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MICROBIAL QUALITY OF FARMED FISH IN MARKETS OF LAHORE, PAKISTAN: A HEALTH CONCERN

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Microbial quality of farmed fish from metropolitan city of Lahore was examined to check acceptability and food safety of fish for human consumption. Samples of four important carps species were collected from main fish market and retailers' shops for analyze to aerobic plate count (APC), total coliform count (TCC), faecal coliform count (FCC), *Escherichia coli* (*E. coli*) and to detect *Vibrio cholera* (*V. cholera*) and *Staphylococcus aerous* (*S. aerous*). APC, TCC, FCC and E. coli were positive in all the samples while *Vibrio cholera* (*V. cholera*) and *Staphylococcus aerous* (*S. aerous*) were found absent. The highest level of APC ($4.82\times10^6\pm1.149$ cfu g^{-1}) was found in *Ctenopharyngodon idella*, and of TCC (149.38±18.940 MPN), FCC (148.24±79.127 MPN) and *E. coli* (23.40 MPN) in *Hypophthalmichthys molitrix*. Microbial loads increased from main fish market to retailers' shops. All the tested samples were beyond the permissible limits for APC (5×10^5 g^{-1}), TCC and FCC (nil); while for *E. coli* (< 10MPN g^{-1}), sample of *C. mrigala* (8.32 MPN) and *C. idella* (6.98 MPN), collected from main fish market were under permissible limits. Time series increase in microbial loads was indicative of poor sanitary conditions and the fish available in the markets was unacceptable and unsafe for human consumption.
Keywords: Microbial quality, APC, coliforms, *E. coli*, farmed carps, main fish market, retailers' shops

INTRODUCTION

Fish is increasingly becoming an important human diet component due to its nutritional value with digestive proteins and other microelements (Gandotra *et al.*, 2013). Fish, however, being an extremely perishable food commodity, has quite shorter shelf life in comparison to other meats (Lauzon *et al.*, 2010), and sometimes, its consumption can cause food poisoning or other diseases due to infection and intoxication (Can, 2010; Begum *et al.*, 2010). Fish spoilage and deterioration instigates immediately as the fish is caught or slaughtered (Popovic *et al*., 2010; Gandotra *et al.*, 2013), which is a time and temperature dependent phenomenon attributed to the culture environment, harvest, transport and post-harvest management tools and techniques (Boyd, 1984). This quality deterioration is activated by development of microbes, inherent enzymatic activities and decomposing bacteria present in slime and on skin of fish (Auborg, 2008). These bacteria, after loss of fish immune system can invade rapidly the fish muscle to spoil it (Jianadasa *et al.*, 2014).

These microbial loads, enzymatic activities and lipid oxidation leads to the formation of aldehydes, ketones, alcohols, amines and sulfides etc. (Jan *et al.*, 2014) which make fish off-flavoured having bad smell and the taste (Gram and Dalgaard, 2002) causing even disease to human (Brigitte *et al.*, 2004). This fish spoilage besides a health concern can sometimes cause significant economic losses also (Abbas, 2014).

Pakistan falls between 33°N and 20°N, and is bestowed with vast fresh and marine water resources. Attributed to its specific geographical location it acts as drainage basin for the Himalayas; consisting of a gigantic network of rivers, streams, lakes, reservoirs, canals, and waterlogged areas comprising about 8.60 million hectares of area. Above 0.60 Million tons of fish is annually produced from capture fisheries and the aquaculture in Pakistan. As per FAO report (2012), fisheries sub-sector is providing direct livelihood to about 400,000 people of the country, while other 600,000 folks are involved in subsidiary activities. Thus, this sub-sector is making an important contribution in economy of Pakistan.

