

Microbiological Quality Assessment of Cultured Fish Samples Collected from Ponds and Different Local Markets of Bangladesh

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ABSTRACT: The bacteriological quality of Pangas (*Pangasianodon hypophthalmus*), Mrigal (*Cirrhinus cirrhosus*), Carfu (*Cyprinus carpio*) of cultured ponds and different wholesale and retail markets of Dhaka city were analysed. Microbial load was highest in local retail markets samples and was lowest in cultured ponds samples. In total 27 samples were considered for analysis and they differ from species to species and with environment. Total Viable Bacterial Counts ranged from 4.32×10^5 to 5.92×10^6 cfu/g. Total Coliform count was always larger than 240 MPN/g. Total Pseudomonas and Staphylococcus spp. ranged from 1.19×10^3 to 3.28×10^5 and 1.14×10^2 to 6.56×10^3 respectively. Salmonella spp. were absent in all cultured pond fish samples and were present in all wholesale and local retail market samples. Overall, the present study reveals that three species of the fishes under this study were contaminated with different types of bacteria and cultured pond fish samples were comparatively good in quality.

KEYWORDS: Microbiological, Pangas, Mrigal, Carfu, Bangladesh

I. INTRODUCTION

Fish play a significant role in the Bangladeshi diet, providing more than 60% of animal source food, representing a crucial source of micro-nutrients, and possessing an extremely strong cultural attachment [1]. The total production of fish in Bangladesh was 3410254 Metric ton in 2012-13 [2]. Bangladesh where malnutrition remains a significant development challenge, Fish is an irreplaceable diet for the people [3]. Unfortunately, a large amount of fish spoils every year in the country due to the growth and activity of pathogenic microorganisms [4]. The quality of fish mainly depends on the time between the harvesting and processing periods. At the time of being captured, fresh fish contain on their bodies a wide variety of microorganisms, also known as the microflora of the fish [5]. Fish or frozen fish may be contaminated with different types of bacteria, such as Salmonella, coliform, fecal coliform, streptococci and Staphylococcus aureus, and these are responsible for causing different food borne diseases [6]. Microbial hazards causing infections are poor health are closely related to food safety concerning with animal proteins derived from marketed food such as fish. This creates a burning question for all consumers with a high risk commodity with regard

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to pathogenic bacterial contaminations alarming to food safety challenge [7]. Pangas (*Pangasianodon hypophthalmus*), Mrigal (*Cirrhinus cirrhosus*), Carfu (*Cyprinus carpio*) are very common fishes in Bangladesh and many people consume these species of fishes regularly in Bangladesh. So, the microbiological qualities of these species are important to consumers as well as fish processors and public health authorities.

So, the present investigation was carried out to determine the bacteriological status of the Pangas, Mrigal and Carfu that we consumed regularly.

II. MATERIALS AND METHODS

We collected fish samples from 3 different cultured ponds of Comilla, 3 wholesale market of Dhaka city, and 3 retail local market of Dhaka city. We took a sterile aseptic container together with ice to maintain the temperature (4°C to 6°C) and stored at -20°C at the laboratory until use. We maintain ICMSF [8] rules for sampling fishes. All of the samples were carried out to the laboratory for microbiological investigations. The analysis was performed to the laboratory immediately after sample reach. Fish was cut into small pieces and we take skin and muscle parts of the fish for analysis. All samples were analysed in duplicate. The microbiological parameters- Total bacterial count (TBC), Total Coliforms (TC), Salmonella, *Staphylococcus aureus* and Total Pseudomonas count were carried out according to (APHA, 2001)[9].

Microbiological Analysis:

Total Viable Bacterial Count:

Total Viable Bacterial Count was enumerated by pour plate method on plate count agar medium. The plates were incubated at 37°C for 24 hours. Total bacterial count was measured in colony forming unit per gram (cfu/g).

Total Coliforms (TC):

Coliform count was determined by using MacConkey agar. Typical pink colonies were counted for Total Coliform after incubation of plates at 37°C for 24 hours.

Total Pseudomonas:

Total Pseudomonas count was enumerated by pour plate method on pseudomonas agar medium. The plates were incubated at 37°C for 24 hours and measured in colony forming unit per gram (cfu/g).

