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MICROBIOLOGICAL ASSESSMENT OF SIX TYPES OF SELECTED FISHES COLLECTED FROM FOUR DIFFERENT MARKETS IN DHAKA CITY

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Abstract

Fish is the major source of protein and one of the popular food sources in Bangladesh. The consumption of fish is 14kg/ year for each person in Bangladesh. This study performed to identify the microbial quality of fish, the present study was conducted for microbiological assessment of six selected fishes in different market conditions. For this, Total Viable Bacterial Counts (TVBC), Total Coliform Counts (TCC), Total Fecal Coliform Counts (TFCC) and presence of *Salmonella* and *Vibrio* spp. were determined in Rohu (*Labeo rohita*), Yellowtail catfish *Pangasius pangasius*), Wallago (*Wallago attu*), Striped dwarf catfish (*Mystus vittatus*), Climbing perch (*Anabas testudineus*) and Spotted snakehead (*Channa punctatus*). Microbial load was highest in Rohu and lowest in Tangra . Total coliform count was highest in Wallago and lowest in Striped dwarf catfish. Fecal coliform was also isolated from the locally available fish. The highest and lowest count was found in Spotted snakehead from different market. *Salmonella* spp was identified only in two samples whereas *Shigella* spp was found among most of the samples .*Vibrio* spp was also isolated, *Vibrio cholerae* was found in the samples of Wallago and in Spotted snakehead. *Vibrio parahemolyticus* was found only in one of the samples of Climbing perch. Almost all the values exceeded the ICMF limits (10_2 cfu/g for total coliform and nil for the fecal ones-last).There was a significant reduction in counting after washing the samples. It was observed that there had been maximum three log reduction in case total heterotrophic count (found in Spotted snakehead) and a minimum of one log reduction in all the other cases of sampling. Moreover in times there was total elimination of the indicator or pathogenic organisms after the samples were washed thoroughly and if the load also was not very high. So, this indicates that, if proper cleaning, washing, sanitation or hygiene maintaining facilities is available then the consumers will be able to take safe and quality fish and fishery products.

Key words: Fish, microbiological contamination, Bangladesh, *E. coli*, Total Coliform Counts, *Vibrio cholerae.*

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INTRODUCTION

Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people (Chowdhury & Baqluis, 1997). It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis (Altinok *et al*., 2006). Fish is regarded a healthier meat option due to the high content of Long Chain Polyunsaturated Fatty acids (LCPUFAs), which are associated with improving health and preventing diseases of old age (Shewan,1970). Fisheries sector plays a vital role in the economic development of Bangladesh contributing about 4.92 % of the country's GDP. About 1.28 million fishermen and 3.08 millon fish farmers are associated with this sector for their livelihood, the fish consumption is 14kg/year for each person in Bangladesh where its need average 18kg/year (DoF, 2003). The number of existing fish processing plants in our country is

129 and Bangladesh exported 63,377 metric tons of fishery products in the year 2003-04 (DoF, 2003). Maintenance of appropriate quality of the products is considered vital for achieving desired success in the global trade of this product. Government Fish Inspecting and Quality Control (FIQC) Department therefore has been well equipped with modern laboratory facilities. And Department of Fisheries (DOF) have legal authority to inspect farms dealing with fish business. In Bangladesh, fish are grown mostly under natural environment maintaining the original taste and texture of the products. Both salt water and fresh water fish contain comparatively high levels of proteins and other nitrogenous constituents. The carbohydrate content of fish is nil, while fat content varies from very low to rather high values, depending upon the species (Boyd, 1984). Today more and more people are turning to fish as a healthy alternate of red meat. The low fat content fish species and the effects on coronary heart disease of polyunsaturated fatty acids found in fatty fish species are extremely important aspects for health conscious people, particularly in affluent countries where cardiovas cular disease mortality is high (Rashid & Chowdhury, 1996). Consumption of fish may also cause disease due to infection or intoxication. It is believed to be the reflection of the general contamination in the aquatic environments (Regenstein & Regenstein.1997). It is generally recognized that the internal flesh of healthy live fish is sterile, although a few reports to the contrary exist (Rubbi *et al.*, 1978).The fish contains normal flora which are of varied type. They are usually nonpathogenic and contains about 108/gram. Bacteria that exist on fresh fish are generally found in 3 places: the outer slime, gills and the intestine of the feeding fish. The most susceptible part is the gill region, including the gills itself. Fresh water fish tend to have lower counts than marine species, (Shewan, 1961) .The micro floras of freshwater fish may include most salt water genera, and in addition *Aeromonas, Lactobacillas, Alcaligenes, Streptococcus* and Enterobacteriaceae (Koutsoumanis & Nycha, 2000). Warm water fish often have large numbers of coryneforms and Enterobacteiaceae may even include *Salmonella*. Fresh water fish are more likely to be contaminated by fecal organisms fecal coliforms, *E.coli*, *Enterobacter*, *Citrobacter*, *Aeromonas*, and *Pseudomonas.* Contamination of fishes can carry out in diffresnt stage of transport, processising and handling. Water quality is a big factor for good quality fish, if water that used for fish processing it may contaminant the whole food processing plant.

