



Evaluation of Microbial Quality of Fishes and Sanitary Condition of Wholesale and Retail Fish Markets in Peri-urban Area of Kolkata, India


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ABSTRACT

The present study conducted during October, 2019 to January, 2020 was aimed to assess the microbial quality of selected fish species *viz.*, *Labeo rohita*, *Lates calcarifer* and *Johnius belangeri* and check the sanitary condition of two selected fish markets *i.e.*, Baghajatin wholesale fish market and Garia retail fish market in Kolkata, India. The results of TPC of fish muscle never crossed the limit of 4.70 log cfu g⁻¹ as specified by ICMSF. Total plate count (TPC) of fish muscle were well within the limit of acceptability suggesting to be fit for consumption. The Total plate count value of water, ice, and swab samples were found higher in the month of October and lower in the month of January. The high values of TPC were recorded in water, ice and swab samples suggesting poor sanitation, improper handling and use of contaminated water in ice production. All the three species collected from two different selected fish markets were detected to contain the pathogenic microorganisms like *Salmonella* sp., *Clostridium* sp., *Listeria* sp. and *Staphylococcus aureus*. The results also indicated the presence of faecal coliform in water, ice and swab samples. It is recommended that potable water must be used for manufacturing of ice and handling should be done under good hygiene and sanitary conditions.

KEYWORDS: Freshness, pathogenic bacteria, quality, sanitary condition, TPC

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1. INTRODUCTION

Fisheries sector makes a fundamental commitment to improve the healthy livelihoods of human through food and nourishment security, as well as providing basic nutrition to millions of people throughout the world. Considering the high nutritional value, fish are the significant carrier of pathogenic microorganisms (Prasad and Murugadas, 2019). Fish can be corrupted by aquatic environment and post harvesting condition (Ikbal et al., 2023, Cochrane, 2021). Food security is a comprehensive description of dealing with, preparation, processing, transportation, and capacity conditions that prevent food-borne infections and provide the good quality products to the consumers (Ikbal et al., 2020, Carthy et al., 2018, Cheng et al., 2013). Fish quality may be deteriorated by the aerobic spore forming bacteria which causes physical, chemical, and microbiological spoilage (Jalal et al., 2017). Microbial nature of fish relies upon the nature of water, ice and furthermore the transportation system through which they are shipped off the market. After catching, the fish shifts a few hands beginning from the fishers through the middle man mainly fish sellers, then wholesalers and at last to the retailers (Pal, 2010). Intrinsic characteristics such as fragile muscle tissue, activity of endogenous protease, inappropriate handling methods and storage conditions can be prone to physical, chemical and microbial changes in fish which affects its freshness quality (Singh et al., 2019, Cheng et al., 2015). Everybody should guarantee that food is protected and appropriate for human utilization (Kapute et al., 2012). Raw fish usually contains a variety of bacteria, which can be harmful and cause foodborne infections quickly if left at room temperature for an extended period of time without keeping in low temperature (Nowshad et al., 2021, Jaaskelainen et al., 2019, Sharif et al., 2018). Microbial population which is essentially present in the external surfaces (skin and gills) is mainly liable for the deterioration of freshly caught fishes. Pathogenic microbes particularly *Salmonella* spp. and *Vibrio* spp. are the essential worry of food security concerning fish (Beyari et al., 2021, Helmi et al., 2020, Pal, 2012). At the point when fish is handled with uncontaminated water and cooked appropriately, it brings down the chances of food contamination. Appropriate temperature is a useful factor that can inhibit microbial development and foodborne illness (Nur et al., 2020). Particular kind of microscopic organisms are liable for the deterioration of fish muscles after death (Begum et al., 2010). It mainly relies upon the different type of fishes and its chemical composition, feeding habit, and the region where the fish is harvested and also the type of fishing gear used during the time of catching (Francoise, 2010). Other major external sources

of microbial contamination are ice, water and fish container which is known to carry heavy bacterial loads (Sanjee and Karim, 2016). Quality and sanitation propaganda mainly depend on the cleanliness of fish market, quality of water used and the handling practices during the time of transportation of fish. Monitoring the microbial quality of fish is an integral part of enhancing food safety (Onjong et al., 2018). The aim of the fish processing industry is to provide safe, wholesome and acceptable product to the consumer (Pal and Mahendra, 2015). Microbiological quality evaluation means to assess the hygienic conditions of fishes and the chance of presence of pathogenic microorganisms in fish (Gutema and Hailemichael, 2021, Rasul et al., 2020, Shahriar et al., 2019, Hussain et al., 2018). Utilization of these contaminated fishes might make disease or intoxication to the purchasers. The study targets evaluating month to month profile of microbiological quality of selected fish species collected from Baghajatin wholesale and Garia retail fish market and also evaluate the sanitary condition of selected fish markets.