Staphylococcus aureus:

0.2ml samples were spread into Baird Parker Agar (BPA) medium and incubated for 24 hours at 37°C. Hollow zone surrounded colonies were counted as *Staphylococcus aureus*.

Salmonella:

25g of sample was enriched in 225g Lactose Broth medium. This medium was incubated at 37°C for 24 hours. After 24 hours, a loopful growth of this medium was enriched in selenite broth and incubated again 24 hours incubation at 37°C. Then a loopful growth of this medium was streaked to Bismuth Sulphite Agar (BSA) plate and incubated for 24 hours at 37°C. Typical black centered colonies were counted for salmonella.

III. RESULTS AND DISCUSSIONS

We studied following bacteriological quality analysis of these three species of fish samples-Total Viable Bacterial Counts(TVBC),Total Coliform Counts(TCC),Total Pseudomonas Counts(TPC), *E. coli*, *Staphylococcus aureus*, *Salmonella spp.* The Study reveals that bacterial count was higher in local retail markets fish samples and was lower in ponds fish samples.

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Table1: Bacteriological quality assessments of fish samples collected from cultured ponds

Sampling Sites	Sample (Skin-and-muscle)	Microbial Count					Occurrence
		*TVBC cfu/g	**TCC MPN/g	E. coli MPN/g	***TPC cfu/g	Staphylococcus spp. (cfu/g)	Salmonella /25g
Pond-1	Pangas	3.14X10 ⁵	>240	0.7	1.19x10 ³	2.03x10 ²	Absent
	Mrigal	3.61x10 ⁵	>240	0.9	2.38x10 ³	2.10x10 ²	Absent
	Carfu	3.31x10 ⁵	>240	0.9	2.88x10 ³	2.31x10 ²	Absent
Pond-2	Pangas	2.97X10 ⁵	>240	0.9	2.31x10 ³	2.37x10 ²	Absent
	Mrigal	3.60x10 ⁵	>240	0.7	2.94x10 ³	2.72x10 ²	Absent
	Carfu	3.91x10 ⁵	>240	0.9	3.11x10 ³	2.54x10 ²	Absent
Pond-3	Pangas	3.55X10 ⁵	>240	0.4	2.18x10 ⁴	1.14x10 ²	Absent
	Mrigal	4.22x10 ⁵	>240	0.7	3.10x10 ⁴	1.84x10 ²	Absent
	Carfu	4.62x10 ⁵	>240	0.7	2.40x10 ⁴	2.66x10 ²	Absent

*Total Viable Bacterial Counts(TVBC),**Total ColiformCounts(TCC),***Total *Pseudomonas* Counts(TPC)

From this study, it shows that among the 9 samples of different ponds of Comilla, the value of Total Viable Bacterial counts TVBC ranging from 2.97X10⁵ to 4.62x10⁵. It complies with the International Commission on Microbiology Specifications for Foods [10] limit value of 5x10⁵cfu/g. The highest value of bacterial presence was shown in Pond-3 Carfu samples and lowest value was shown in Pond-2 Pangas sample. All samples contain Total *Coliform* and the value is always <240 MPN/g. *E. coli* also presents in all samples and the value difference is from 0.4 MPN/g to 0.9 MPN/g. Total *Pseudomonas* and *Staphylococcus spp.* are contained in all 9 samples of ponds ranging from 1.19x10³ to 3.11x10³ and 1.14x10² to 2.72x10² respectively. All 9 samples of ponds do not contain *Salmonella spp.*