The true incidence of food-borne disease by fish in Bangladesh yet unknown although present per capita annual fish consumption in Bangladesh stands at about 14 kg/year (DoF 2003). So it's important to find out the quality of fish we consumed. This study was carried out to investigate the microbiological quality of the locally available fresh water fish and characterize several important indicators and pathogenic bacteria.

MATERIALS AND METHODS

All the experiments of this investigation were carried out at the laboratory of the Department of Microbiology, Stamford University Bangladesh from 27 March to 9 May, 2011.

Sample collection

Fish samples such as Rohu (*Labeo rohita*), Yellowtail catfish (*Pangasius pangasius*) ,Wallago (*Wallago attu*), Striped dwarf catfish (*Mystus vittatus*), Climbing perch (*Anabas testudineus*), Spotted snakehead (*Channa punctatus*) were collected from four local markets in Dhaka city: Area A, B, C and D.

Sample preparation

All equipments were aseptically sterile before use. Sterile Normal saline (0.85% NaCl) used for homogenization of the fish samples. 10g of fish of each sample was measured very carefully in the weighing machine and dissolved into 90 ml of normal saline. Each sample was homogenized separately. Strict aseptic technique was followed to avoid any cross contamination. Ten fold serial dilutions of the fish samples were made up to 10-8 in sterile normal saline.

Isolation microbiological culture

In this study culture media used for isolation for different types of bacteria. All media are sterilized by autoclaving at 121°C for15 minutes. MacConkey agar is a differential medium for enterobacteriaceae was used to isolate and enumerate coliform bacteria. XLD medium was used for the selective isolation of *Salmonella* spp and the Enterobacteriaceae. The mFC agar medium was used for the isolation of fecal coliforms. Mannitol Salt Agar (MSA) was used for isolation of *Staphylococcus* spp. Cetrimide for *Pseudomonas* spp. Eosin Methyline Blue (EMB) agar especially selective for *E.coli.* Thiosulfate-Citrate-Bile-Sucrose Agar (TCBS) selective for *Vibrio spp*. The incubation period ranged

from 24 to 48 hours, The MFC plates were incubated at 44.0±0.5 °c for 24 hours. The rest of the inoculated media were incubated at 37.0±0.5 °c. The total bacterial count was performed by standard method (Baron, 1985).

Bacterial Characterization and Identification

Bacterial colonies were observed after 24-48 hours of incubation for their colonial characteristics such as shape, colour, size, edge elevation, transparency and surface texture. Similarly, the isolates were Gram stained to differentiate the organisms into Gram negative and Gram positive by microscopic examination of stained preparation. Biochemical tests were performed according to the methods described in Microbiology Laboratory Manual (Cappuccino *et al*., 1996). Some of the important tests are performed includes Triple Sugar Iron Agar (TSI) Test, Oxidase test, Catalase test, Indole production test, Methyl Red (MR) test, Voges-Proscauer (VP) test, Citrate utilization test and Motality Indole Urea (MIU) tests.

Stock Culture of Pure Bacteria Isolats

Stock culture was preserved in small vials containing a non-selective media having buffering capacity, with sterile paraffin oil and preserved at 24 °c Miniamal media used for the preservation of pure culture for future study.

