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Studies on the quality of fresh water and marine fish sold in and around Greater Hyderabad Municipal Corporation

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ABSTRACT

Physico-chemical and micro-biological quality assessment of some selected fresh water and marine fish varieties available in Greater Hyderabad Municipal Corporation (GHMC), Telangana, India Study was undertaken on few varieties of freshwater (catla, rohu, tilapia and murrel) and marine (mackerel, tuna, pomfret and hilsa) fishes sold at retail outlets of GHMC, Telangana for their freshness, some basic physico-chemical characteristics, microbial load and to know the incidence and load of critical food borne pathogens of the listed fish. The sensory analysis (E – for excellent freshness) showed that 100% murrel & 100% tilapia fishes are E-Grade, then the sequence of occurrence for E-Grade in Catla & Rohu Fishes are 83.33 & 66.66 respectively. Marine fishes are all falls A or B grade for the above parameter, no E grade fishes are reported for these categories. Mean values for pH ranged between 6.65-6.84 (fresh water fishes) and 5.84-6.37 (marine fishes). The WHC varied from 74.79 to 75.33 in freshwater fishes and 70.74 to 70.82 in marine fishes. ERV values ranges from 28.90-29.73 in freshwater fishes and 23.43-23.93 in marine fishes. The fish muscle lipid content TBA values ranges between 0.94 to 1.01 and 1.05 to 1.55 in fresh and marine fishes respectively. PV and FFA was absent in freshwater fishes while they were present in marine fishes. However, TVC, TCC and FCC were considerably higher in freshwater fishes while yeast and mould count in marine fishes. Presence of some critical pathogens such as *Escherichia coli* was reported to be maximum (83.33%) in tilapia Fish whereas marine fishes exhibited higher prevalence (83.33%) for *Vibrio* and other spp. Such as *Pseudomonas* & *Aeromonas* were found more common in marine fishes than in freshwater. All the fish varieties used in this study were found reasonably fit with good freshness and physico-chemical qualities except higher microbial load observed at most of the samples, suggesting better and careful storage and handling of these fishes so that the safety for the end consumers will improve and to make it free of various contaminations.

Keywords: Fish quality, Freshness, Physicochemical quality, Microbial contamination, Pathogens, GHMC, Freshwater fish, Marine fish

Introduction

Fish constitute an essential source of first class protein, essential fatty acids, vitamins, mineral elements and other micronutrients. Growing awareness of healthy diet is enabling increased consumer acceptance and demand for fish and fishery products. India is one among the largest fish-producing countries of world with contribution of fisheries to food security, employment, economic prosperity and trade. Despite its importance, fish spoil much more rapidly than many other food items derived from animal protein source, due to its high moisture content, relatively high water activity, slightly alkaline near neutral pH, presence of abundant endogenous enzymes and nutrient-rich muscle that furnish ample conditions suitable for deteriorative biochemical and microbiological processes after catching. Therefore the factor of freshness in fish quality is of paramount important. Degradation processes include the breakdown of lipids (oxidation) and proteins (autolysis, degradation) by endogenous enzymes and the spoilage by microorganisms that grow on fish meat post-mortem. These factors have the tendency of deteriorating the sensorial properties, nutritional value and hence market life and food safety. Contamination of fish occurs at various stages in the supply chain such as landing, transportation, processing, handling, icing, storage and retail display. Maintaining good sanitation practices and appropriate temperature management helps limiting the microbial spoilage and the growth of food-borne pathogens. Key food-borne microorganisms that are often implicated in fish and fishery products include *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp., *Clostridium* spp. And *Shigella* spp. The presence of these organisms in fish may be the indicator of unsanitary food handling practices, Public health risks. Psychochemical parameters including pH, water holding capacity, E.R.V, (extract released volume), T.B.A (thiobarbituric acid), peroxide value and

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FFA (free fatty acids) indicate fresh quality, protein functional properties, oxidation of lipids and thus deteriorate. Microbiological parameters which helps in determining hygienic and microbiological quality included: Total viable bacterial count, total coliform count, faecal The Greater Hyderabad Municipal Corporation has fish from various freshwater and marine environments to meet consumer demand in a densely populated area of millions of individuals. Variations in the handling and storage procedure coliform count, yeast and mould count.res practiced in retail markets may have the potential to affect its quality and safety attributes. So this study was conducted to analyze the physicochemical and microbiological quality of certain commercially available freshwater and marine fish fillets available in the fish markets of Greater Hyderabad Municipal Corporation, Telangana state and also the incidence and load of some important foodborne pathogens.

Materials and methods

Sample collection

A total of eight fish varieties were selected for the study, comprising four freshwater species catla, rohu, tilapia, and murrel and four marine species mackerel, tuna, pomfret, and hilsa. Samples were collected from retail fish markets located within the Greater Hyderabad Municipal Corporation, including Gudimalkapur, Budwel, and Ramnagar.

Table 1. Details of sample collection

Sl No	Fish sample (common name)	Fish sample (scientific name)	Habitat
1	Catla	<i>Catla catla</i>	Fresh water
2	Rohu	<i>Labeo rohita</i>	
3	Tilapia	<i>Oreochromis niloticus</i>	
4	Murrel	<i>Channa striata</i>	
5	Mackerel	<i>Rastrelliger kanagurta</i>	Marine water
6	Tuna	<i>Thunnus albacares</i>	
7	Pomfret	<i>Parastromateus niger</i>	
8	Hilsa	<i>Tenualosa ilisha</i>	

Samples were collected aseptically during the early morning in sterile polyethylene bags and transported under chilled conditions in insulated ice boxes to the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Rajendranagar, Hyderabad. Samples were analysed immediately after arrival.

