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Comparative Microbiological Assessment of Export Oriented Fishes and Locally Marketed Fishes of Bangladesh

Mst. Khadiza Begum[1], Mohammad Mamun Chowdhury[1*], Wahida Haque[1], Tanzinah Nasrin[2]

[1](*Department of Fisheries, University of Dhaka, Dhaka-1000, Bangladesh*)
[2](*Department of Fisheries (DoF), Fish Inspection & Quality Control (FIQC) Laboratory, Ministry of Fisheries and Livestock, Dhaka-1000, Bangladesh*)
[*](*Corresponding author. E-mail: mmchowdhury@du.ac.bd*)

Abstract: *For this study five species of fish were selected. The selected fish samples were Magur (Clarias batrachus), Mola (Amblyopharyngodon mola), Pabda (Ompok pabda), Rohu (Labeo rohita) and Koi (Anabas testudineus). Fish samples were collected from four fish processing plants, four local fish markets and four super shops. The study revealed that SPC in fish processing plant samples ranged from 2.32×10^4 to 4.95×10^5 cfu/g, TCC from <3 to15 MPN/g and TFC from <3 to 7.4 MPN/g. In samples of local fish markets SPC, TCC, TFC ranged from 2.89×10^5 to 2.98×10^7 cfu/g, 15 to 460 MPN/g, 11 to 240 MPN/g, respectively. In fish samples of super shops SPC, TCC, TFC ranged from 5.96×10^4 to 5.16×10^6 cfu/g, <3 to 240 MPN/g, <3 to 120 MPN/g respectively. Salmonella spp. was detected in most of the samples of local fish markets and few samples of super shops and only two samples of fish processing plant. Vibrio cholerae was absent in all fish samples of fish processing plants and five samples of local fish markets and three samples of super shops were contaminated with this organism. In most of the fish samples, the mean value of SPC, TCC and TFC were significantly different ($p<0.05$) among fish processing plants and local fish markets, fish processing plants and super shops. The study also revealed that most of the isolates of Salmonella spp. and Vibrio cholerae were sensitive to chloramphenicol, tetracycline and erythromycin. From the results, it might be concluded that almost all the fish samples of fish processing plants were of good quality for export and consumption, whereas fish samples of local fish markets were highly contaminated thus reflects the unhygienic condition of the markets and super shop samples were less contaminated but not as good as fish processing plant samples.*

Key words: *Export oriented fishes, Locally marketed fishes, Microbiological assessment.*

I. Introduction

Bangladesh is one of the world's major inland fisheries producers and has a huge water resource all over the country. Production of fish contributes to the livelihoods and employment of millions of people of Bangladesh. It contributes 4.37% of our GDP, 23.37% of agricultural sector and 2.01% to foreign exchange earnings [1].

Fisheries sector is the second largest export earning sector of Bangladesh. Major export items of fish products are raw shrimp, block frozen, Individual Quick Frozen (IQF) shrimp and white fish, Peeled and Undeveined (PUD) and Peeled and Deveined (P&D) block frozen shrimp, dry, salted and dehydrated fish, eel, crab and a little quantity of value added fish and shrimp products. European Union, USA, Japan, Middle East countries, UAE and Gulf States are major countries where fish products exported [2]. To export fishes (processed or frozen) in these countries, exporting countries need to comply with the EU standard and USFDA standard. On July 30, 1997, the EU banned imports of fishery products from Bangladesh after the inspection of seafood processing plants [2]. Currently out of 100 fish processing plants, there are 75 plants which have EU approval [1]. The major goal of the fish processing industries is to provide safe, wholesome and acceptable products to the consumer. Control of microorganisms is essential to meet this goal. The control is partly exerted through processing and preservation techniques that eliminate microorganisms or prevent their growth.