In Pakistan, aquaculture, especially for last two decades is developing with an annual growth rate of above ten percent (Khan and Chatta, 2015). Indigenous major carps (rohu, mrigal and catla) and Chines carps (grass carp and silver carp) are widely cultured in ponds under a semi-intensive polyculture system; using modern techniques. However, the area of food safety and quality control, which is of key importance, both, for export marketing as well as for local fish consumption, is still almost ignored in the country; especially in freshwater farmed fish. Presently, two Fish Quality Control Labs are working in the country, exclusively for fish and seafood products, but due to poor legislation, the fish marketing system is quite outmoded. Fish harvesting, transportation and the post-harvest handling is more unhygienic with insanitary practices, resulting erratic and unpredictable fish quality in domestic markets. The seafood exports from Pakistan to European Union (EU) are banned sporadically from, due to poor sanitary and phytosanitary (SPS) measures and other quality related issues. This study was planned to examine the microbial quality of commercially important farmed freshwater carps sold in various marketing channels in Lahore, the main metropolitan city of the Punjab Province, to assess food safety level of the produce as per international standards set for human consumption.

MATERIALS AND METHODS

Sample collection**:** Four food fish species of commercially cultured carps namely i.e. *Labeo rohita* (Rohu), *Cirrhinus mrigala* (Mori), *Ctenopharyngodon idella (*Grass carp) and *Hypophthalmichthys molitrix* (Silver carp), five specimens each, of edible size were sampled randomly, both form main fish market and the retailers shops of metropolitan city, Lahore, following standard sampling procedures (Steel *et al.*, 1997). The degutted samples were subjected to necessary biological parameters like wet body weight and total length, preserved in ice boxes and transported to Fish Quality Control Labs, of the Department of Fisheries Punjab, at Fisheries Complex Manawan, Lahore, for detection of various microbial quality parameters in fish.

Microbial load assessment**:** To assess microbial load, Aerobic Plate Counts (APC), Total Coliform Counts (TCC), Faecal Coliform Counts (FCC), *Escherichia coli* (*E. coli*) were determined and the quantitative analysis of *Vibrio cholera* and *Staphylococcus aerous* were made. Fifty (50) g of fish flesh from each fish sample was taken in aseptic blender jar and added with 450 ml of sterile Butterfield's phosphate buffer solution. After homogenization, its serial dilutions were prepared in reagent bottles. To assess APC, 1 ml of each dilution was poured into petri dish, added 12-15 ml hot molten plate count agar and incubated for 48 (±2) h at 35°C for growth. After incubation, the number of colonies (cfu/g) was counted. To detect *Vibrio cholera*, and *Staphylococcus aerous* TCBS agar and Baird-Parker agar were used respectively. For *Vibrio cholera* 25g test sample and 225 ml AP broth was added in 500 ml flasks and prepared suspended homogenate by swirling the mixture and incubated at 42°C (±0.2) in water bath for 6-8 h. 3mm loops from this broth was then incubated on TCBS agar plate at 35°C for 18-24 h and *V. cholera* colonies were identified. Similarly for *Staphylococcus aerous*, food homogenate was prepared by taking 50 g unthawed sample and 450 ml phosphate buffered dilution H_2O in blender jar and homogenizing it for 2 min. the serial dilutions were prepared until 10^{-6} and incubated in tubes at 35°C for 48 h 3 mm loops were transferred to dried medium plates and by streaking isolated colonies were obtained.

For Total Coliform Counts (TCC), these serial dilutions were shifted in Lauryl tryptose broth (LST broth) tubes and incubated for production of gas. The Coliforms presenting positive reactions were further incubated in EC medium to detect Faecal Coliform Counts (FCC) and among these, the cultures showing results for gas production in EC medium were further transferred in L-EMB agar and examined by IMViC reaction for presence of *E. coli.* The counts for TCC, FCC and *E. coli* are denoted by Most Probable Number (MPN/g). All these microbiological tests were performed following AOAC (2012)**.**

Statistical analysis**:** The data collected for various microbial quality parameters was analyzed statistically by Two Way Analysis of Variance (ANOVA) and data of APC was further subjected the LSD test following Steel *et al.* (1997) to find out significance levels at P= 0.05 among different fishes at both the fish marketing points, using statistical package R.

RESULTS

Aerobic plate counts**:** Means and ranges of Aerobic Plate Counts of all four experimental fish species from both fish marketing points, i.e. main fish market and the retailers shops are presented in Table 1. Aerobic Plate Counts (APC) in fish flesh of all four species were higher in samples collected from retailers shops when compared with the samples collected from main fish market of Lahore (Fig. 1).