Determination of freshness and physicochemical quality

Freshness of the samples was studied using an EU freshness grading scale developed by the European Union, EU (1996) [1]. Samples were graded using organoleptic qualities into three grades of freshness, namely: Extra-E (good quality); A-Good; B-Satisfactory; and-Unsuitable.

The physicochemical quality of the samples

The physicochemical quality indices were determined using pH, WHC, ERV and the level of lipid oxidation of the fish. Water holding capacity (WHC), extract release volume (ERV) were determined using standards methods according to FSSAI Manual (2012) [2], a level which has been established to indicate optimum shelf life, The TBA

level was determined according to Witte *et al.* (1970) [3], expressed as mg malondialdehyde (MDA)/kg of fish. The peroxide value (PV) and free fatty acids (FFA) were estimated using standard methods described in FSSAI and Koniecko.

Microbiological quality determination

Standard methods of WGU (2014) were used for the estimation of total viable count (TVC), total coliform count (TCC), faecal coliform count (FCC) and Total Yeast & Mould count (TYMC). Fish samples were screened for presence and loads of selected food borne pathogens like *E. coli*, *Salmonella* spp., *Clostridium* spp., *S. aureus*, *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp and *Shigella* spp.

Isolation of pathogenic organisms

Selective Isolation of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Clostridium* spp., *Vibrio* spp., *Shigella* spp., *Aeromonas* spp. And *Pseudomonas* spp was performed from the fish samples by BAM (1998) [4]. *Staphylococcus aureus* spp; The inoculum were plated on Voges Johnson agar and incubated at 37C overnight for the appearance of black colonies. These colonies are typical of *S.aureus* and result was recorded as log CFU/g. *Escherichia coli* The inocula (10-fold dilution) were spread on Levine's EMB agar and incubated at 37C. Bluish-purple colonies with green metallic shine were designated as *E. coli*; *Salmonella* spp. The samples were first pre-enriched in buffered peptone water, enriched in tetrathionate broth and sub-plated on XLD agar. Presumptive *Salmonella* colony are usually pink with or without black center; *Clostridium* spp. The sample were applied on SPS selective medium and incubated at 37C under anaerobic condition to obtain Black cotton-wool type colony (*Clostridium* spp); *Vibrio* spp; The samples were directly enriched in Alkaline peptone water, then plated on TCBS agar and characteristic large yellow and green colonies were selected for further biochemical characterization ;*Shigella* spp. The selected samples were pre-enriched in *Shigella* broth then directly inoculated on *Salmonella-Shigella* agar. Colourless colony was picked; *Aeromonas* spp. The enrichment of Samples was performed in Tryptic Soy Broth. The diluted inoculum were spread on A.hydrophila and P.aeruginosa Selective medium A.hydrophila and P.aeruginosa selective media. Translucent colonies of A.hydrophila and blue-green colonies of P.aeruginosa were presumptively considered positive.

Identification and characterisation of isolates

All the presumptives isolates obtained on the selective media were identified on the basis of morphology, Gram reaction and bio-chemical characteristics by referring to the schemes of Cowan and Steel (1993) [5]. The following bio-chemical tests viz. Methyl red, Voges-Proskauer (VP), indole, citrate utilization, nitrate reduction, oxidase and catalase, motility tests and 3% NaCl tolerance test were conducted to confirm the isolates and additional testes like coagulase and DNase test was perform for confirmation of *S. aureus*, Congo red binding and haemolysis on 5% sheep blood agar was performed to confirm *E. coli*.

Results

Freshness assessment

Freshness assessment showed clear differences between freshwater and marine fish. All murrel and tilapia samples were classified as E-grade (100%), indicating excellent freshness. *Catla* samples comprised 83.33% E-grade and 16.66% A-grade, whereas rohu showed 66.66% E-grade and 33.33% A-grade.

Marine fish were predominantly classified as A- and B-grade. Mackerel, tuna, and pomfret each showed 50% A-grade and 50% B-grade samples. Hilsa showed 66.66% A-grade and 33.33% B-grade samples. No marine fish sample was classified as E-grade.

Table 2. Results of Freshness of Fish

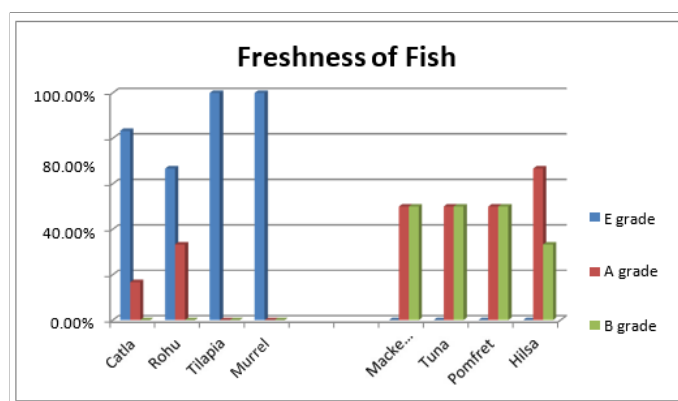
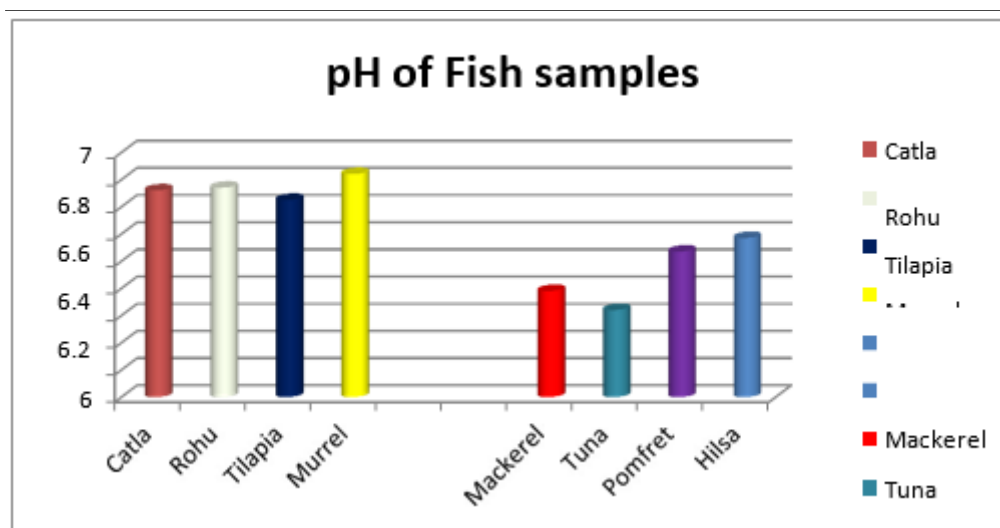
Sl No	Source	Fish sample	E grade	A grade	B grade
1	Fresh Water	Catla	83.33%	16.66%	
2		Rohu		33.33%	
3		Tilapia	100%		
4		Murrel	100%		
5	Marine water	Mackerel		50%	50%
6		Tuna		50%	50%
7		Pomfret		50%	50%
8		Hilsa		66.66%	33.33%

