Microbiological quality of two freshwater fishes from different markets of Lahore, Pakistan

Sibgha Farooq, Hafiz Abdullah Shakir*

Fish Microbial Biotechnology Laboratory, Institute of Zoology, University of the Punjab, Lahore, Pakistan.

Abstract- Present study was carried out to assess the microbiological quality of two freshwater fishes (*Labeo rohita* and *Wallago attu*) which are available for sale at three local markets (site A: whole sale market, site B: retailer market and site C: supers stores) of Lahore**.** Mean bacterial count was higher in fishes purchased from site A as compared to site B and site C. Among fish species, higher mean colony forming units (CFU/g) was recorded in *L. rohita* as compared to *W. attu*. In comparison of different organs, higher mean bacterial load was measured in skin followed by gills and muscles. Bacterial genera isolated from fishes were food-borne pathogens which include *Aeromonas***,** *Bacillus***,** *Enterobacter***,** *Shigella***,** *Staphylococcus***,** *Yersinia***,** *Streptococcus* and *Klebsiella* which were significantly different in all sites except *Moraxella*. These genera not only involve in spoilage but also linked with human illness. On the basis of results, it is suggested that harvesting, handling, transportation and storage of fishes especially at site A should be performed hygienically to reduce the microbial exposure.

Index Terms**:** *Labeo rohita*, *Wallago attu*, fish organs, sampling sites, food-borne pathogens.

I.INTRODUCTION

Food safety is a major issue of the world. On the other hand, the demand of highly nutritious food has been increasing with gradual increase in world population [1]. Fishes due to presence of good quality macromolecules are considered as highly risk commodity [2]. Microbes are major cause of spoilage in fishes. These microbes are present naturally on outer surfaces of fishes such as skin and gills and in gastrointestinal system [3]. Furthermore, the microbial contamination occurs due to inappropriate handling and storage during harvesting, transportation, marketing, processing till serving as a meal [4].

The most common pathogens are *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Clostridium* spp., *Shigella* spp., *Aeromonas* spp., and *Yersinia* spp [5]. Globally, these are considered as major meat pathogens that are responsible for causing 42% of food-borne outbreaks [6]. For example, *Aeromonas* is widely distributed pathogen in aquatic environments which not only cause the diseases in aquatic animals such as fish but also affect humans directly and increase the levels of histamine, which poses a chemical hazard to the human health [7]. Hence, fish meat provides a major vehicle for the proliferation of microbial pathogens which are responsible for causing food-borne illness in human [8]. The knowledge about microbiological quality of fishes is very important due to the importance of health and welfare of human.

The present study aimed to assess the microbiological quality of two freshwater fishes, *Labeo rohita* and *Wallago attu* sold in three different marketing channels of Lahore, Punjab, Pakistan. Both fish species are available from every fish shop and have high market demand in Punjab, Pakistan due to its taste.

II.MATERIALS AND METHODS

A. Sampling site:

The present study was conducted on three different markets of Lahore. The brief description of sampling sites (Fig.1) is described below.

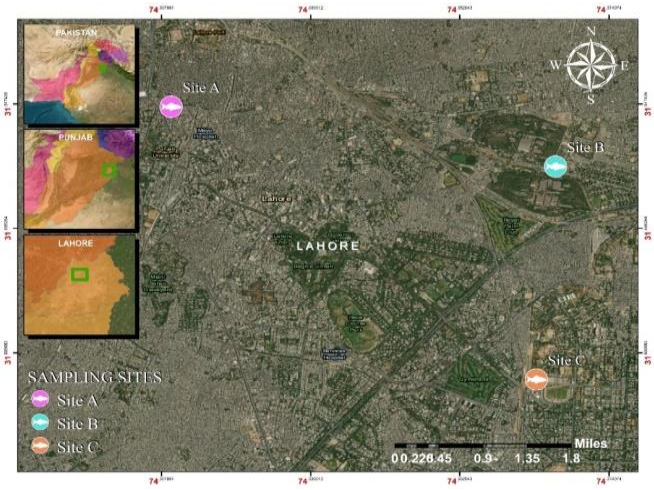

Fig 1: Sampling sites of Lahore fish market.

Site A: Fish whole sale market/ Fish mandi:

It is situated near Anarkali Bazar/Urdu Bazar, Lahore. This site characterized with whole sale area where fish sold in large quantity/bulk at low cost to fish vendors and consumers. These vendors sale the fishes in different areas of Lahore. Fish came at site A through a long chain from the distant rivers and fish farms. The ice is used during transportation and at storage place (floor) at site A. Microbial contamination in fishes may occur from capturing gears, transportation, storage place *etc.* Normally fishes arrived at site A late night and sale of fishes are carried out in early morning. The sales of fishes almost finish till 8 am.