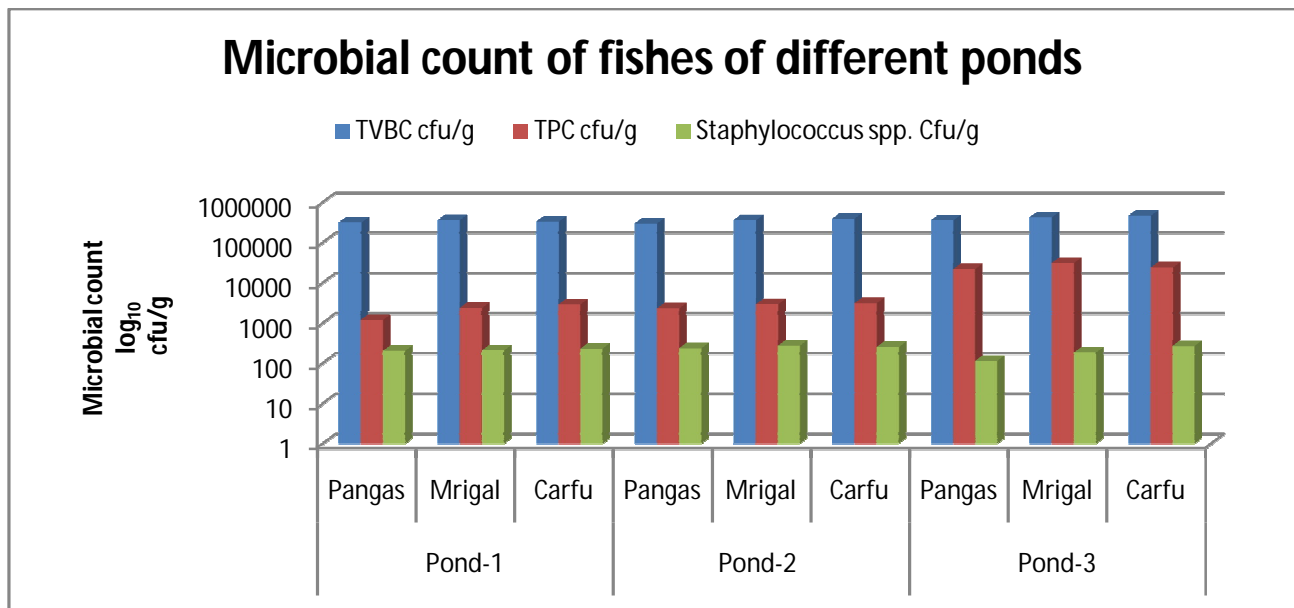


Fig-1:Microbial count of fish samples collected from cultured ponds

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Fig-1 shows log₁₀ base microbial count of different fishes of cultures ponds. It shows that fishes from pond-3 have comparatively high microbial load than other two ponds. Carfu of Pond-3 have highest value of TBVC and lowest in Pangas of pond-2. Other bacterial counts also varied with different species and ponds.

Table 2: Bacteriological quality assessments of fish samples collected from Wholesale Market

Sampling sites	Sample (skin-and-muscle)	Microbial Count					Occurrence Salmonella spp. (cfu/g)
		*TVBC cfu/g	**TCC MPN/g	E coli MPN/g	**TPC cfu/g	Staphylo-Coccus spp. (cfu/g)	
Wholesale Market -1	Pangas	6.50X10 ⁵	>240	2.3	3.78x10 ⁴	1.30x10 ³	positive
	Mrigal	5.32x10 ⁵	>240	2.3	3.21x10 ⁴	2.63x10 ²	positive
	Carfu	6.10x10 ⁵	>240	2.3	4.63x10 ³	3.30x10 ³	positive
Wholesale Market-2	Pangas	7.13X10 ⁵	>240	2.8	1.77x10 ⁴	1.29x10 ³	positive
	Mrigal	6.26x10 ⁵	>240	2.3	1.33x10 ⁴	5.64x10 ²	positive
	Carfu	4.32x10 ⁵	>240	2.3	3.27x10 ³	1.18x10 ³	positive
Wholesale Market-3	Pangas	4.58X10 ⁵	>240	1.5	2.64x10 ⁴	7.26x10 ²	positive
	Mrigal	5.22x10 ⁵	>240	0.9	1.32x10 ⁵	3.82x10 ³	positive
	Carfu	7.45x10 ⁵	>240	1.5	1.08x10 ⁵	3.68x10 ³	positive

**Total Viable Bacterial Counts(TVBC), **Total ColiformCounts(TCC), **Total *Pseudomonas* Counts(TPC)

According to the results among 9 samples of wholesale market samples, highest value of TVBC found in Wholesale Market-3 Carfu 7.45x10⁵ and lowest value detect in Wholesale Market-2 Carfu 4.32x10⁵. In all the cases Total Coliform was every time >240 MPN/g. E. coli varies from 0.9 to 2.8 MPN/g. In all the samples of Wholesale markets Total Pseudomonas was present and the value ranges from 4.63x10³ to 1.08x10⁵. The highest value of Staphylococcus spp. found in Wholesale Market-2 Mrigal 5.64x10² and lowest value detect in Wholesale Market-2 Carfu 1.18x10³. Salmonella detect in all the samples that were collected from wholesale markets.