Freshness loss is primarily associated with endogenous biochemical changes in fish muscle and generally precedes microbial spoilage (Ehira *et al.*, 1987) [6]. The comparatively lower freshness grades observed in marine fish may be associated with differences in handling, transportation, storage duration, and temperature conditions. Prolonged holding and high ambient temperatures are known to accelerate fish quality deterioration.

pH

Freshwater fish showed higher pH values than marine fish. The mean pH of freshwater fish ranged from 6.65 ± 0.04 to 6.84 ± 0.03 , while marine fish ranged from 5.84 ± 0.04 to 6.37 ± 0.03 . Among freshwater species, murrel recorded the highest pH (6.84 ± 0.03), followed by rohu (6.74), catla (6.72), and tilapia (6.65). Among marine fish, hilsa showed the highest pH (6.37 ± 0.03), followed by pomfret (6.27), mackerel (5.98), and tuna (5.84 ± 0.04).

The observed differences may be associated with species, muscle composition, season, diet, physical activity, and stress during capture, and other physiological factors. The values obtained were generally comparable with earlier reports.

**Figure 1.** Freshness of milk samples collected**Figure 2.** pH of fish samples collected

Water-holding capacity

Water-holding capacity was significantly higher in freshwater fish than marine fish. Freshwater fish showed WHC values ranging from $74.79 \pm 0.01\%$ to $75.33 \pm 0.13\%$, while marine fish ranged from $70.74 \pm 0.01\%$ to $70.82 \pm 0.02\%$. Murrel recorded the highest WHC ($75.33 \pm 0.13\%$) among all species, whereas tuna recorded the lowest ($70.74 \pm 0.01\%$). Among marine fish, hilsa had the highest WHC

(70.82%), followed by pomfret (70.80%), mackerel (70.79%), and tuna (70.74%).

Reduced WHC may be associated with changes in myofibrillar protein functionality, which plays an important role in water retention. Loss of WHC may also contribute to changes in fish texture during deterioration.

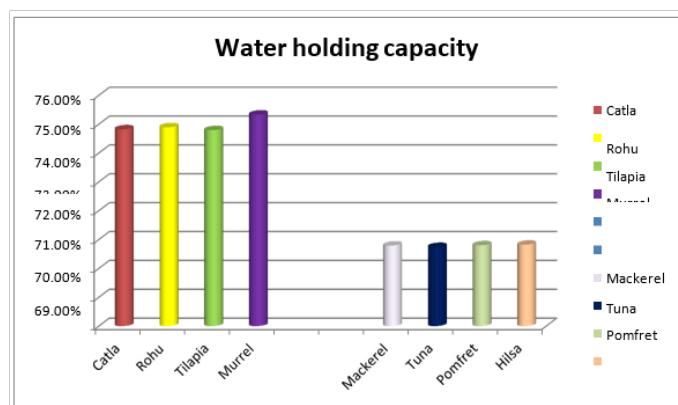


Figure 3. WHC of fish samples collected

Extract release volume

ERV, which can be used as an indicator of incipient spoilage, was higher in freshwater fish than marine fish. Freshwater fish showed values ranging from 28.90 ± 0.23 to 29.73 ± 0.24 mL, while marine fish ranged from 23.43 ± 0.35 to 23.93 ± 0.38 mL. Murrel showed the highest ERV (29.73 ± 0.24 mL), followed by catla (29.67 mL), tilapia (29.36 mL), and rohu (28.90 mL). Among marine fish, pomfret recorded the highest value (23.93 ± 0.38 mL), followed by hilsa (23.59 mL), mackerel (23.53 mL), and tuna (23.43 ± 0.35 mL). Higher ERV values generally indicate better-quality muscle, whereas declining ERV may occur with spoilage.