Domestic markets of Bangladesh support demand of fish of country people. In Bangladesh, super shop has a special site for selling of where modern facilities like sufficient ice, better quality water supply, uniform salesmen, electric balance, attractive packaging system etc. are available, whereas, local fish markets have dirty, damp and unhealthy place, unhealthy fish holding baskets, insufficient ice, poor quality water supply, poor display and packaging facilities etc. and this unhealthy conditions encourages microbial contamination from different sources [3]. The bacterial flora on fish reflects the aquatic environment which affects the quality and storage life of fishery products [4]. Bacteriological quality is directly related to the spoilage of fish and can be the cause of food poisoning.

The widespread use of antibiotics in the aquaculture systems and agricultural sectors in Bangladesh may act as the source of antibiotic diffusion into the sediment. The uncontrolled antibiotics will remain in the sediment and an alteration of microflora composition of the sediment and antibiotic resistant bacteria may occur with exerting of selective pressure [5].

This study was aimed for comparative analysis of microbiological assessment of export oriented fishes collected form fish processing plants and fishes collected from local fish markets and super shops and to observe antibiotic susceptibility of pathogenic isolates.

II. Materials and Methods

2.1 Selection of fish samples

For the assessment of microbial status, five types of commercially important fish species were selected because these species have high demand in foreign markets as well as in local markets. The selected fish samples were- Magur (*Clarias batrachus* Linnaeus, 1758), Mola (*Amblyopharyngodon mola* Hamilton-Buchanan, 1822), Pabda (*Ompok pabda* Hamilton-Buchanan, 1822), Rohu (*Labeo rohita* Hamilton-Buchanan, 1822) and Koi (*Anabas testudineus* Bloch, 1795). Fish species were identified according to Rahman [6] and Shafi and Quddus [7].

2.2 Collection of fish samples

Fish samples were collected from four fish processing plants, four local fish markets and four super shops. Export oriented fish samples from processing plants were collected as block frozen & transported to the laboratory using insulated icebox & stored at -18˚ C. Samples from local fish markets and super shops were collected in sterilized plastic bags asceptically in the morning and transported to the laboratory using icebox and then stored at -18˚ C in freezer.

2.3 Microbiological assessments

Bacteriological parameters for examination of fish samples were- Standard Plate Counts (SPC), Total Coliform Counts (TCC), Total Fecal Coliform Counts (TFC) and qualitative analysis of *Salmonella* spp. and *Vibrio cholerae*.

2.4 Preparation of test samples

Fish samples were taken out of the refrigerator & thawed at room temperature. Then samples were cut & 20 g of each sample was blended with 180 ml of sterile bacteriological peptone water in a stomacher blender. Each sample was serially diluted [8].

2.5 Enumeration of SPC

SPC was enumerated according to ISO 4833:2003 [9].

2.6 Enumeration of TCC

TCC was enumerated according to ISO 4831:1991 [10].

2.7 Enumeration of TFC

TFC was enumerated according to ISO 7251:1993 [11].

2.8 Detection of *Salmonella* spp.

Presence of *Salmonella* spp. was detected according to ISO 6579:2002 [12].

2.9 Detection of *Vibrio cholerae*

Presence of *Vibrio cholerae* was detected according to ISO/TS 21872-1:2007 [13].

2.10 Antibiotic Susceptibility Test

In this study the pathogenic isolates were tested against seven antimicrobial agents to observe whether they were sensitive or resistant to those agents. Antibiotic susceptibility test of the isolated organisms was done by disc diffusion method using the Kirby-Bauer technique [14] and as per recommendation of NCCLS [15].

2.11 Statistical Analysis

The mean values of bacterial load were compared using ANOVA followed by Tukey's post hoc test for multiple comparisons.

III. Results and Discussion

Microbiological assessment was conducted on five types of freshwater fishes collected from four fish processing plants, four local fish markets and four super shops. The results are presented in tables 1, 2 and 3. All counts of bacteriological parameters showed great variation from species to species as well as among three different market conditions. According to the guideline of ICMSF acceptable limit of standard plate count (SPC) for white fish is 5×10^5 cfu/g and acceptable limit of total coliform and fecal coliform are 100 MPN/g and <3 MPN/g respectively. *Salmonella* spp. and *Vibrio cholerae* should be absent [16].