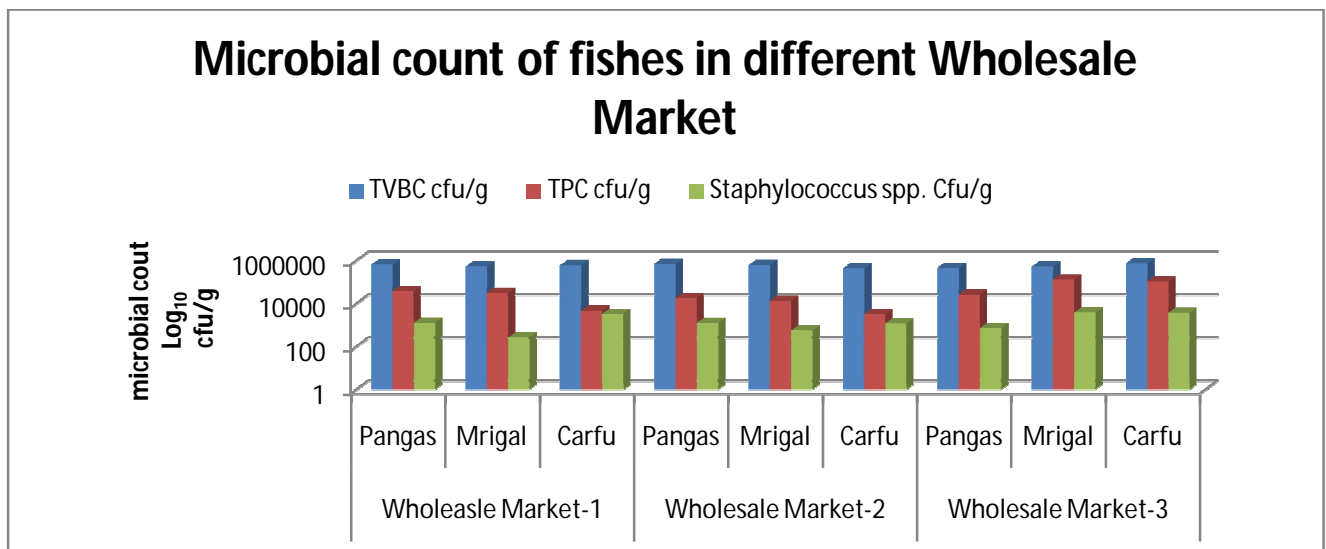


Fig-2: Microbial count of fish samples collected from Wholesale Market

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Fig-2 shows log₁₀ base microbial count of different fishes of Wholesale market. It shows that fishes from Wholesale market-3 have comparatively high microbial load than other two wholesale market. Carfu of Pond-3 have highest value of TBVC and lowest in Mrigal of pond-2. Bacterial counts also varied with different species and wholesale market.

Table 3: Bacteriological quality assessments of fish sample from Local Retail Market

Sampling sites	Sample (skin-and-muscle)	Microbial Count					Occurrence Salmonella spp. (cfu/g)
		*TVBC cfu/g	**TCC cfu/g	E coli MPN/g	***TPC cfu/g	Staphylo coccus spp. (cfu/g)	
Local Retail Market -1	Pangas	2.39X10 ⁶	>240	2.3	1.31x10 ⁴	3.66x10 ³	positive
	Mrigal	3.06x10 ⁶	>240	1.5	2.01x10 ⁴	4.26x10 ³	positive
	Carfu	3.52x10 ⁶	>240	2.8	2.35x10 ⁵	6.56x10 ³	positive
Local Retail Market -2	Pangas	3.66X10 ⁶	>240	2.3	1.42x10 ⁴	2.29x10 ³	positive
	Mrigal	3.92x10 ⁶	>240	2.8	1.02x10 ⁵	2.98x10 ³	positive
	Carfu	4.12x10 ⁶	>240	1.5	1.22x10 ⁵	3.17x10 ³	positive
Local Retail Market-3	Pangas	5.60X10 ⁶	>240	2.3	1.33x10 ⁵	2.25x10 ³	positive
	Mrigal	4.71x10 ⁶	>240	2.3	3.28x10 ⁵	4.29x10 ³	positive
	Carfu	5.92x10 ⁶	>240	2.3	3.01x10 ⁵	3.20x10 ³	Positive