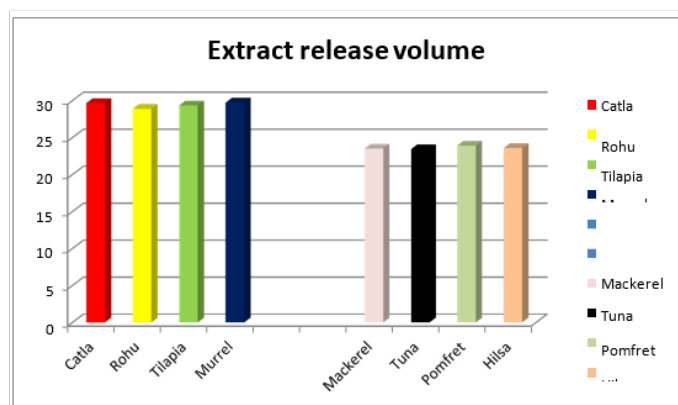


Figure 4. ERV of fish samples collected

Thiobarbituric acid value

TBA values were higher in marine fish than freshwater fish. Freshwater fish recorded values ranging from 0.94 ± 0.05 to 1.01 ± 0.01 mg MDA/kg, whereas marine fish showed values from 1.05 ± 0.01 to 1.55 ± 0.15 mg MDA/kg. Among freshwater fish, catla and murrel showed the lowest values at approximately 0.94 mg MDA/kg, followed by rohu (0.98) and tilapia (1.01). Among marine fish, pomfret showed the highest TBA value (1.55 ± 0.15 mg MDA/kg), followed by hilsa (1.26 ± 0.13), tuna (1.24 ± 0.07), and mackerel (1.05 ± 0.01). The higher TBA values in marine fish indicate greater lipid oxidation compared with freshwater fish.

Peroxide value and free fatty acids

Peroxide value was not detected in any of the freshwater fish samples. In marine fish, PV ranged from 1.37 ± 0.00 to 3.58 ± 0.06 mg%, with the highest value in tuna (3.58 ± 0.06), followed by mackerel (3.47 ± 0.06), pomfret (2.80 ± 0.00), and hilsa (1.37 ± 0.00). Similarly, FFA were not detected in freshwater fish, whereas marine fish showed measurable levels. Tuna recorded the highest FFA value

(3.19%), followed by mackerel ($2.51 \pm 0.10\%$), pomfret ($2.16 \pm 0.01\%$), and hilsa ($1.26 \pm 0.13\%$). The presence of PV and FFA in marine fish indicates greater lipid degradation and oxidation compared with freshwater fish.

SI No	Source	Fish	chemical parameters		
			TBA (mg MDA/kg)	PV (mg%)	FFA (mg%)
1	FRESH WATER	Catla	0.94 ± 0.05^c	ND	ND
2		Rohu	0.98 ± 0.38^c	ND	ND
3		Tilapia	1.01 ± 0.01^{bc}	ND	ND
4		Murrel	0.94 ± 0.06^c	ND	ND
5	MARINE	Mackerel	1.05 ± 0.01^{bc}	3.47 ± 0.06^b	2.51 ± 0.10^b
6		Tuna	1.24 ± 0.07^b	3.58 ± 0.06^a	3.19 ± 0.00^b
7		Pomfret	1.55 ± 0.15^a	2.80 ± 0.00^c	2.16 ± 0.01^c
8		Hilsa	1.26 ± 0.13^b	1.37 ± 0.00^d	1.26 ± 0.13^d

Table 3. Results of Chemical Parameters of fish sample

(a,b,c,d differ significantly at $p < 0.05$)
ND-not detected

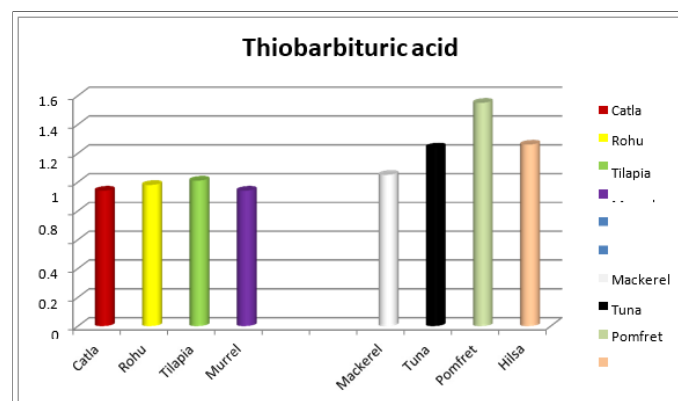


Figure 5. TBA values of Fish samples collected

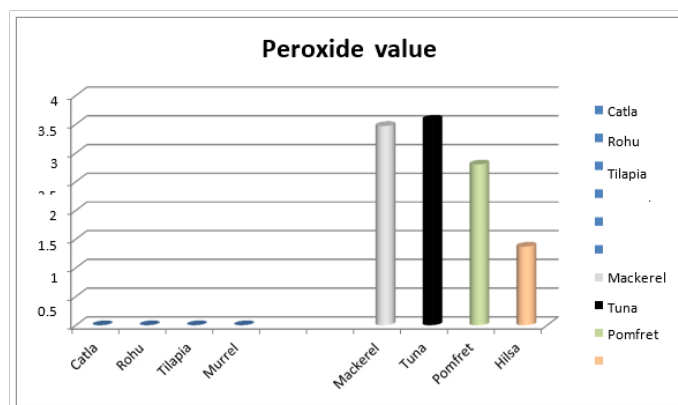


Figure 6. PV values of fish samples collected

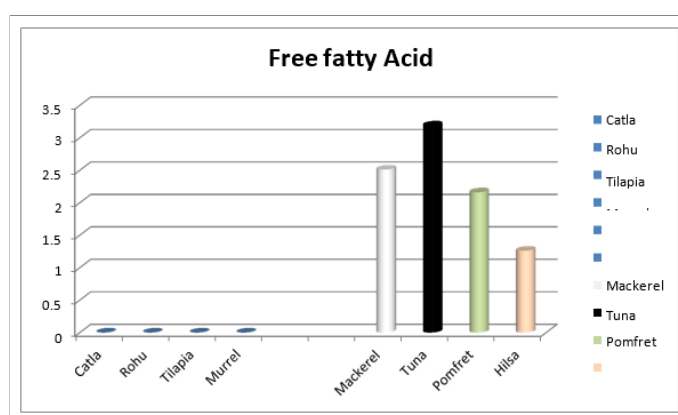


Figure 7. FFA values of fish samples collected

Microbiological quality

Total viable count

The TVC was higher in freshwater fish than marine fish. Freshwater fish showed counts ranging from 6.45 to 7.10 Log CFU/g, while marine fish ranged from 5.73 to 6.13 Log CFU/g. Among freshwater