Site B: Fish retailer market

The one of fish retailer market is situated near Mughulpura, Lahore. The shops in this market sold fishes to consumers between 8 am to 4 pm. Fishes came in this market from long distant rivers/fish farms or from site A. The ice is used during transportation and at storage place (non-working old refrigerator) in shops. Contamination in retailer shops may occur due to unhealthy storage place, use bare hands to weigh

and lack of appropriate packaging commodities for fish transactions. The fishes sold to consumers on reasonable prices.

Site C: Fish superstores

The samples of site C were collected from three superstores belonging to multi-national chains. These stores sold fishes to consumers with good hygienic practices, better preserving facilities with good cost, less cross-contamination, better sanitary conditions, proper handling, availability of ice and cooling set up but with high price.

B. Fish Sampling and Isolation of bacteria

The present study was conducted to assess the microbiological quality of two freshwater fishes. Total 54 fishes (9 fish specimen from different shops of each market x 3 sites x 2 fish species) of comparable wet body weight was purchased, kept in separate sterile containers with sterile gloves and transported in insulated boxes under ice to Fish Microbial Biotechnology Laboratory within 3 hours. Known weight samples of skin, gills and muscles from each fish specimen were cut using sterile instruments under aseptic conditions to prepared homogeneous sample (original solution) in saline (0.89% NaCl) solution. The serial dilutions were prepared from original solution. Different known dilutions (100 µl) of each sample were spread on Tryptic soy agar (TSA) and Maconkey agar and incubated the Petri plates at 30°C and 37°C, respectively for 24 hours [9].

The Petri plates showed colonies within the range of ≥30 to 300 were considered and bacterial colonies were categorized based on different morphological characters (shape, size, elevation, structure, surface, color, and opacity *etc.*). The morphological different bacterial colonies corresponding to skin, gills and muscles of each *L. rohita* and *W. attu* of different sites were calculated and were expressed as colony-forming units per gram (CFU/g) of sample.

The representatives of each colony were streaked and re-streaked on TSA and Maconkey agar to obtain pure culture. The slant of each pure culture was prepared and stored for future use.

C. Identification of bacterial isolates:

For identification of different bacterial strains at genus level, different biochemical tests were performed such as Gram's staining, catalase, citrate, oxidase test, indole, methyl red and vogues-proskeur [10].

D. *Statistical analysis*:

The data were statistically analyzed by General linear model (GLM) in Minitab-16 software to find the effect of sampling sites x fish species x organs of fish species interaction. Tukey's post-hoc test at $P< 0.05$ was used if there were more than two means to compare for their significant differences. The effect of this parameter was considered highly significant if $P<0.001$, very significant if $P<0.01$ and significant if $P<0.05$.

III.RESULTS

In present study, total fifty-nine bacterial isolates were recovered from sampling sites. These isolates up to genus level were identified employing biochemical tests. Fifty-three isolates were isolated on TSA medium belonging to seven genera (*Aeromonas, Bacillus, Moraxella, Shigella, Staphylococcus, Streptococcus* and *Yersinia)*. Six isolates were isolated on Maconkey agar medium belonging to two genera (*Enterobacter and Klebsiella)* (Table 1).

Table 1: Identification of isolates up to genus level based on different biochemical tests.

Bacterial Genus	Biochemical tests						
	Gram staining	Catalase	Citrate	Oxidase	Indole	Methyl red	Vogues Proskeur
TSA medium							
Aeromonass	-	+	+	+	+	-	+
Bacillus	+	+	+	-	-	-	+
Moraxella	-	+	-	+	-	-	-
Shigella	-	+	-	-	-	+	-
Staphylococcus	+	+	+	-	-	+	+
Streptococcus	+	-	+	+	-	+	-
Yersinia	-	+	-	-	-	+	-
Maconkey agar medium							
Enterobacter	-	+	+	-	-	-	+
Klebsiella	-	+	+	-	-	-	+

Abbreviations: TSA: Tryptone Soy Agar, -: negative test, +: positive test

In present study, the bacterial load of all genera recovered on TSA medium was highly significantly different ($P<0.001$) in all organs (skin, gills and muscles) of both fish species purchase from three markets except genus *Moraxella.* The highest mean bacterial load (148.0±1.6 x10^4 CFU/g) of genus *Streptococcus* was recorded in skin of *L. rohita* purchased from site A with genus wise order was *Streptococcus* > *Shigella* > *Aeromonas* > *Staphylococcus*> *Bacillus*>

Moraxella> Yersinia (Table 2).