Table-1: Bacteriological assessment of five types of fish samples collected from four fish processing plants (FPP).

Sampling sites	Fish sample	SPC (cfu/g)	TCC (MPN/g)	TFC (MPN/g)	*Salmonella* spp.	*Vibrio cholerae*
FPP-1	Magur	4.20×10^4	<3	<3	Absent	Absent
	Mola	6.04×10^4	15	7.4	Absent	Absent
	Pabda	4.95×10^5	3.6	<3	Absent	Absent
	Rohu	9.13×10^4	9.2	<3	Absent	Absent
	Koi	4.70×10^4	<3	<3	Absent	Absent
FPP-2	Magur	2.70×10^5	<3	<3	**Present**	Absent
	Mola	2.32×10^4	<3	<3	Absent	Absent
	Pabda	1.67×10^5	3.6	<3	Absent	Absent
	Rohu	5.75×10^4	<3	<3	**Present**	Absent
	Koi	1.59×10^5	3	<3	Absent	Absent
FPP-3	Magur	3.09×10^4	<3	<3	Absent	Absent
	Mola	2.58×10^5	9.2	<3	Absent	Absent
	Pabda	2.68×10^4	7.4	<3	Absent	Absent
	Rohu	3.00×10^4	9.2	3.6	Absent	Absent
	Koi	3.18×10^5	7.4	<3	Absent	Absent
FPP-4	Magur	5.18×10^4	3.6	<3	Absent	Absent
	Mola	5.36×10^4	3.6	<3	Absent	Absent
	Pabda	6.30×10^4	3.6	<3	Absent	Absent
	Rohu	1.80×10^5	<3	<3	Absent	Absent
	Koi	3.41×10^5	<3	<3	Absent	Absent

In this study, SPC was found to be the highest in Pabda collected from fish processing plant-1 and that was 4.95×10^5 cfu/g, the lowest value was found in Mola sample of processing plant-2. But SPC of all samples were within the acceptable limit. All values of TCC were within the acceptable limit. The highest value of TFC was 7.4 MPN/g which was found in Mola sample of processing plant-1 and the lowest value was <3 MPN/g. *Salmonella* spp. was absent in almost all the samples except Magur and Rohu of processing plant-2. *Vibrio cholerae* was absent in all the samples (Table-1).

Table 2: Bacteriological assessment of five types of fish samples collected from four local fish markets (LFM).

Sampling sites	Fish sample	SPC (cfu/g)	TCC (MPN/g)	TFC (MPN/g)	*Salmonella* spp.	*Vibrio cholerae*
LFM-1	Magur	2.65×10^6	93	43	**Present**	Absent
	Mola	3.12×10^6	150	150	Absent	**Present**
	Pabda	6.38×10^5	75	43	Absent	Absent
	Rohu	5.53×10^6	43	15	**Present**	Absent
	Koi	3.84×10^6	460	240	Absent	**Present**
LFM-2	Magur	1.55×10^7	210	64	Absent	Absent
	Mola	4.56×10^6	120	75	**Present**	Absent
	Pabda	3.52×10^6	460	93	**Present**	Absent
	Rohu	4.76×10^6	93	15	**Present**	Absent
	Koi	8.69×10^5	210	150	**Present**	Absent
LFM-3	Magur	4.07×10^6	240	150	**Present**	Absent
	Mola	2.89×10^5	240	240	**Present**	Absent
	Pabda	6.02×10^5	43	11	Absent	Absent
	Rohu	9.81×10^5	21	21	Absent	**Present**
	Koi	7.03×10^6	120	43	Absent	**Present**
LFM-4	Magur	5.21×10^5	75	75	Absent	Absent
	Mola	6.84×10^5	27	21	**Present**	Absent
	Pabda	3.09×10^5	75	23	**Present**	Absent
	Rohu	6.94×10^6	15	9.2	**Present**	Absent
	Koi	2.98×10^7	210	21	Absent	**Present**

