Presence of *Salmonella* spp.

Salmonella belongs to Enterobacteriaceae family. They are common water borne bacterium which may be apparently present in the tissues of normal fishes (Bibi *et al*., 2015). The injury or environmental stress in fishes causes serious outbreaks of the diseases with mortality. The transmission of *Salmonella* in the raw fish may be the reason for the spread of the diseases. The impact of this pathogen in human, particularly in the very young or older, includes gastroenteritis, abdominal cramps, enteric fever resulting from ingestion of uncooked fishes. In spite of these unhealthy impacts, their epidemiology is still poorly understood (Bibi *et al*., 2015). *Salmonella* in freshwater fishes has been usually related to the fecal contamination of water from where fish were harvested (Mhango *et al*., 2010). Fish works as a passive carrier of *Salmonella* that may excrete *Salmonella* spp. without apparent symptoms and represent no clinical disease. In this study the presence of *Salmonella* spp. was found in fish and prawn samples might be due to the improper handling and storage (Table 5).

Table 4: Biochemical test results of bacteria collected from fish and prawn

	***Vibrio* spp.**	***Aeromonas* spp**	***Salmonella* spp.**	***E. coli***
Colony characteristics	Yellow and Green colony on TCBS	Yellow colony on RS	Black colony on SSA	Dark centre with green sheen colony on EMB
Biochemical tests				
O/F Test	+	+	+	+
Gram staining	Negative Rod	Negative Rod	Negative Rod	Negative Rod
Citrate test	+	+	-	-
Gas test	-	-	+	+
Motile	+	+	+	+
Oxidase	+	+	-	-
Catalase	+	+	+	-
Indole	+	+	-	+
Urease	+	-	-	-
Methyl Red	+	-	+	+
Voges Proskear's	-	+	-	-

Presence of *Aeromonas* spp.

Several reports supported the presence of Aeromonas in fish samples of market (Sharma and Kumar, 2011; Gowda *et al*., 2015). Bardhan *et al*. (2021) reported the high frequency of antibiotic-resistant motile aeromonads in retail market in Kolkata that might be spread through the food chain and could be serious for public health concern. This study also corroborates with those previous findings (Table 5).

Table 5: Presence of bacteria in skin and muscle sample of fish and prawn (n=3).

Name of the Market	Name of the species	Number of positive samples (Skin / Muscle) out of 3 samples of each species from all markets			
		Vibrio spp.	*Aeromonas* spp	*Salmonella* spp.	*E. coli*
New Garia fish market	*M. rosenbergii*	2 / 1	2 / 1	1 / 1	3 / 2
	Mugil cephalus	3 / 3	1 / 1	0 / 0	3 / 3
	Labeo rohita	1 / 0	2 / 1	0 / 0	3 / 1
Jadavpur fish market	*M. rosenbergii*	1 / 1	2 / 1	0 / 1	3 / 2
	Mugil cephalus	3 / 2	0 / 0	1 / 0	3 / 2
	Labeo rohita	0 / 0	1 / 0	0 / 0	3 / 2
Mukundapur fish market	*M. rosenbergii*	2 / 2	1 / 1	0 / 1	3 / 2
	Mugil cephalus	2 / 2	1 / 1	1 / 0	3 / 3
	Labeo rohita	1 / 0	1 / 1	0 / 0	3 / 2
	Total	15 / 11	11 / 7	3 / 3	27 / 19
	(%)	56% / 41%	41% / 26%	11% / 11%	100% / 70%

Conclusion

Out of three fish markets the most bacterial contamination was observed in New Garia Market. Raw fish have been recognized as a major carrier of food borne pathogens. It would therefore be recommended that the local fish sellers should be familiarized with the Good Hygienic Practice (GHP) and HACCP for better services. The fish sellers of those three markets should play significant role by giving hygiene raw fish to people for their good health. There is need for strict monitoring by competent authorities for improvement the quality of ice used for fish preservation.

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