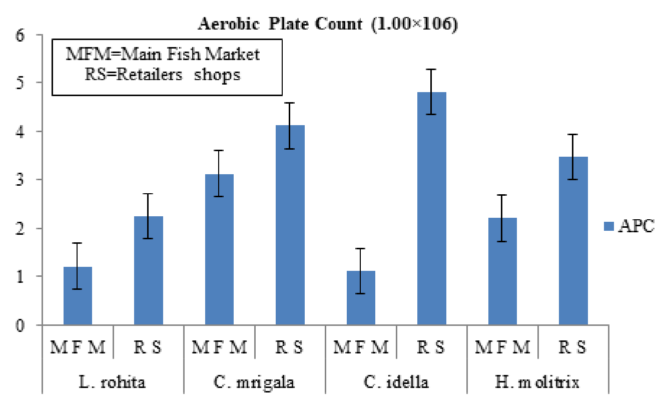

Figure 1. Increase in Aerobic Plate Counts (APC) from main fish market to retailer shops.

APC in samples of *L. rohita* and *C. idella* collected from retailers shops (2.25×10^6 and 4.82×10^6 cfu g^{-1}) were significantly higher than the samples collected for these fish species from main fish market (1.22×10^6 and 1.12×10^6); while in samples of *C. mrigala* and *H. molitrix* collected from retailers shops (4.12×10^6 and 3.47×10^6 cfu g^{-1}) were though higher than samples of these fish species collected from main fish market (3.13×10^6 and 2.21×10^6 cfu g^{-1}) but were non-significant. The acceptable limits set for Aerobic Plate Counts

Table 1. Mean Aerobic Plate Counts (APC) found in samples of farmed carps collected from main fish market and retailer shops in Lahore.

Fish marketing point	Fish species	APC (cfu g^{-1})			
		Mean	**SE**	**Min.**	**Max.**
Main Fish Market	*Labeo rohita*	$1.22\times10^{6\,a}$	1.931	8.49×10^5	1.91×10^6
	Cirrhina mrigala	$3.13\times10^{6\,a}$	4.849	2.04×10^6	4.78×10^6
	Ctenopharyngodon idella	$1.12\times10^{6\,a}$	1.017	8.56×10^5	1.43×10^6
	Hypophthalmichthys molitrix	2.21×10^{6a}	3.915	1.27×10^6	3.32×10^6
Retailers Shops	*Labeo rohita*	$2.25\times10^{6\,b}$	0.238	1.75×10^6	3.07×10^6
	Cirrhina mrigala	$4.12\times10^{6\,a}$	1.227	4.46×10^5	7.12×10^6
	Ctenopharyngodon idella	$4.82\times10^{6\,b}$	1.149	2.09×10^6	8.40×10^6
	Hypophthalmichthys molitrix	$3.47\times10^{6\,a}$	1.097	5.60×10^5	7.17×10^6

Note: The same species having different superscripts at two marketing points have significantly different mean values of APC

Table 2. Post Hoc Test (LSD) to find mean APC Difference among various species.

(I) Fish species	(J) Fish species	Mean difference (I-J)	SE	Sig.
L. rohita	*C. mrigala*	-1.8880*	0.752	0.017
	C. idella	-1.2370	0.752	0.110
	H. molitrix	-1.1030	0.752	0.152
C. mrigala	*L. rohita*	1.8880*	0.752	0.017
	C. idella	0.6510	0.752	0.393
	H. molitrix	0.7850	0.752	0.304
C. idella	*L. rohita*	1.2370	0.752	0.110
	C. mrigala	-0.6510	0.752	0.393
	H. molitrix	0.1340	0.752	0.860
H. molitrix	*L. rohita*	1.1030	0.752	0.152
	C. mrigala	-0.7850	0.752	0.304
	C. idella	-0.1340	0.752	0.860

Note: The values having * as superscript have significant APC Mean Difference

Table 3. Mean Total Coliform Counts (TCC) found in samples of farmed carps collected from main fish market and retailer shops in Lahore.