In the samples of local fish markets, the highest value of SPC was 2.89×10^7 cfu/g which was found in Koi sample collected from local fish market-4 and the lowest value was found in Mola sample of local fish

market-2 that was 2.89×10^5 cfu/g. Other samples exceeded the limit of 5×10^5 cfu/g. TCC was highest in Koi fish sample of local fish market-1 and Pabda sample of local fish market-2, that was 460 MPN/g and the lowest 15 MPN/g was found in Rohu sample collected from local fish market-4. The highest TFC was found in Koi and Mola samples of local fish market-1 and local fish market-3 which was 240 MPN/g and the lowest value was 9.2 MPN/g found in Rohu sample of local fish market-4. *Salmonella* spp. was present in most of the samples of all the local fish markets. *Vibrio cholerae* was present in Mola, and Koi samples collected from local fish market-1, Rohu and Koi samples of local fish market-3 and Koi fish samples of local fish market-4 (Table-2).

Table 3: Bacteriological assessment of five types of fish samples collected from four super shops (SS).

Sampling sites	Fish sample	SPC (cfu/g)	TCC (MPN/g)	TFC (MPN/g)	*Salmonella* spp.	*Vibrio cholerae*
SS-1	Magur	2.50×10^5	<3	<3	Absent	Absent
	Mola	3.32×10^5	240	36	**Present**	Absent
	Pabda	3.26×10^5	9.2	3.6	Absent	Absent
	Rohu	2.56×10^5	<3	<3	Absent	Absent
	Koi	2.91×10^5	29	15	**Present**	Absent
SS-2	Magur	3.36×10^5	43	23	Absent	Absent
	Mola	5.70×10^5	120	120	**Present**	Absent
	Pabda	7.81×10^5	27	15	Absent	Absent
	Rohu	4.49×10^5	11	11	**Present**	Absent
	Koi	5.16×10^6	150	23	Absent	**Present**
SS-3	Magur	2.44×10^6	36	29	**Present**	Absent
	Mola	3.23×10^5	75	27	Absent	Absent
	Pabda	5.96×10^4	14	9.2	**Present**	Absent
	Rohu	2.50×10^5	35	21	Absent	Absent
	Koi	1.76×10^6	120	38	Absent	Absent
SS-4	Magur	5.20×10^5	36	21	Absent	Absent
	Mola	5.04×10^5	75	21	**Present**	Absent
	Pabda	4.44×10^5	64	23	Absent	**Present**
	Rohu	3.60×10^6	29	11	**Present**	Absent
	Koi	6.57×10^5	240	11	Absent	**Present**

The highest value of SPC in samples of super shops was 5.16×10^6 cfu/g which was found in Koi sample of super shop-4 and the lowest was 5.96×10^4 cfu/g in Pabda sample collected from super shop-3. TCC was highest in Mola sample of super shop-1 and Koi of super shop-4, which was 120 MPN/g and the lowest value was <3 found in Magur and Rohu samples collected from super shop-1. TFC was highest in Mola sample of super shop-2, which was 120 MPN/g and the lowest value was <3 found in Magur and Rohu samples collected from super shop-1. *Salmonella* spp. was present in Mola and Koi samples of super shop-1, Mola and Rohu samples of super shop-2, magur and Pabda samples of super shop-3 and Mola and Rohu samples of super shop-4. In this study *Vibrio cholerae* was present in Koi sample of super shop-2 and Pabda and Koi samples collected from super shop-4 (Table-3).

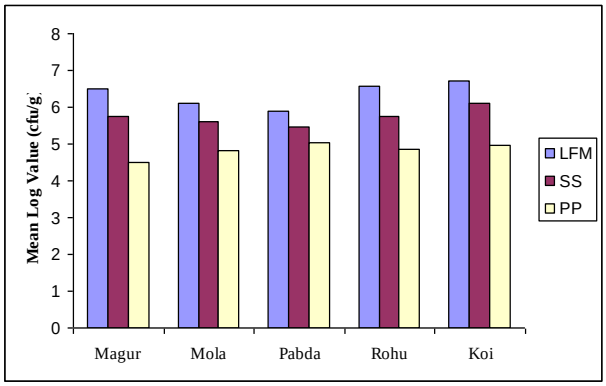

Figure 1: Showing comparative analysis of SPC (mean log value) in fish samples collected from local fish markets (LFM), super shops (SS) and fish processing plants (PP).