species, murrel recorded the highest TVC (7.10 Log CFU/g), followed by catla (6.99), rohu (6.76), and tilapia (6.45). Among marine fish, tuna showed the highest count (6.13 Log CFU/g), followed by mackerel (5.91), hilsa (5.73), and pomfret. The higher bacterial load in freshwater fish indicates greater microbial contamination and may be related to source water quality, handling, transportation, and retail conditions.

Sl No	Source	Fish	Microbial quality of fish (Log CFU/gm)			
			TVC	TCC	FCC	TYMC
1	FRESH	Catla	6.99±0.09	3.13±0.27	2.66±0.12	1.96±0.04
2		Rohu	6.76±0.11	3.10±0.29	2.58±0.13	1.93±0.04
3		Tilapia	6.45±0.10	3.06±0.22	2.53±0.12	1.91±0.04
4	WATER	Murrel	7.10±0.11	3.25±0.19	2.74±0.12	1.96±0.05
5		Mackerel	5.91±0.05	2.95±0.20	2.48±0.12	2.48±0.17
6		Tuna	6.13±0.04	2.98±0.21	2.51±0.10	2.51±0.17
7	MARINE	Pomfret	5.78±0.13	2.91±0.24	2.45±0.10	2.38±0.15
8		Hilsa	5.73±0.08	2.84±0.19	2.41±0.07	2.36±0.12

(TVC- Total Viable counts, TCC-Total coliform count, FCC- Faecal coliform counts, TYMC-Total yeast and mould counts)

Table 4. results of microbial assessment of fish

Total and faecal coliform counts

TCC ranged from 3.06–3.25 Log CFU/g in freshwater fish and 2.82–2.95 Log CFU/g in marine fish. FCC ranged from 2.53–2.70 Log CFU/g in freshwater fish and 2.41–2.51 Log CFU/g in marine fish. The higher coliform and faecal coliform counts in freshwater fish may indicate contamination from water, handling, transportation, icing, or inadequate hygienic practices. Coliform organisms are useful indicators of sanitary quality and possible faecal contamination.

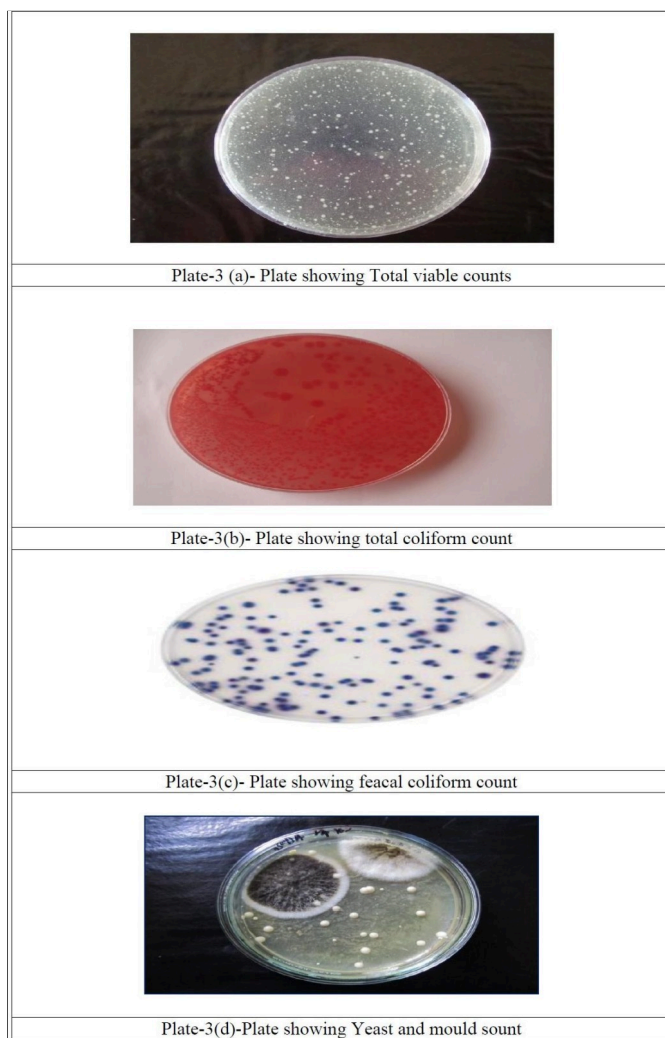


Figure 8. Representative culture plates showing microbiological enumeration of food samples: a) Total viable count b) Total coliform count c) Faecal coliform count, d) Yeast and mould count

Unlike bacterial indicators, yeast and mould counts were higher in marine fish. Freshwater fish showed counts ranging from 1.91–1.96

Log CFU/g, while marine fish ranged from 2.36–2.51 Log CFU/g. Yeasts and moulds contribute to deterioration and decomposition of fish and may also produce metabolites associated with food spoilage and health concerns.

Incidence of pathogenic organisms

Escherichia coli: *E. Coli* occurred much higher frequency in freshwater fish (83.33%) than marine fish (50%). Higher incidence was observed in tilapia (83.33%), followed by catla, rohu & Murrel (66.66%). incidence on different fishes was mackerel & pomprfet (50%) & tuna & hilsa (33.33%). Occurrence of *E. Coli* in different species may indicate a transfer of contamination during handling operation and food preparation, handling processing transport, exposure of water and floor to the source of contamination.