Bacterial isolates which were isolated on Maconkey agar includes *Enterobacter* and *Klebsiella*. The genus *Klebsiella* was recorded significantly high (P<0.001) in skin (217.2±10.8 x10^4 CFU/g) of *L. rohita* purchased from site A in comparison of other organs of *L. rohita* and *W. attu.* In general, *Klebssiella* was dominant as compared to *Enterobacter* in all samples (Table 3).

Table 2: Mean viable count (CFU/g x10^4±SD) of different bacterial genus isolated from organs of different species from different sampling sites on Tryptone Soy Agar.

Fish Species	Sites	Organ	Bacterial Genus						
			Aeromonas	*Bacillus*	*Moraxella*	*Shigella*	*Staphylococcus*	*Yersinia*	*Streptococcus*
L. rohita	A	Skin	66.0±5.2^{a}	46.0±0.4^{a}	28.0±1.6^{a}	105.6±7.2^{a}	59.6±4.4^{a}	26.4±4.8^{a}	148.0±1.6^{a}
	B		50.0±3.6^{b}	28.4±2.8^{b}	23.6±2.8^{ab}	28.8±2.4^{c}	25.6±2.4^{cd}	18.4±2.4^{b}	54.8±3.6^{d}
	C		15.6±2.8^{c-f}	28.0±2.4^{b}	13.6±4.8^{cde}	16.4±1.2^{de}	15.2±3.2^{f-h}	11.2±0.8^{f}	16.4±1.2^{gh}
	A	Gills	20.8±2.4^{c}	25.2±2.8^{bc}	23.2±4.8^{ab}	46.4±0.8^{b}	31.6±2^{c}	18.0±2^{b}	119.6±6^{b}
	B		16.4±1.2^{c-e}	20.8±2.4^{cd}	17.6±2.4^{bc}	17.6±0.8^{de}	22.4±4de	15.6±3.6^{bc}	28.0±3.2^{g-i}
	C		12.0±3.2^{d-f}	17.6±4de	12.4±2.8^{c-f}	14.8±2^{ef}	08.0±1.6^{ij}	07.6±2^{b-e}	14.0±5.2^{g-j}
	A	Muscles	18.8±2.8^{cd}	16.0±1.6^{e-g}	16.0±1.6^{b-d}	31.6±6^{c}	20.8±2.4^{d-f}	12.4±2^{b-e}	70.8±1.2^{c}
	B		11.2±1.6^{ef}	14.4±2.4^{d-f}	11.6±3.6^{c-g}	10.8±1.2^{e-h}	12.8±0.8^{g-i}	07.2±0.8^{d-f}	16.0±2.4^{ef}
	C		10.8±0.4^{e-g}	10.8±2.8^{f-i}	05.2±2^{fg}	06.8±2^{f-h}	03.6±1.2^{j}	02.0±0.4^{d-f}	05.2±2^{k}
W. attu	A	Skin	16.8±1.6^{c-e}	15.6±2^{d-f}	27.6±2.8^{a}	28.8±2.4^{c}	40.8±2.4^{b}	16.7±6.4^{bc}	51.6±1.2^{e}
	B		12.8±2.4^{d-f}	14.0±1.2^{e-g}	17.2±2.8^{bc}	24.8±2.4^{cd}	16.8±1.6^{e-g}	13.6±1.6^{b-d}	21.2±4.4^{fg}
	C		10.4±1.6^{f-h}	10.0±1.2^{f-j}	12.8±2.4^{c-f}	13.2±4.4^{e-g}	09.2±1.2^{h-j}	11.2±2.4^{b-e}	06.4±0.8^{jk}
	A	Gills	16.0±0.8^{ef}	12.0±1.6^{e-h}	12.4±2.8^{c-f}	16.4±2.8^{de}	25.2±2.8^{cd}	16.4±2.8^{bc}	31.2±3.2^{g-j}
	B		11.2±0.8^{e-g}	07.6±1.2^{h-k}	11.2±1.6^{c-g}	11.2±2.4^{e-h}	08.0±0.8^{ij}	12.8±0.8^{b-e}	10.4±1.6^{i-k}
	C		08.8±1.6^{c-e}	04.0±1.6^{jk}	08.0±1.6^{d-g}	09.6±0.8^{e-h}	04.4±1.2^{j}	06.4±0.8^{d-f}	02.8±0.4^{k}
	A	Muscles	04.0±1.6^{g-i}	08.8±1.6^{g-k}	08.8±1.6^{d-g}	05.2±0.4^{gh}	14.8±1.2^{f-i}	10.0±2^{c-e}	14.8±2.8^{d}
	B		03.2±0.8^{hi}	05.6±0.8^{i-k}	07.6±1.2^{e-g}	04.0±0.8^{h}	03.2±0.8^{j}	05.2±1.2^{ef}	07.6±1.2^{h-k}
	C		01.6±0.8^{i}	02.8±1.2^{k}	04.0±1.6^{g}	02.8±1.2^{h}	02.8±1.2^{j}	01.6±0.8^{f}	02.4±1.6^{k}
Significance level (p)			***	***	NS	***	***	***	***