2. MATERIALS AND METHODS

2.1. Sampling and experimental fish

Fish samples were collected in the early morning hours at monthly intervals during October, 2019 to January, 2020 from both the Baghajatin wholesale fish market (22°29'00.2"N; 88°22'30.7"E) and Garia retail fish market (22°28'57"N; 88°23'06"E) of peri-urban Kolkata, West Bengal, India. The experimental fish included Rohu (*Labeo rohita*), Asian seabass (*Lates calcarifer*) and Croaker (*Johnius belangeri*) of size 250–350 g, originating from unknown culture systems, were randomly selected from the mentioned fish markets. Water and ice used in fish markets were also sampled into sterile conical flasks and transported to the laboratory within 2 hours. Fish contact surfaces like the platforms where the fishes were kept and the crates were swabbed (25 cm² area is swabbed using a square 5×5 cm²) thoroughly with sterile cotton swabs and transported to laboratory within 2 hours. Fish, Water, Ice and Swab samples were placed in the insulated box to avoid further contamination. Proper sealing help to prevent the chance of contamination. Sample box was carefully carried up to laboratory of Fish Processing Technology, Faculty of Fishery Sciences within two hours of collection. Assessment of microbiological quality of fish samples was done at a monthly span all through the time of October 2019 to January 2020 with a sample size of 10 fishes each month. In the present study, the microbial quality of fish samples including Total plate count and detection of pathogenic microorganisms and the sanitary condition of selected fish markets including the total plate count and faecal coliform count of Water, Ice, and Swab



samples were measured.

2.2. Total plate count

TPC of muscle was done by following the standard method given by Anonymous, 2001, with some modifications. In brief, 10 g of fish sample was taken and it was homogenized with 90 ml of physiological saline. Serial decimal dilutions of 10^{-2} , 10^{-3} , 10^{-4} , etc were prepared using 9 ml of 0.85% physiological saline. 0.1 ml of inoculum from each of the dilutions were spread plated onto Nutrient agar (NA) plates using a sterile glass spreader. The Petri plates were incubated at 37°C for 24 hours.

2.3. Faecal coliform (MPN method)

The three tubes MPN (Most Probable Number) of indicator bacteria were analysed following standard procedures and the results were expressed as MPN ml⁻¹ (Anonymous, 2001).

2.4. Pathogen detection

HiFast™ food pathogen detection kit was used in this study for the detection of *Salmonella*, *Listeria*, *S. aureus*, and *Clostridium* spp. Enrich fish samples in stomacher bags on adding the medium. For this, suspend sterile enrichment medium bud in distilled water. Shake and mix evenly dissolve the bud completely. Add sample dilutions to be enriched and incubate the prepared suspensions in stomacher bags at 35–37°C for 4–6 hours. Inoculate enriched sample into testing medium. Incubate at 35–37°C for 4–8 up to 12 hours. Interpret for colour change, blackening of medium, clot formation, and stormy fermentations in respective media.

2.5. Statistical analyses

All the data were checked for normal distribution with normality plots prior to analysis of variance (ANOVA) to determine significant differences among means at $\alpha=0.05$ level, using statistical tools of Microsoft Office Excel (2019) and R software (4.0.2). Tukey HSD was used to determine significant differences between samples.

3. RESULTS AND DISCUSSION

Marine or freshwater fishes are transported from landing centres to wholesale markets and retail fish markets which may likewise infect the associate people by handling. A wide possibility of destruction of fish can be occurred due to the attack of various pathogens, which unfavourably influences the human wellbeing and financial condition. At the point when the buyers buy these types of fishes, the related microorganisms are moved to the people who convey those (Begum et al., 2010). In this way, there is generally a gamble of weakening of value because of poor or unhygienic handling, transportation and storage. Microbiological quality assessment aims to measure the

hygienic quality of fish and the presence of pathogenic microorganisms in fish.