Fish marketing point	Fish species	TCC (MPN)			
		Mean	**SE**	**Min.**	**Max.**
Main Fish Market	*Labeo rohita*	78.00^{a}	12.810	43.00	118.00
	Cirrhina mrigala	65.76^{a}	8.348	42.80	93.00
	Ctenopharyngodon idella	41.76^{a}	8.183	15.00	64.00
	Hypophthalmichthys molitrix	70.76^{a}	11.720	42.00	102.50
Retailers Shops	*Labeo rohita*	143.88^{b}	21.485	93.40	209.10
	Cirrhina mrigala	83.38^{a}	7.408	64.00	106.50
	Ctenopharyngodon idella	103.80^{a}	29.495	39.00	210.00
	Hypophthalmichthys molitrix	149.38^{b}	18.940	113.40	220.00

Note: The same species having different superscripts at two marketing points have significantly different mean values of TCC. MPN = Most Probable Number

APC) in fresh and frozen fin fish as per Pakistan Fish Quality Control Rules, 1998 are 5×10^5 cfu g^{-1}. Thus, APC in samples of all four experimental carps fish species collected from both fish marketing points were higher than the prescribed permissible limits for human consumption.

APC data was then pooled and analyzed for Two Way Analysis of Variance (ANOVA), to find out significance levels, both among fish marketing points and among species (Table 2). The APC were found significantly higher in samples collected from retailers shops of Lahore than fish samples collected directly from main fish market (P=0.002). In pooled data, however, among species there was no significant difference found in APC levels (P=0.110). When data was further subjected to Post Hoc Tests (LSD), it revealed that APC were significantly higher in *C. mrigala* than *L. rohita* (P=0.017); but there was no significant difference seen among other fish species (Table 3).

Table 4. Mean Faecal Coliform Counts (FCC) found in samples of farmed carps collected from main fish market and retailer shops in Lahore.

Fish marketing point	Fish species	FCC (MPN)			
		Mean	SE	Min.	Max.
Main Fish Market	*Labeo rohita*	35.60 [a]	10.241	15.00	72.40
	Cirrhina mrigala	26.18 [a]	3.957	16.60	35.20
	Ctenopharyngodon idella	19.96 [a]	3.876	7.30	31.50
	Hypophthalmichthys molitrix	30.38[a]	8.228	15.00	56.00
Retailers Shops	*Labeo rohita*	56.48 [a]	10.358	25.30	78.30
	Cirrhina mrigala	31.68 [a]	4.423	15.60	39.20
	Ctenopharyngodon idella	40.20 [a]	9.784	21.00	75.00
	Hypophthalmichthys molitrix	148.24 [a]	79.127	39.00	463.00

Note: The same species having different superscripts at two marketing points have significantly different mean values of FCC. MPN = Most Probable Number

Table 5. Mean *Escherichia coli* (*E. coli*) found in samples of farmed carps collected from main fish market and retailer shops in Lahore.

Fish marketing point	Fish species	*E. coli* (MPN)			
		Mean	SE	Min.	Max.
Main fish market	*Labeo rohita*	13.66 [a]	3.344	7.20	26.00
	Cirrhina mrigala	8.32 [a]	2.173	3.00	16.00
	Ctenopharyngodon idella	6.98 [a]	1.136	3.00	9.20
	Hypophthalmichthys molitrix	11.20[a]	4.247	3.00	22.00
Retailers shops	*Labeo rohita*	22.64 [a]	4.544	8.70	35.60
	Cirrhina mrigala	16.68 [b]	2.672	11.00	23.00
	Ctenopharyngodon idella	17.44 [b]	3.043	11.60	28.00
	Hypophthalmichthys molitrix	23.40 [b]	2.682	15.00	29.40

Note: The same species having different superscripts at two marketing points have significantly different mean values of *E. coli*. MPN = Most Probable Number

Total coliform counts and faecal coliform counts: Means and ranges of Total Coliform and Faecal Coliform counts from both fish marketing points are presented in Table 4 and 5. TCC and FCC also revealed similar increasing trend from main fish market to retailers' shops (Fig. 2).