Salmonella spp was found in 16.66% of catla, rohu, tilapia, tuna & hilsa sample, there was no isolate of *Salmonella* from murrel, mackerel & pomprfet. *Salmonella* spp poses considerable public health risk. Fish can serve as passively carriers. The contaminated surface and water may be the source of salmonella.

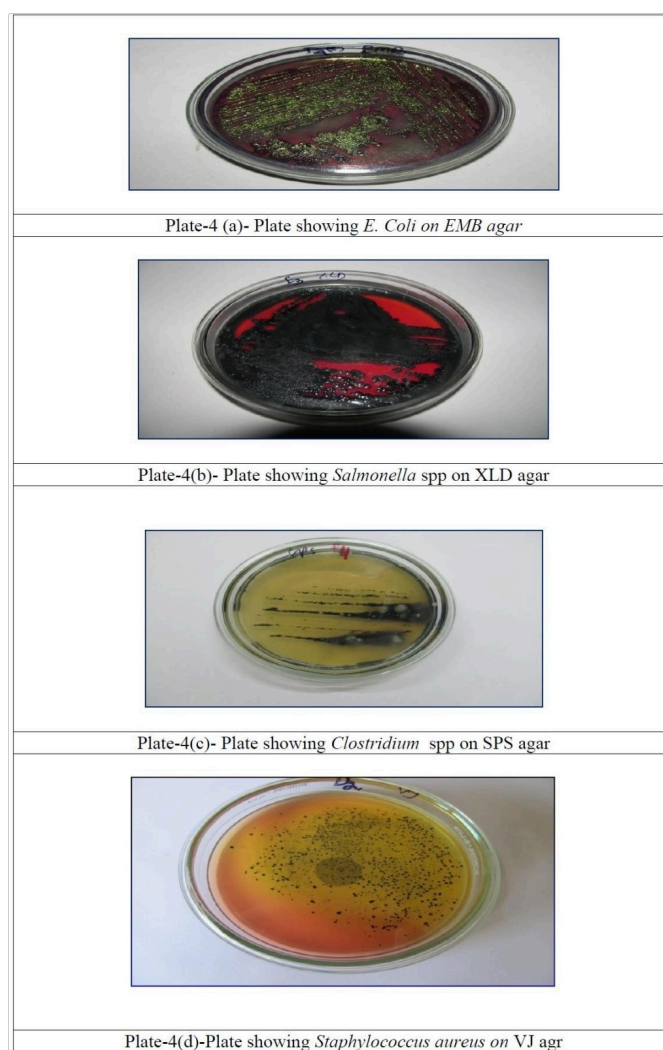
Clostridium spp.: incidence of *Clostridium* Spp was the highest in rohu (50%); incidence of contamination was also high in catla, tilapia, murrel, tuna, pomprfet & hilsa (33.33%). Whereas mackerel contain 16.66% *clostridium* Spp. Contamination of these bacteria could occur from environment like dust water soil container, handlers etc. Since *Clostridium* spp. is spore bearing in nature it remains viability for a prolonged time under unfavourable condition.

Staphylococcus aureus: *S. Aureus* was observed in both marine (33.33%) and freshwater fishes (33.33% & 50%). Higher incidence was found in both fresh water fish *Catla* and rohu (50%) where as murrel and tilapia (33.33%), incidences on Marine fish were; mackerel (16.66%), Tuna & pomprfet & hilsa (33.33). The incidence is associated with food handlers & equipments or surfaces in immediate surroundings, which have been soiled either with food debris, faeces or on the surfaces harbour the microorganism.

Vibrio spp: Marine fish (83.33%) reported for presence of *V. spp.* than freshwater fish (50%). Presence of *V. Spp.* might have attributed to factors of environmental importance as temperature; salinity of the water as these parameters favour proliferation, survival and presence.

Aeromonas spp: marine fish reported presence of more *A. Spp* than freshwater fish. *A. spp.* detected highest on marine fish tuna & pomprfet (66.66%), mackerel & hilsa (50%), of freshwater; cat la & murrel (33.33%), rohu & tilapia (16.66%).

Sl No	Source	Fish	Incidence of Pathogenic organisms (%)							
			<i>E coli</i>	<i>Salmonella spp</i>	<i>Clostridium spp</i>	<i>Staphylococcus aureus</i>	<i>Vibrio spp</i>	<i>Aeromonas spp</i>	<i>Pseudomonas spp</i>	<i>Shigella spp</i>
1	FRESH WATER	Catla	66.66	16.6	33.33	50	50	33.33	16.66	16.66
2		Rohu	66.66	16.6	50	50	50	16.66	16.66	0
3		Tilapia	83.33	16.66	33.33	33.33	50	16.66	16.66	16.66
4		Murrel	66.66	0	33.33	33.33	50	33.33	33.33	0
5	MARINE	Mackerel	50	0	16.66	16.66	83.33	50	50	16.66
6		Tuna	33.33	16.66	33.33	33.33	83.33	66.66	50	0
7		Pomfret	50	0	33.33	33.33	83.33	66.66	50	16.66
8		Hilsa	33.33	16.66	33.33	33.33	83.33	50	66.66	0

Table 5. Results of incidence of pathogenic organisms in fish**Figure 9.** Representative culture plate showing the isolation and presumptive identification of selected bacterial pathogens on selective and differential media: a) *Escheria coli* on EMB agar, b) *Salmonella* spp on XLD agar, c) clostridium spp. on SPS agar, and d) staphylococcus spp. On VJ agar