3.1. Enumeration of TPC in selected fish species

The three species collected from Baghajatin wholesale fish market were analysed for total plate count (TPC) as shown in Figure 1 and Table 3. The TPC of *L. rohita* muscle with skin was found to be high in the month of October (3.9 ± 0.2 log cfu g⁻¹) and low in the month of January (3.2 ± 0.04 log cfu g⁻¹) with significant ($p < 0.05$) variation over different sampling months. Similarly, the TPC value for *L. calcarifer* and *J. belangeri* was recorded highest during the month of October (4.1 ± 0.09 and 4.19 ± 0.05 log cfu g⁻¹) and lowest during the month of January (3.48 ± 0.07 and 3.7 ± 0.06 log cfu g⁻¹) respectively with significant ($p < 0.05$) variation over different sampling months. The TPC value found in October was significantly higher than the values obtained in the month of January may be due to the temperature variations. Similarly, three fish species were also collected from Garia retail fish market for total plate count (TPC) as shown in Figure 2 and Table 3. The TPC values of

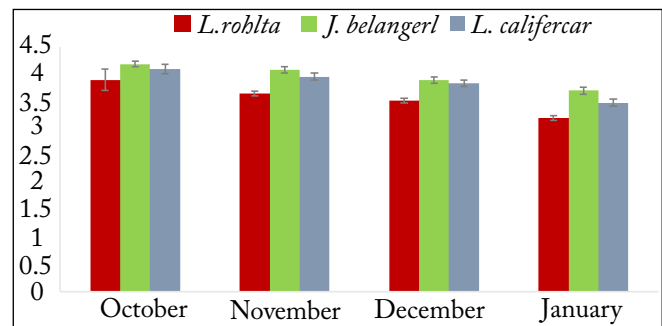


Figure 1: Variation in TPC of *Labeo rohita* (log cfu g⁻¹), *Lates calcarifer* (log cfu g⁻¹) and *Jobnius belangeri* (log cfu g⁻¹) collected from selected wholesale fish market; *: Results are mean of three determinations (n=3) with s.d.; #: Values of means varied significantly ($p < 0.05$) with months.

L. rohita muscle with skin were found 4.8 ± 0.35 log cfu g⁻¹ in the month of October, 4.6 ± 0.5 log cfu g⁻¹ in the month of November, 3.6 ± 0.45 log cfu g⁻¹ in the month of December and 3.5 ± 0.4 log cfu g⁻¹ in the month of January respectively. The TPC value for *L. calcarifer* was recorded highest during the month of October (4.4 ± 0.4 log cfu g⁻¹) and lowest in the month of January (3.97 ± 0.17 log cfu g⁻¹) respectively, but the changes were not significant ($p < 0.05$). On the other hand, the TPC value of *J. belangeri* was recorded highest during the month of October (4.63 ± 0.14 log cfu g⁻¹) and lowest in the month of January (4.24 ± 0.13 log cfu g⁻¹) respectively with significant ($p < 0.05$) variation over different sampling months. Bordoloi (2013) reported that the total plate count (TPC) of the muscle of fresh *Labeo rohita* varied from 3.1×10^5 to 6.8×10^6 cfu g⁻¹ which fairly coincides with the finding of the present study. On

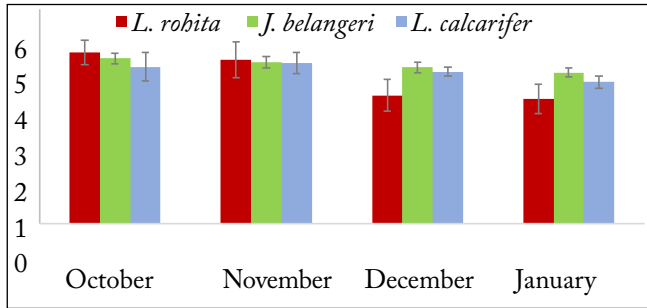


Figure 2: Variation in TPC of *Labeo rohita* (log cfu g⁻¹), *Lates calcarifer* (log cfu g⁻¹) and *Jobnius belangeri* (log cfu g⁻¹) collected from selected retail fish market; * Results are mean of three determinations (n=3) with s.d.; #: Values of means varied significantly (p<0.05) with months

the other hand, Dey and Dora (2010) recorded that fresh croaker showed TPC value of 5.4±0.02 log cfu g⁻¹ which is in accordance with the findings of the present study.