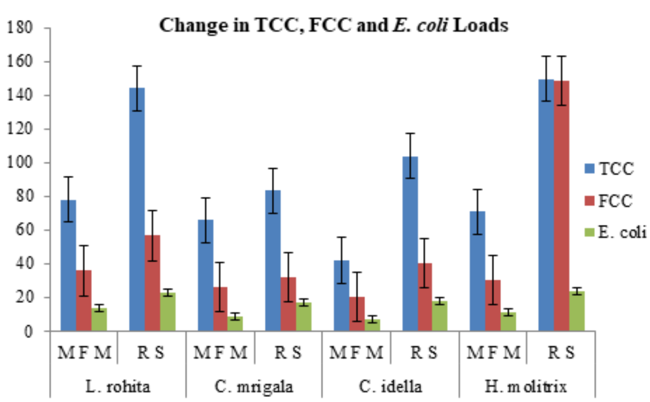

Figure 2. Increase in TCC, FCC and *E. coli* loads from main fish market to retailer shops.

TCC in samples of *L. rohita* and *H. molitrix* collected from retailers shops (143.88 MPN and 149.38 MPN) were significantly higher than the samples collected for these fish species from main fish market (78.00 MPN and 70.76 MPN). TCC in other fish species and FCC in all fish species collected from retailers' shops were non-significantly higher than samples of these fish species collected from main fish market (Table 4 and 5). The highest TCC (210 MPN) was found in *C. idella* and the highest FCC (463 MPN), in *H. molitrix*; both found in samples collected from retailers shops (the sale point for end consumers). All the samples collected for all four fish species, were positive for TCC and FCC while the permissible limits safe for human consumption, as given by FAO are zero g^{-1}.

E. coli, Staphylococcus aureus and Vibrio cholera: *E. coli* (Table 6) was also found in almost all samples and was with a similar trend of higher side in the samples collected from retailers' shops when compared with samples from main fish market (Fig. 2). *E. coli* in samples of *C. mrigala, C. idella* and *H. molitrix*, collected from retailers shops were significantly higher than the samples collected from main fish market; while of *L. rohita*, were though higher but non-significant. E. coli was also beyond the permissible limits prescribed by FAO (<10 MPN g^{-1}) in all the samples collected from both types of marketing points except of *C. mrigala* (8.32 MPN g^{-1}) and *C. idella* (6.98 MPN g^{-1}) collected from main fish market. The *V. cholerae,* and *S. aureus* were not found in any sample of any fish species collected from both marketing points. Thus, overall microbial quality of farmed carps, sold in metropolitan city of Lahore; however, was not fit for human consumption.

Table 6. Bacteriological criteria of permissible limits for finfish whole, dressed, fillets in fresh /frozen /chilled products.

S.#	Parameter	Admissible level
1.	Aerobic Plate Counts	<500000 cfu/g *
2.	Total Coliform Counts	As defined by the Competent Authority
3.	Faecal Coliform Counts	<1000MPN/g *
4.	*Escherichia coli*	<20MPN/g *
5.	*Vibrio cholera*	Nil/g **
6.	*Staphylococcus aureus*	<100/g *

* Pakistan Fish Inspection and Quality Control Rules 1998, ** Food Administration Manual, 1995, cfu = Colony forming units, MPN = Most Probable Number

DISCUSSION

***Aerobic plate counts*:** In any food commodity, the level of microorganisms is indicated by aerobic plate counts (APC) which designate the quality, contamination and shelf life of the product (Maturin and Peeler, 1998) and fresh fish and fish products normally contain APC, 10^4-10^5 cfu g^{-1} (Nickelson and Finne, 1992). Mean APC in all the four experimental fish species of this study was calculated between 1.12×10^6-4.82×10^6 cfu g^{-1} which is almost in line with findings of Begum *et al.* (2010), who noted APC of *L. rohita*, 3.6×10^5-3.5×10^7 cfu g^{-1} and of Tilapia, 4.3×10^5-5.84×10^6cfu g^{-1}. Jeyasekaran *et al.* (2003), Dhanapal *et al.* (2013) and Arefin *et al.* (2014), however, observed APC of farm produced *L. rohita*, 4.70×10^4, 6.60×10^2 cfu g^{-1} and 5×10^5 cfu g^{-1} which were quite lesser then present study depicting better quality of fish than this one.