RESULTS

Total Count of Organisms Found in various selective and differential media Selective media usually allows or encourages the growth of certain types of organism in preference to others. Whereas, differential medium is a solid medium in which different types of organism may be distinguished by their different forms of growth. It was observed that, the Minimal media is a non-selective basal medium, which, without supplement can support the growth of various nutritionally undemanding species. In case of the XLD medium (table 1&2), pink with black centered colonies (suspected as *Salmonella*) were found from Wallago and Striped dwarf catfish before washing the samples. But after washing no colonies were found .Whereas pink colonies (suspected as *Shigella)* were found almost from all samples except Climbing perch. Here also a remarkable lessening of the count was found after cleaning the samples properly.

The range of pink colonies in MaC (probably, *E.coli*) varied from as low as 1 □103 cfu/g to 4.9□105 cfu/g before washing but after washing this counting reduced to 6.1□103 cfu/g and even nil in some of the samples. Whereas, the light pink colonies (probably, *Klebsiella*) was also found in case of Rohu, Striped dwarf catfish and Spotted snakehead(Table 1&2). Among these different types of medium, the TCBS helps to isolate between sucrose fermenting and non-fermenting *Vibrio* Spp (table 1&2) and mFC helps to detect the presence of fecal coliforms (Table 1&2).

Table 1: Bacterial concentration at different types of media before washing the fish sample.

Media	Coloni Color	Bacterial count cfu/gm Fish sample						City market
		Rohu	Yellowtail catfish	Wallago	Striped dwarf catfish	Climbing perch	Spotted snakehead	
Minimal media		$9.4X10^6$	$6X10^6$	NA	$2.9X10^6$	NA	$7.1X10^5$	A
		NA	$5.5X10^6$	$4.6X10^7$	NA	$8.1X10^6$	NA	B
		$5X10^5$	NA	$8.9X10^5$	NA	NA	$3.2X10^7$	C
		NA	NA	NA	$3.5X10^6$	$5.8X10^4$	NA	D
MacConkey Agar	Pink	$2X10^4$	$5X10^3$	NA	$8.2X10^4$	NA	$2X10^3$	A
		NA	$9.9X10^4$	$4.9X10^5$	NA	$2X10^5$	NA	B
		$7X10^3$	NA	$3.4X10^3$	NA	NA	$6X10^4$	C
		NA	NA	NA	$1X10^3$	$7.2X10^3$	NA	D
mFC agar	Blue	$1.4X10^4$	$9.5X10^2$	NA	$5X10^2$	NA	$2.1X10^2$	A
		NA	$3X10^3$	$4.1X10^4$	NA	$8.3X10^5$	NA	B
		$7.1X10^2$	NA	NA	NA	NA	$3.1X10^5$	C
		NA	NA	NA	$3.6X10^4$	NA	NA	D
XLD	Pink	$2.6X10^2$	$5X10^5$	NA	NA	NA	$2X10^4$	A
		NA	NA	$8X10^7$	NA	NA	$4.9X10^5$	C
	Black	NA	NA	NA	$5X10^3$	NA	NA	A
		NA	NA	$9X10^3$	NA	NA	NA	C
TCBS	Yellow	NA	NA	$6X10^5$	NA	NA	NA	B
		NA	NA	NA	NA	NA	$4.6X10^4$	C
	Green	NA	NA	NA	NA	$5X10^5$	NA	B

Keyword: City market (A), (B), (C) and (D) NA- Not available

Table 2: Bacterial concentration at different types of media after washing the fish sample.