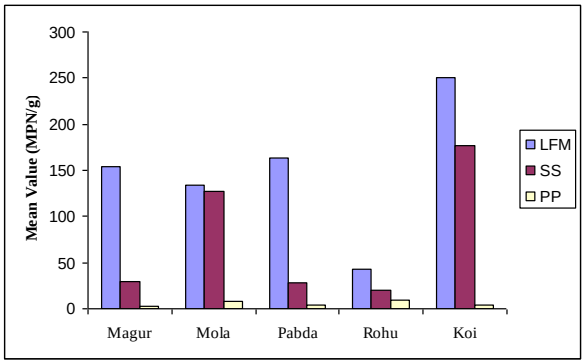

Figure 2: Showing comparative analysis of TCC (mean value) in fish samples collected from local fish markets (LFM), super shops (SS) and fish processing plants (PP).

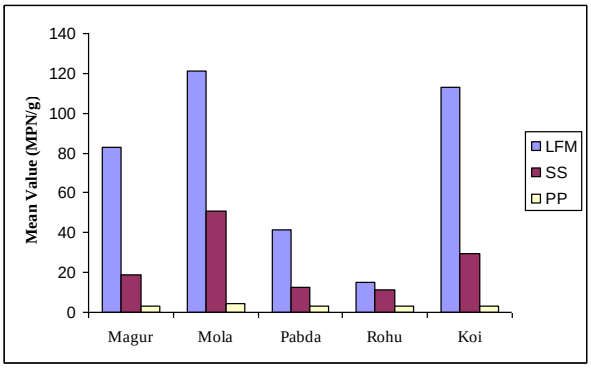

Figure 3: Showing comparative analysis of TFC (mean value) in fish samples collected from local fish markets (LFM), super shops (SS) and fish processing plants (PP).

In this study, the mean value of SPC, TCC and TFC in most of the fish samples were significantly different ($p<0.05$) among fish processing plants and local fish markets, fish processing plants and super shops. But in case of some fish samples there were no statistically significant difference among local fish markets and super shops.

It is obvious that in all types of fish samples collected from local fish markets, SPC, TCC and TFC were higher than super shops and fish processing plants and in all types of fish samples collected from fish processing plants, these values were lowest in comparison with the fish samples collected from super shops and local fish markets (Fig. 1, 2 & 3).

In the present study the export oriented fish samples from different fish processing plants were of good quality. But *Salmonella* spp. was present in Magur and Rohu fish samples collected from processing plant-2. Salmonella is highly pathogenic and this is the major reason for isolation of such bacteria from fish samples. Most of the samples of local fish markets and super shop were contaminated by *Salmonella* spp. The environment acts as main source of this organism in aquaculture products rather than poor standards of hygiene and sanitation. But external contamination may also be the source of the occurrence of these bacteria in fish [17]. As the processing plants follow proper EU guidelines for handling, processing and storage of fish, polluted aquaculture environment might be responsible for the presence of Salmonella spp. According to ICMSF guidelines, other bacteriological parameters were within the acceptable limit. On the other hand, from the result it was found that the samples of local fish markets were highly contaminated as they showed higher counts in all the bacterial parameters, whereas, the bacterial load and occurrence of pathogenic isolates were comparatively lower in super shop samples. From the result it might be said that the processing, handling and storage condition of super shops is better and the quality of fish is better than the local fish markets. It might also indicate better quality preservation, handling and hygiene and sanitary maintenance of super shops than local markets but not as much satisfactory as fish processing plants. This high quality rate of fish processing plants might be the reflection of their compliance with HACCP and EU guidelines which are not followed by any local fish market or super shop.