Microbial load predominantly depends upon the aquatic environment, where the fish grows and post-harvest handling (Shewan, 1977). Fish spoilage is mainly attributed to microbial growth; hence APC can be a good fish quality index (Mohammad and Hamid, 2011). Higher levels of APC in all four fish species, sampled from retailers shops in comparison to main fish market advocate poor post-harvest handling of fish as instrumental of this time series rise in microbial load. Under pooled conditions, APC in samples of *C. mrigala* were found significantly higher than *L. rohita* which may be attributed to feeding habits of *C. mrigala* as this is a bottom dwelling fish where the substrate possibly becomes extra contaminated with bacteria (Quaiyum *et al.,* 2012).

***Total coliform counts and faecal coliform counts*:** Coliforms are a group of bacteria which indicate presence of pathogens, parasites and viruses in a sample (Shankar *et al*., 2010). In the present study, T.C.C and F.C.C were present in all the fish samples collected from both the marketing points while the FAO permissible limits for fish are nil; this indicates the potential health concerns for the consumers. Like APC, T.C.C and F.C.C also exhibited similar intensifying trend from main fish market to retailers' shops, which is a characteristic of the pond environment and sanitary conditions prevailed during harvest and post-harvest fish handling (Hossain *et al.*, 2015). The highest T.C.C (210 MPN g^{-1}) was found in *C. idella* while the highest F.C.C (463 MPN g^{-1}), in *H. molitrix*, both in samples collected from retailer's shops. The present finding of T.C.C. is almost similar to Begum *et al.* (2010), who observed T.C.C., >240 MPN g^{-1} both in *L. rohita* and Tilapia; however, F.C.C in their study was much lower than the present study (> 15 MPN g^{-1}). Hasan *et al.* (2013) detected T.C.C. in *H. molitrix* and *L. rohita,* 515 MPN g^{-1} and 603 MPN g^{-1}, respectively which were much higher than the present study.

***E. coli, Staphylococcus aureus and Vibrio cholera*:** *E. coli* were also found almost in all fish samples and showing similar increasing trend from main fish market to retailers' shops ranging from 3.00 MPN g^{-1} to 29.40 MPN $g^{-1}$and were beyond acceptable limits of FAO for human consumption (< 10 MPN g^{-1}) in samples collected from both marketing points except of *C. mrigala* (8.32 MPN g^{-1}) and *C. idella* (6.98 MPN g^{-1}) of main fish market. *E. coli* was reported as a good sanitary indicative of fish quality in 1930s (Griffiths and Fuller, 1936) and is now commonly applied as strong parameter of microbial fish quality especially related to fecal contaminations (Silva and Hofer, 1993; Jeyasanta *et al.*, 2012). This advocates the poor sanitary conditions during transport as well at the retailer market and the consumption of this fish again can be a potential health risk for the consumer. *V. cholera* and *S. aureus* were not detected in any fish sample collected from both fish marketing points which is in line with the findings of Jan *et al.* (2014) who did not find *S. aureus* in *Labeo rohita* and *Wallogo attu* sampled from Peshawar fish markets, Pakistan.

***Conclusion*:** So, it can be said that overall microbial quality of fish sold in fish markets of Lahore was not good and was a health risk for the consumers. Increase in microbial load from main market to retailers' shops was indicative of unhygienic handling and storage facilities at the markets. Besides introduction of good aquaculture practices (GAqP), some improvements in post-harvest management with better practices, marketing infrastructure & marketing system, introduction of cold chain in fisheries business and adoption of proper hygiene all along the chain from fish farm to the retailers are also required to provide good quality fish not only to the domestic consumers and to start export fish marketing to the neighboring countries.

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