Media	Coloni Color	Bacterial count cfu/gm Fish sample						Area
		Rohu	Yellowtail catfish	Wallago	Striped dwarf catfish	Climbing perch	Spotted snakehead	
Minimal media		$6.2X10^5$	$4X10^4$	NA	$2.7X10^5$	NA	$9.2X10^3$	A
		NA	$3.7X10^5$	$3.3X10^6$	NA	$7.0X10^5$	NA	B
		$2X10^3$	NA	$7.2X10^3$	NA	NA	$3.3X10^4$	C
		NA	NA	NA	$6.8X10^3$	$3.9X10^2$	NA	D
MacConkey Agar	Pink	$1.1X10^2$	$3.5X10^2$	NA	$2X10^2$	NA	Nil	A
		NA	$6.1X10^3$	$3 X10^3$	NA	Nil	NA	B
		Nil	NA	Nil	NA	NA	$9.2X10^1$	C
		NA	NA	NA	Nil	$8 X10^2$	NA	D
mFC agar	Blue	$5.2X10^2$	Nil	NA	Nil	NA	Nil	A
		NA	670	$2X10^2$	$2.2X10^2$	$2.2X10^2$	NA	B
		Nil	NA	NA	NA	NA	$2.9X10^3$	C
		NA	NA	NA	5.3	NA	NA	D
XLD	Pink	Nil	6X1	NA	NA	NA	2.5X103	A
		NA	NA	6.5	NA	NA	Nil	C
	Black	NA	NA	NA	Nil	NA	NA	A
		NA	NA	Nil	NA	NA	NA	C
TCBS	Yellow	NA	NA	2.1	NA	NA	NA	B
		NA	NA	NA	NA	NA	8X102	C
	Green	NA	NA	NA	NA	2X1	NA	B

Keyword: City market (A), (B), (C) and (D) NA- Not available

Gram Staining was performed with all the different types of colonies obtained from the selective and differential medium. This helps in a presumptive isolation of the organism suspected. It divides bacterial cells into two major groups, gram positive and gram negative. It is based on the difference in the chemical composition of the bacterial cell walls. The Gram reaction was performed for all the different types of colonies observed. Referring to the Table 3, all the isolates from the different media gave a Gram negative reaction and rod shape, except the isolates from the TCBS, which appeared as comma shape.

Table 3: Gram reaction test of different media culture.

Test Preformed	MAC		mFC	XLD		TCBS	
	Dark pink	Light pink		Pink	Black	Yellow	Green
Gram reaction	Gram negative, short rod	Gram negative, rod	Gram negative, short rod	Gram negative, rod	Gram negative, rod	Gram negative, Coma Shaped	Gram negative, rod

Biochemical test helps to identify the suspected organisms by comparing the results with the identification chart **(Cappuccino, 1999)**. The isolates from MacConkey were identified as Escherichia spp and *Klebsiella* spp as the results of the biochemical tests were similar to that of the identification chart **(Laboratory Manual, Cappuccino 1999)**, e.g., in case of *E.coli* positive results were found in indole and MR reactions, whereas VP reaction, citrate and urease gave negative results and in case of *Klebsiella* Indole, Citrate utilization, Urease showed positive result.

Table 4: Summary of biochemical tests performed

Biochemical tests		**Suspected**						
		E. coli	*Klebsiella*	*E. coli*	*Salmonella sp.*	*Shigella sp*	*Vibrio cholera*	*Vibrio parahemolyticus*
Motility		+	-	+	+	+	+	+
TSI	Slant	A	A	A	A	A	A	A
	Butt	A	A	A	K	A	K	A
	Gas	+	-	-	+	+	-	-
	H2S	-	-	-	+	-	-	-
Catalase test		+	+	+	+	+	+	+
Oxidase test		-	+	-	-	-	-	+
Nitrate		-	+	-	+	+	-	+
Indole		+	-	+	-	+	+	?
MR		+	-	+	+	+	+	+
VP		-	-	-	-	-	-	+
Citrate		-	+	-	+	-	+	+
		M	M	m	X	X	T	T
		Colonies on						

The XLD media gave two different types of colonies, pink and black. These two were suspected to be *Shigella* and *Salmonella* spp, differentiated on the basis of motility, citrate test. The former gave negative result on the contrary to the latter. in Triple Sugar Iron Agar both the slant and butt turned black and the citrate gave a positive result by changing the color of the media to Prussian blue from green.

Vibrio spp was also identified by observing the biochemical tests. It gave positive results in oxidase, citrate, motility tests and utilized sucrose or lactose in the KIA test.

Isolates from maC and mFC, those which showed all the biochemical characteristics of *Escherichia* spp were further confirmed by inoculating in BGLB broth and EMB agar. They produced gas in the former and characteristic metallic sheen in the latter. Afterwards, again the biochemical tests were performed for further confirmation.