Total Viable Bacterial Counts(TVBC), **Total ColiformCounts(TCC),*Total Pseudomonas Counts (TPC)

This experiment shows among 9 Local Retail Market samples Pangas sample of Local Retail Market-1 defines lowest TVBC count 2.39X10⁶ and Local Retail Market-3 Carfu shows highest TVBC count 5.92x10⁶. Every sample has Total Coliform Count which was >240MPN/g. E coli was present in all the samples of local retail market varied between 1.5-2.8. The value of Total Pseudomons Count and Stapylococcus spp. count were differed from 1.02x10⁵ to 3.28x10⁵ and 2.25x10³ to 4.29x10³ correspondingly. In this case all the samples contain Salmonella.

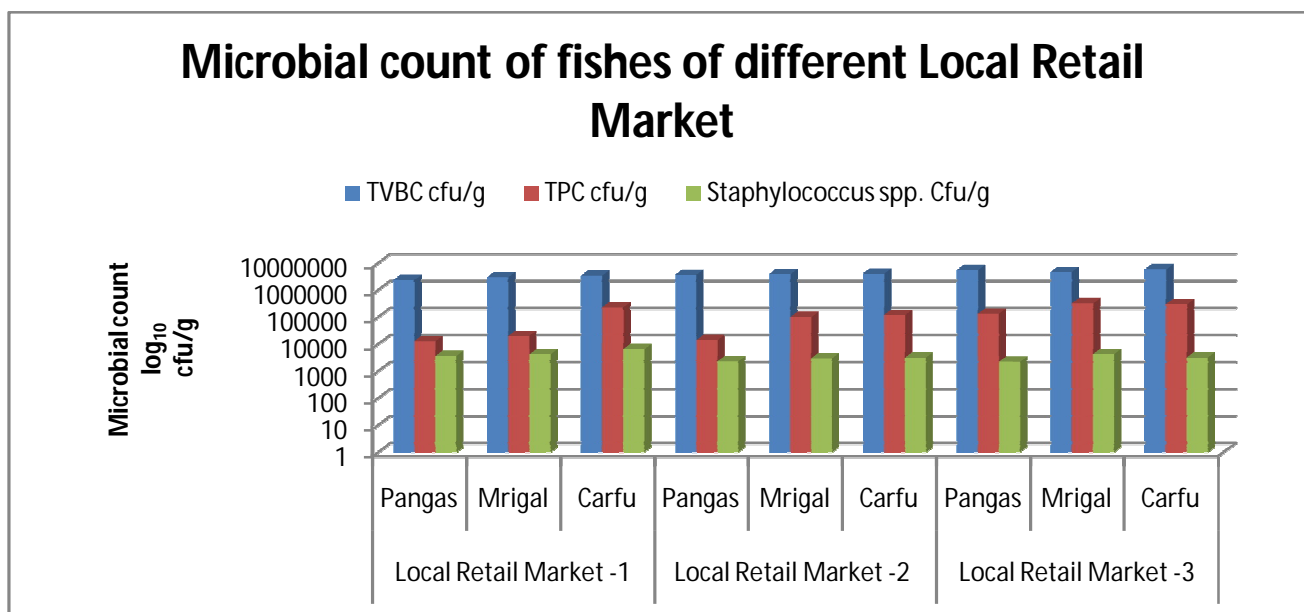


Fig-3: Microbial count of fish samples collected from Local Retail Markets

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Fig-3 shows log₁₀ base microbial count of different fishes of Local Retail Markets. It shows that fishes from local retail markets-3 have comparatively high microbial load than other two wholesale market. Carfu of Pond-3 have highest value of TBVC and lowest in pangas of pond-1. In this case Bacterial counts also varied with different species and different local retail markets.

IV. CONCLUSION

The study provides a clear perspective of the bacterial profusion in ponds and different markets of Bangladesh of these three species. We can see that fish samples from local retail markets are more contaminated with bacterial than those of ponds samples. This may be due to rough handling and sorting or lack of sanitation. So we have to maintain proper handling and transportation system by giving proper training on the aspect to avoid the health risks and cross contamination.

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