3.2. Enumeration of TPC in collected water, ice and swab samples

Water and ice are the most important factor for the good quality of fish. The ice made under poor sanitary conditions may become a risk factor to the consumers. The microorganisms present in the environment enable it to enter the food chain through raw material which affects the quality of fresh fishes (Cruz-Romero et al., 2008). The TPC value of the water samples collected from Baghajatin wholesale fish market varied from 4.66±0.05 log cfu ml⁻¹ in October to 4.06±0.11 log cfu ml⁻¹ in January which showed significant variations (p<0.05) over the sampling months as shown in Figure 3 and Table 4. On the other hand, TPC value of water samples collected from Garia retail fish market ranged between 5.01±0.21 log cfu ml⁻¹ in October to 4.27±0.13 log cfu ml⁻¹ in January with significant (p<0.05) variation over different sampling months as shown in Figure 4 and Table 5. Ice samples used in the

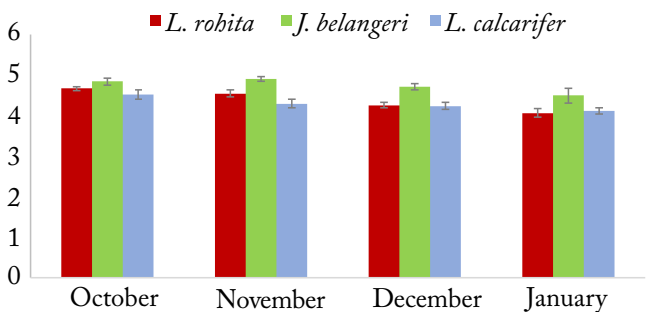


Figure 3: Variation in TPC in Ice (cfu ml⁻¹), Water (cfu ml⁻¹) and Swab (cfu cm⁻²) collected from selected wholesale fish market; * Results are mean of three determinations (n=3) with s.d.; #: Values of means varied significantly (p<0.05) with months

Baghajatin wholesale fish market over different sampling months contained a higher value of TPC in the month of October (4.83±0.08 log cfu ml⁻¹) and least (p<0.05) in the month of December and January (4.71±0.08 and 4.49±0.18 log cfu ml⁻¹) respectively as shown in Figure 3 and Table 4. Similarly, the TPC value of Ice samples collected from Garia retail fish market ranged from (5.41±0.21 log cfu ml⁻¹) in October to (4.71±0.09 log cfu ml⁻¹) in January with significant (p<0.05) variation over different sampling months as shown in Figure 4 and Table 5. Sanjee and Karim, (2016) reported that the total viable aerobic count (TVAC) in ice samples ranged between 3 to 18 cfu ml⁻¹.

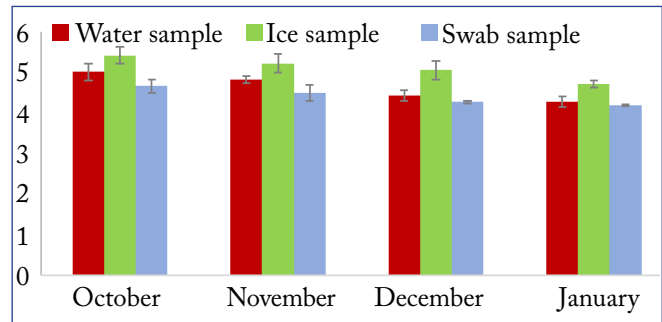


Figure 4: Variation in TPC in Ice (cfu ml⁻¹), Water (cfu ml⁻¹) and Swab (cfu cm⁻²) collected from selected retail fish market; * Results are mean of three determinations (n=3) with s.d.; #: Values of means varied significantly (p<0.05) with months