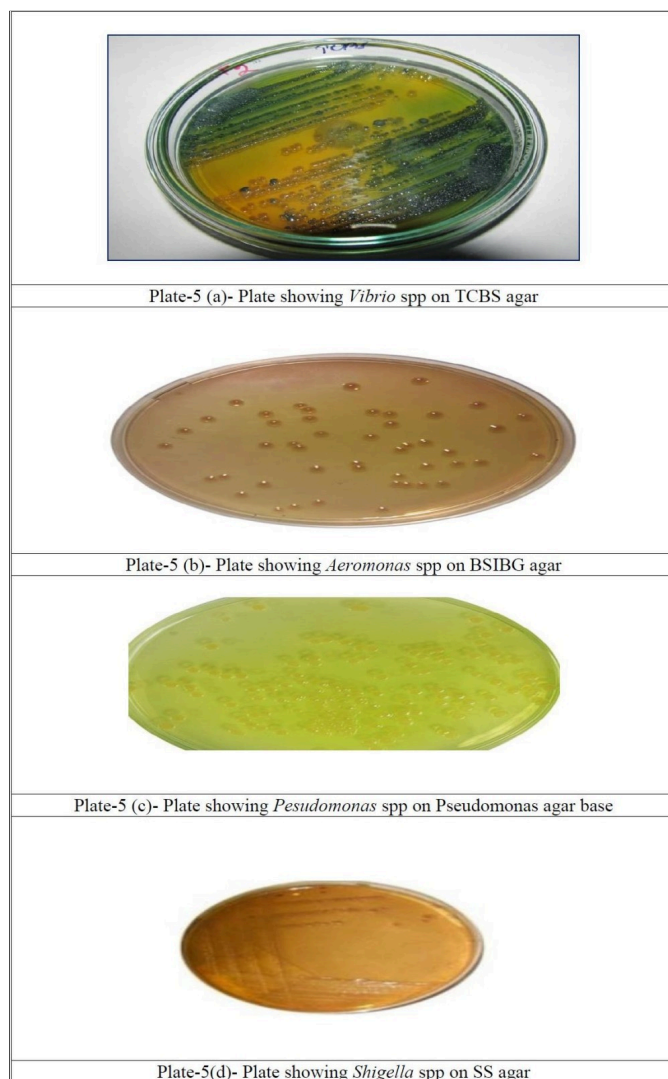


Figure 10. Representative culture plates showing the isolation and presumptive identification of selected bacterial pathogens on selective media: a) *Vibrio* spp. on TCBS agar, b) *Aeromonas* .on BSIBG agar, c) *Pseudomonas* spp. on Pseudomonas agar base, and d) *Shigella* spp. on SS agar

The incidence of *Pseudomonas* spp was higher in the case of marine fishes, where Hilsa has the highest incidence (66.66 %), it was followed by Mackerel, Tuna, and Pomfret (50 % each). In fresh water fishes, the incidence in Murrel was 33.33 %, in *Catla*, Rohu and *Tilapia* was 16.66 %.

Shigella spp. Was identified in 16.66 % samples of *Catla*, tilapia, mackerel and pomfret, whereas it was not observed in rohu, murrel, tuna and hilsa. Presence of these bacteria suggests that contamination might occurred due to harvesting, handling, processing and storage. Pathogenic organism loads Loads of individual pathogenic bacteria showed marked difference between fresh and marine water fishes; loads for *E.coli* recorded was between 5.47-5.84 Log CFU/g for freshwater fishes and 3.69-4.77 Log CFU/g for marine fishes, more loads were found in the former which is in accord with the incidence. Loads for *Salmonella* spp recorded was between 0-2.47 Log CFU/g. No *Salmonella* spp detected in Murrel, Mackerel, Pomfret. Whereas in the positive samples, the loads varied between 2.00 and 2.47 Log CFU/g. The loads for *Clostridium* spp recorded was between 1.00-2.32 Log CFU/g and 1.00-2.30 Log CFU/g for fresh and marine water fish respectively.

For *Staphylococcus aureus* higher loads was observed for marine fish (5.30-5.90 Log CFU/g) compared to freshwater fish (4.69-4.87 Log CFU/g). Loads of *Vibrio* spp recorded was 2.00-2.47 Log CFU/g and 3.00-3.47 Log CFU/g for fresh and marine water fish. Also loads of *Aeromonas* spp was significantly higher for marine fish (5.47-5.84 Log CFU/g) compare to fresh water fish (2.47-2.77 Log CFU/g).

Loads of *Pseudomonas* spp was between 2.47-3.69 Log CFU/g for fresh and 5.60-5.84 Log CFU/g for marine fish, and that of *Shigella* spp ranged between 2.00-2.47 Log CFU/g for positive samples; the bacteria was absent from rohu, murrel, tuna and hilsa.

Sl No	Source	Fish	Count (Log CFU/gm)							
			<i>E coli</i>	<i>Salmonella</i> spp	<i>Clostridium</i> spp	<i>Staphylococcus aureus</i>	<i>Vibrio</i> spp	<i>Aeromonas</i> spp	<i>Pseudomonas</i> spp	<i>Shigella</i> spp
1	FRESH WATER	Catla	5.77-5.81	2.30-2.47	1-2	4.77-4.81	2-2.30	2.60-2.69	2.69-2.77	2-2.30
2		Rohu	5.69-5.74	2-2.47	2.30-2.32	4.69-4.77	2-2.47	2.69-2.77	2.84-2.90	0
3		Tilapia	5.69-5.84	2.30-2.47	2-2.2	4.69-4.74	2.30-2.47	2.60-2.69	2.47-3.69	2.30-2.47
4		Murrel	5.47-5.60	0	1-2.30	4.84-4.87	2-2.30	2.47-2.60	3.60-3.69	0
5	MARINE	Mackerel	3.69-3.84	0	2-2.02	5.69-5.81	3-3.30	5.47-5.60	5.69-5.79	2-2.30
6		Tuna	3.77-3.90	2-2.47	1-2.01	5.30-5.69	2-3.30	5.47-5.69	5.60-5.69	0
7		Pomfret	3.77-4.30	0	1-2.30	5.60-5.77	3-3.30	5.60-5.69	5.69-5.74	2-2.47
8		Hilsa	3.84-4.77	2.30-2.47	1.30-1.32	5.69-5.90	3.32-3.47	5.77-5.84	5.77-5.84	0