In most of the samples of local fish markets and super shops, all bacteriological parameters were beyond the acceptable limit. Higher SPC value might be the reflection of inadequate use of ice or cross contamination. The presence of coliform group (E. coli) in higher range suggests contamination of the samples before or during handling, processing and marketing. Lower load of TCC and TFC in samples of super shops

indicates low range of contamination than local fish markets. Fecal coliform is present highly in diarrheal stools of infected persons. So, the unwashed hands of infected food handlers forgetting to wash hands with soap after using the bathroom may also contaminate food [18].

In the present study *Vibrio cholerae* was qualitatively analysed and found in few samples of local fish markets and super shops and was absent in all the samples of fish processing plants. The aquatic environment polluted by external sources can be responsible for this contamination. The handling and processing methods, species variation and also the aquaculture practices in fish farm might be responsible for the reasons of variations in microbial quality. So, proper care should be taken to avoid pollution in culture environment as well as to avoid contamination of fish products during handling, processing and preservation. According to the European Commission Regulation, foodstuffs should not contain microorganisms or their toxins or metabolites in quantities that present an unacceptable risk for human health [19]. The safety of foodstuffs is mainly ensured by a preventive approach, such as implementation of good hygiene practice and application of HACCP principles.

From Table-4 it can be said that most of the isolates of *Salmonella* spp. were resistant to ampicillin and ciprofloxacin, and sensitive to Chloramphenicol, Ceftriazone, Tetracycline, Erythromycin and Trimethoprime-sulfomethoxazole and most of the isolates of *Vibrio cholerae* were resistant to ampicillin, Ceftriazone and Trimethoprime-sulfomethoxazole, and sensitive to Ciprofloxacin, Chloramphenicol, Tetracycline and Erythromycin.

Table-4: Antibiotic susceptibility of pathogenic isolates.

Antibiotics	***Salmonella* spp. n=21**		***Vibrio cholerae* n=8**	
	R(%)	**S(%)**	**R(%)**	**S(%)**
Ampicillin	17(81%)	4(19%)	6(75%)	2(25%)
Ciprofloxacin	12(57.14%)	9(42.86%)	1(12.5%)	7(87.5%)
Chloramphenicol	8(38%)	13(62%)	2(25%)	6(75%)
Ceftriazone	6(28.6%)	15(71.4%)	5(62.5%)	3(37.5%)
Erythromycin	7(33.33%)	14(66.67%)	3(37.5%)	5(62.5%)
Tetracycline	10(47.62%)	11(52.38%)	2(25%)	6(75%)
Trimethoprime-sulfomethoxazole	3(14.3%)	18(85.7%)	8(100%)	0(0%)

n= Number of isolates, R= Resistant, S= Sensitive

The uncontrolled and irregular use of antibacterial agents in aquaculture systems and agricultural sectors is responsible for the occurrence of the multiple antibiotic resistance traits among the fish pathogens [20]. This result suggests that commercially available fish may facilitate the dissemination of the antibiotic resistant bacteria as they are contaminated with some pathogenic strains [21]. For this reason, effective medication might be restricted during fish born disease outbreaks and antibiotic sensitivity might be helpful for effective medication.

IV. Conclusion

From the result it might be concluded that the export oriented fish samples collected from four different fish processing plants were of better quality, whereas the fish samples collected from local fish markets were more contaminated with high bacterial load than those of the super shops due to unhygienic condition of the handling and marketing area. The fish samples of super shops were also contaminated in comparison with fish processing plant samples. Contaminated fish could be the potential reservoirs for transmission of pathogenic bacteria and rapid spoilage of the fishes. So, foodstuffs should not contain microorganisms or their toxins in such quantities that present a risk for human health. It can be recommended that Good Aquaculture Practices, HACCP principles, Good Hygiene Practices, Good Manufacture Practices etc. should be followed to produce better quality fish and fish products and to avoid public health risks due to fish born disease outbreaks.

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