Personal hygiene practices and hygienic production processes are equally important to ensure fish and fishery products are safe to eat. The swab collected randomly from the crates and the places where the fishes were kept in the Baghajatin wholesale fish market, had TPC values ranging from 4.51±0.11 log cfu cm⁻² in October to 4.11±0.07 log cfu cm⁻² in January. The variation in TPC of swab samples indicated significant variation (p<0.05) among the sampling months as shown in Figure 3 and Table 4. On the other hand, the TPC values of Swab samples collected from Garia retail fish market varied from 4.66±0.16 log cfu cm⁻² in October to 4.18±0.02 log cfu cm⁻² in January which showed significant (p<0.05) variation over different sampling months as shown in Figure 4 and Table 5. Prabakaran et al. (2011) also reported that the TPC of swabs sample taken from the Worker’s hand and Fish handling box at fish processing plant Mandapam, Tamilnadu was 37×10² cfu 25 cm⁻² and 39×10² cfu 25 cm⁻² respectively.

3.3. Enumeration of faecal coliform

Coliform detection is basically employed as an indicator of sanitary conditions in fish processing environment or as an indicator of water quality. For that the Most Probable Number approach is used as a multi-step statistical test. The faecal coliform count of water sample collected from

Baghajatin wholesale fish market was found to have the highest value in the month of October (11.93 ± 0.4 MPN ml^{-1}) and least in the month of January (5.89 ± 0.48 MPN ml^{-1}) with significant variations ($p < 0.05$) over the sampling months. The ice samples had the highest faecal coliform count of 10.05 ± 0.44 MPN ml^{-1} in October and lowest of 4.06 ± 0.91 MPN ml^{-1} in January and the values were significant ($p < 0.05$) among the sampling months. In case of swab samples, faecal coliform count was recorded 13.39 ± 0.84 MPN ml^{-1} in the month of November and 7.95 ± 1.18 MPN ml^{-1} in the month of January with significant variations ($p < 0.05$) over the sampling months as shown in Figure 5 and Table 4. On the other hand, faecal coliform count of water samples collected from Garia retail fish market was found highest in the month of October (13.01 ± 0.7 MPN ml^{-1}) and least in the month of January (3.8 ± 0.7 MPN ml^{-1}) with significant variations ($p < 0.05$) over the sampling months. The ice samples had the highest faecal coliform count of 11.5 ± 0.3 MPN ml^{-1}

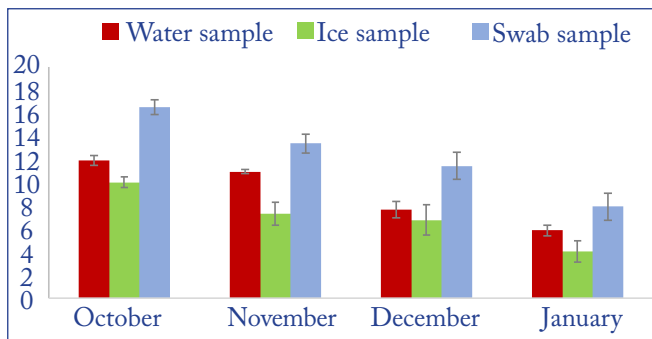


Figure 5: Variation in Faecal coliform in Ice (cfu ml^{-1}), Water (cfu ml^{-1}) and Swab (cfu cm^{-2}) collected from selected wholesale fish market; * Results are mean of three determinations ($n=3$) with s.d.; # Values of means varied significantly ($p < 0.05$) with months

in October with lowest significant value ($p < 0.05$) was recorded in the month of January (4.7 ± 0.6 MPN/ml). In case of swab samples, the faecal coliform count was recorded 19.8 ± 0.6 MPN ml^{-1} in the month of October and 12.5 ± 1.1 MPN ml^{-1} in the month of December with significant variations ($p < 0.05$) over the sampling months as shown in Figure 6 and Table 5. The faecal coliform count of water samples collected from retail fish market of Vashi, Navi Mumbai was 50 MPN 100 ml^{-1} as stated by Visnuvinayagam et al. (2019).