Table 6. Result of microbial count of pathogenic organisms

Discussion

Freshness serves as the primary indicator of fish quality, which diminishes mainly due to biochemical changes before microbial decay sets in. Within the scope of this study, all samples of murrel and tilapia

were classified as excellent (*E*-grade) on the freshness scale. Freshness among rohu and catla was predominantly excellent, whereas the majority of marine species fell into the acceptable to good (A- and B-grade) range, suggesting lower freshness compared to freshwater samples. Elevated ambient temperatures and extended holding periods likely contribute to this decline in freshness.

The pH, water-holding capacity (WHC) and extract release volume (ERV) of freshwater fish were significantly higher than that of marine fish. Fresh samples of freshwater fish displayed pH values ranging from 6.65–6.84 with highest pH value in murrel (6.84). Similarly, WHC and ERV values of freshwater fish (29.73 mL highest in murrel) indicate better physical quality compared to marine fish (pH 5.84–6.37) but these can vary with factors such as fish species, seasons, diet, activity level, level of stress, and body muscle characteristics. Marine fish indicated greater degree of chemical spoilage. TBA value in marine fish ranges from 1.05 to 1.55 mg MDA/ kg with highest in pomfret (1.55 mg MDA/kg), whereas in freshwater fish it was within range 0.94 to 1.01 mg MDA/ kg and highest in murrel. Fresh samples of freshwater fish recorded no values for peroxide value and FFA which were within range in marine fish from 3.58% (tuna to 0.81%) and 3.19% (tuna to 0.6%) respectively, whereas tuna registered the highest values for PV and FFA Showing higher lipid oxidation and hydrolytic degradation in marine species. Microbiologically, marine fish had low count of TVC Coiform counts and faecal coliform with respect to that of freshwater fish. Lowest TVC was (7.1log CFU/g) observed for murrel (fresh water fish).

Fresh water species of Fish species have relatively high counts on Total coliform and faecal coliforms compared to marine species. These can result from contaminated water sources, handling, processing, transportation and storage. Freshness levels of marine species were generally found to be lower than fresh water species which may be caused by local environmental, physiological and biological factors (Osborne, 1985). Fresh and chilled samples of Fish showed poor hygiene practices and possible spoilage in terms of high aerobic count and coliform presence.

Total Aerobic counts (TAC) of all samples were high. Total coliform (TC) counts also were high in some fresh and chilled marine fish samples. However fresh water species were more dominated in case of presence of coliform and faecal coliform as expected because of being found in stagnant polluted water body. Total yeast and mold counts were in a higher range in marine fish compared to freshwater fish.

Among the selected pathogenicity *E.coli* was more found in freshwater species (83.33% in case of *Tilapia*), Whereas in case of Marine species *Vibrio* were found (83.33%), *Aeromonas* and *Pseudomonas* (66.66%), Where as in case of *Catla* and Rohu the counts were highest for *S. Aureus*, (50% in each). Total count for *Clostridium* in Rohu and *Catla* (50% in each case) while *Shigella* sp. (0% to 16.66%). Among pathogenicity *E.coli* counts were high in freshwater species (5.47-5.84log CFU/g) as compare to Marine species, while *Aureus*, *Vibrio*, *Aeromonas* and *Pseudomonas* counts were significantly high in case of Marine fish species. Based on this work, freshwater species showed best fresh but greater counts of Total coliform, faecal coliform etc. where as amrineries indicated lower freshness with greater amount of lipid deterioration. Thus, it is important to utilize ice packing, appropriate handling, transportation and rapid marketing methods for maintaining overall quality of fish and to avoid consumer's risk.

Conclusion

The present investigation recorded significant differences in physico-chemical and microbiological quality of the freshwater and marine fish sold in GHMC. Freshwater fish with the exception of catla, Rohu revealed fairly good status of sensory freshness, pH, WHC, ERV than marine fishes. All of the murrel and tilapia samples are *E*-grade, most of the *Catla* and Rohu samples are also *E*-grade as assessed by sensory.

Marine fish had comparatively lower pH, WHC and ERV, but revealed a significantly higher TBA value, PV and FFA than freshwater. Lower values of PV and FFA in freshwater fish may suggest for less lipid breakdown. Microbiological survey of freshwater fishes reported higher value of TVC, TCC and FCC where, marine fish samples had a comparatively larger yeast and mold count. Several pertinent food-borne microorganisms have been isolated from the samples of both marine and freshwater fish. *E.coli* were most frequently isolated from fresh water, being maximum in tilapias; *Vibrio*, *Aeromonas* and *Pseudomonas* are significantly more prevalent and present in higher quantities in marine fish samples.

Most fish samples possess generally good freshness and physico-chemical quality for consumption but presence of several indicator microorganisms and foodborne pathogens may reflect the concern for hygiene. Good hygiene, careful handling, sufficient amount of icing, and maintained controlled temperature at storage and during transport, use of potable water and constant attention toward microbiological scrutiny are necessary to preserve quality of fish and to ensure safety. Hence it can be suggested to follow good fish handled and good retail practice throughout.

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