DISCUSSION

Fish is a product that proper handling and processing in order to preserve nutrients and its function components that promote good health **(Okonta, 2005)**. This study was carried out to evaluate the microbiological quality of the locally available fresh water fish. Samples were collected from four local markets in Dhaka. Two samples of each type of fish were collected from the markets. This study was attempted to assess the bacterial density of the samples with the limit values established by various international bodies and countries for exporting frozen fish. The effect of proper washing of the samples with the purpose to reduce the total heterotrophic bacterial count as well as the harmful indicators and pathogens also performed in this research project. The microorganisms isolate and identified from the fresh fish sample can be said to be normal flora of the fish **(Ola and Oladipo, 2004)**. By detecting this load on the fish surface it apparently gives an idea about the quality of the sample. Therefore, aerobic or heterotrophic plate gives an idea about the quality of the sample. Therefore, aerobic or heterotrophic plate count was performed. The total bacterial viable count was beyond the acceptable range. The highest load was 4.6×10^7 cfu/ ml and the lowest was 5.0×10^5 cfu/ ml. This poor quality may be due to poor handling, improper storage system, and sanitary condition at all the steps in the fish processing and also that of the storage premises. Indicator organisms as the total coliform and fecal coliform were also found in all samples. Almost all the values exceeded the ICMF limits (**Pelczar *et al.*, 1977**). The highest number of total coliform were 4.9×10^5 and the lowest was 1×10^3. Only two of the samples contained the count within the acceptable limits. The presence of total coliforms suggests contamination of the sample before or during the processing. During processing the contaminated process water may also contribute to the contamination by total coliforms. Even the ice used to freeze the fish may be a source of its contamination **(Schauer, 2007)**. Fecal coliform was isolated from the locally available fish. The highest count of fecal coliform in the locally available fish was 3.1×10^5 while the lowest count was 2.1×10^2, which was all alarming. The presence of fecal coliform denotes fecal contamination of the samples. The water used for the processing may have somehow been contaminated fecally or even the workers in the processing plant may contribute to the source. Moreover, the presence of total and fecal coliforms indicates the presence of other harmful pathogenic bacteria as *Shigella*, *Salmonella*, and *Vibrio*. Total and fecal coliforms are also named as indicator organisms, whose presence denotes the presence of other pathogens that are harmful for the human. Therefore, further study was carried out to isolate and identify the pathogens. For the isolation of *Salmonella* and *Vibrio* from the natural environment requires a pre-enrichment step, which was performed; that was followed by selective growth on XLD and TCBS respectively. In study to facilitate the growth of vibrios, alkaline peptone water (APW) was used. A pre-

enrichment period for 4-6 hours was done at 37 ^{0}c. In case of *Salmonella* it was grown on selenite broth first for 4 to 6 hours. Later the enriched samples were plated on the TCBS (vibrios) and XLD (salmonellae). For the isolation of *Salmonella* spp and *Shigella* spp, XLD media was used. These two gave distinctive colonies in this media. The former usually gives a black colony by precipitating hydrogen sulfide, whereas the latter appear as pink color due to their inability to ferment lactose. *Salmonella* was identified only in two of the tested samples (Wallago and Striped dwarf catfish). The locally available sample contained a count of 9×10^3, while the lowest count was 3×10^3 whereas *Shigella* was found among most of the samples. The highest count was 8×10^7 and the lowest was 2.6×10^3 this contamination may have occurred from the workers handling the fish or even from the water used for the processing purposes. *Vibrio* spp was also isolated, that gave yellow colonies on the TCBS agar due to their ability to ferment sucrose. It was found in the samples of Wallago and Spotted snakehead. The yellow colony is a distinctive feature of *V. cholerae* and green colonies indicated the presence of the species of *Vibrio* other than *V. cholerae*. It was found only in one of the samples of Climbing perch.

CONCLUSION

This study revealed that the fish that found in local market are highly contaminated. Indicator organism levels are alarming. *Vibrio* and *Shigella* spp, were assumed to be found from most of the samples whereas *Salmonella* were found in few, these all can be a concern in the health hazard. From the study it was revealed that almost all the sellers were neglecting about the quality of the locally available fresh water fish. This reflects the lack of proper knowledge and carelessness. To overcome this situation, microbiological quality control should receive priority status of research and development.

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