3.4. Pathogens detection of mentioned species

The aim of these hygienic practices is to prevent or reduce fish contamination and microbial growth. Most of the mediums involved in the marketing of fish are unaware that they are potential carriers of pathogenic microorganisms. *L. rohita* samples collected from Baghajatin wholesale fish

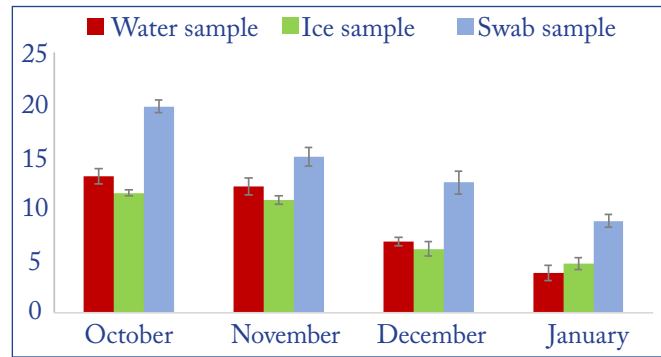


Figure 6: Variation in Faecal coliform in Ice (cfu ml^{-1}), Water (cfu ml^{-1}) and Swab (cfu cm^{-2}) collected from selected retail fish market; * Results are mean of three determinations ($n=3$) with s.d.; # Values of means varied significantly ($p < 0.05$) with months

market were found positive for the presence of *Clostridium* sp. and *Listeria* sp. during the month of October and November. *Salmonella* sp. was detected in the month of October and December in *L. rohita*. *Clostridium* sp. and *Listeria* sp. was detected in the month of October, December and January in *L. calcarifer* and *J. belangeri*. *Salmonella* sp. was detected in the month of November and December for all samples of *L. calcarifer* and *J. belangeri*. Presence and absence of *Listeria* sp., *Clostridium* sp. and *Salmonella* sp. in *Labeo rohita*, *Lates calcarifer* and *Johnius belangeri* is as shown in Table 1. *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella* sp., and *Vibrio cholerae* were among the microorganisms recovered from the three commercially edible tuna fish species, i.e., *Euthynnus affinis*, *Katsuwonus pelamis*, and *Auxis thazard* (Patrata and Aluri, 2021). There are many sources of *E. coli* in the environment including water samples, soil and sediments and treated wastewater effluent (Ishii et al., 2006). Contamination of fish products with *L. monocytogenes* depends on many factors such as cleaning and processing procedures, improper handling at the retail level and using of contaminated raw materials (Zarei, 2012). *L. rohita* samples collected from Garia retail fish market were found positive for presence of *Clostridium* sp. and *Listeria* sp. during the month of October and December. *Salmonella* sp. was detected in the month of October, November and December for *L. rohita*. *Clostridium* sp. and *Listeria* sp. was detected in the month of October, November and January in *L. calcarifer* and *J. belangeri*. *Salmonella* sp. was detected in all samples of *L. calcarifer* and *J. belangeri* in the month of November and December. Presence and absence of *Listeria* sp., *Clostridium* sp. and *Salmonella* sp. in *Labeo rohita*, *Lates calcarifer* and *Johnius belangeri* is as shown in Table 2. The presence of

Table 1: Presence and Absence of *Listeria* sp., *Clostridium* sp. and *Salmonella* sp. in *Labeo rohita*, *Lates calcarifer* and *Johnius belangeri* collected from selected wholesale fish market

+ / -	<i>L. rohita</i>			<i>L. calcarifer</i>			<i>J. belangeri</i>		
	<i>Salmonella</i> sp.	<i>Clostridium</i> sp.	<i>Listeria</i> sp.	<i>Salmonella</i> sp.	<i>Clostridium</i> sp.	<i>Listeria</i> sp.	<i>Salmonella</i> sp.	<i>Clostridium</i> sp.	<i>Listeria</i> sp.
Months									
October	+	+	+	-	+	+	-	+	+
November	-	+	+	+	-	-	+	-	-
December	+	-	-	+	+	+	+	+	+
January	-	-	-	-	+	+	-	+	+

* Results are mean of three determinations (n=3) with s.d.; + Presence; -absence

Staphylococcus aureus indicates the unhygienic condition of the fish market. *S. aureus* can be easily transferred to seafood during handling (Zarei et al., 2012). According to Olgunoglu (2012), the fresh fish, fish meal, farmed and imported frozen shrimp can carry *Salmonella* sp., mainly if they are caught from contaminated areas with faecal pollution or processed, packed, stored, distributed under unsanitary conditions.

Table 2: Presence and Absence of *Listeria* sp., *Clostridium* sp. and *Salmonella* sp. in *Labeo rohita*, *Lates calcarifer* and *Johnius belangeri* collected from selected retail fish market

+ / -	<i>L. rohita</i>			<i>L. calcarifer</i>			<i>J. belangeri</i>		
	<i>Salmonella</i> sp.	<i>Clostridium</i> sp.	<i>Listeria</i> sp.	<i>Salmonella</i> sp.	<i>Clostridium</i> sp.	<i>Listeria</i> sp.	<i>Salmonella</i> sp.	<i>Clostridium</i> sp.	<i>Listeria</i> sp.
Months									
October	+	+	+	-	+	+	-	+	+
November	+	-	-	+	+	+	+	+	+
December	+	+	+	+	-	-	+	-	-
January	-	-	-	-	+	+	-	+	+

* Results are mean of three determinations (n=3) with s.d.; + Presence; -absence

Table 3: Monthly changes in Total plate count (log cfu g⁻¹) of selected fishes

Month	Wholesale fish market			Retail fish market		
	<i>L. rohita</i>	<i>L. calcarifer</i>	<i>J. belangeri</i>	<i>L. rohita</i>	<i>L. calcarifer</i>	<i>J. belangeri</i>
October	3.9±0.2	4.1±0.09	4.19±0.05	4.8±0.35	4.4±0.4	4.63±0.14
November	3.6±0.04	3.96±0.06	4.09±0.06	4.6±0.5	4.5±0.3	4.53±0.16
December	3.5±0.04	3.84±0.06	3.89±0.06	3.6±0.45	4.26±0.12	4.38±0.15
January	3.2±0.04	3.48±0.07	3.7±0.06	3.5±0.4	3.97±0.17	4.24±0.13

Table 4: Monthly changes in total plate count (log cfu ml⁻¹) and faecal coliform count (MPN ml⁻¹) of Water, Ice and Swab sample collected from selected wholesale fish market

Month	TPC (log cfu ml ⁻¹)			Faecal coliform count (MPN ml ⁻¹)		
	Water sample	Ice sample	Swab sample (log cfu cm ⁻²)	Water sample	Ice sample	Swab sample
October	4.66±0.05	4.83±0.08	4.51±0.11	11.93±0.4	10.05±0.44	16.52±0.63
November	4.54±0.09	4.9±0.06	4.29±0.1	10.96±0.18	7.36±0.99	13.39±0.84
December	4.25±0.07	4.71±0.08	4.23±0.09	7.65±0.71	6.79±1.32	11.45±1.18
January	4.06±0.11	4.49±0.18	4.11±0.07	5.89±0.48	4.06±0.91	7.95±1.18

Table 5: Monthly changes in Total plate count (log cfu g⁻¹) and Faecal coliform count (MPN ml⁻¹) of Water, Ice and Swab sample collected from selected retail fish market

Month	TPC (log cfu ml ⁻¹)			Faecal coliform count (MPN ml ⁻¹)		
	Water sample	Ice sample	Swab sample (log cfu cm ⁻²)	Water sample	Ice sample	Swab sample
October	5.01±0.21	5.41±0.21	4.66±0.16	13.1±0.7	11.5±0.3	19.8±0.6
November	4.82±0.08	5.22±0.23	4.49±0.19	12.1±0.8	10.8±0.4	15±0.9
December	4.43±0.13	5.05±0.23	4.27±0.03	6.8±0.4	6.1±0.7	12.5±1.1
January	4.27±0.13	4.71±0.09	4.18±0.02	3.8±0.7	4.7±0.6	8.8±0.6

4. CONCLUSION

The muscle of both the fish species collected from two selected fish markets were considered as safe for human consumption. The hygienic status of Garia retail fish market was very poor than Baghajatin wholesale fish market. This might be due to poor sanitation, improper handling and use of contaminated water in ice production. The crates and platforms must be disinfected with 100–200 ppm of chlorine. Quality of water and ice used in Garia retail fish market should be improved immediately.

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