Means within same column with different alphabet differ significantly from each other (P<0.05). Here *, ** and *** represent significance at and P<0.001, P<0.01 and P<0.05 respectively (Minitab 16 General linear model)

Abbreviations: A: whole sale market, B: retailer market, C: supers stores, NS: non significance (P>0.05)

Table 3: Mean viable count (CFU/g x10^4±SD) of different bacterial genus isolated from organs of different species from different sites on Maconkey agar.

Fish Species	Sites	Organ	*Enterobacter*	*Klebsiella*
L. rohita	A	Skin	78.8±6.8^{a}	217.2±10.8^{a}
	B		21.6±3.2^{bc}	185.6±8.8^{b}
	C		21.2±3.6^{bc}	50.0±2.8^{f}
	A	Gills	31.2±6.4^{b}	202.0±2.8^{ab}
	B		16.0±1.6^{c-e}	120.8±16^{d}
	C		15.6±1.2^{c-e}	39.2±8^{f}
	A	Muscles	16.8±1.6^{cd}	195.2±8^{b}
	B		14.4±5.6^{c-e}	112.8±1.6^{d}
	C		10.4±1.6^{d-g}	30.4±6.4^{f}
W. attu	A	Skin	17.2±2^{cd}	141.6±9.6^{c}
	B		15.6±2.8^{c-e}	116.8±2.4^{d}
	C		13.6±4^{c-f}	40.8±1.6^{f}
	A	Gills	13.2±3.6^{c-f}	114.4±4.8^{d}
	B		12.8±2.4^{c-f}	66.4±4^{e}
	C		08.4±1.2^{d-g}	35.6±4.4^{f}
	A	Muscles	06.0±1.2^{e-g}	112.8±1.6^{b}
	B		04.0±0.8^{fg}	07.6±2^{g}
	C		01.6±0.8^{g}	07.2±1.6^{g}
Significance level (p) <0.001			***	***

Means within same column with different alphabet differ significantly from each other (P<0.05). Here *, ** and *** represent significance at and P<0.001, P<0.01 and P<0.05 respectively (Minitab 16 General linear model)

Abbreviations: A: whole sale market, B: retailer market, C: supers store

IV.DISCUSSION

In present study, the bacterial genera (*Aeromonass, Bacillus, Klebsiella, Yersinia, Moraxella, Enterobacter, Streptococcus, Staphylococcus* and *Shegilla)* isolated from different organs of both fishes from different sampling sites were remarkably same. Arif *et al.* [11] reported that isolation of *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas* spp., *Escherichia coli* and *Klebsiella* spp. from fish collected from fish market of Peshawar. Sinha *et al.* [12] also reported the presence of pathogenic bacteria such as *Salmonella, Staphylococcus, Aeromonas, Pseudomonas, E. coli, Micrococcus, Streptococcus, Proteus, Klebsiella* and molds in *L. rohita* purchased from market. Among all vertebrates, fishes are more susceptible to microbial contamination due to its perishable content of protein [13].

In a present study, comparative analysis showed variation in sampling sites, fish species and different organs. Samples of fishes collected from site A (whole sale market/mandi) showed higher mean bacterial count as compared to site B (retailer market) and site C (super stores). The present results are in line with the findings of other researchers [14]. High microbial contamination in the fishes of whole sale market (site A) might be associated with un-availability or delaying of icing, improper handling, compactness, lack of hygiene and contamination of water used for making ice [15]. The high bacterial contamination in whole sale market may due to staying of fish at ambient temperature with very low or absence of ice which enhance the microbial attack on fish [16]. The use of contaminated tap water for making ice in unhygienic boxes may produce bacterial contaminated ice. The contaminated ice is also one of source of bacterial contamination in fishes. One of the major reason is lack of cool chain management especially in whole sale markets [17]. The present study showed less bacterial count in retailer markets (site B) in comparison to whole sale markets due to some extent because of better handling and storage facilities. During present sampling, damping of huge number of fishes were observed at site A and site B. Big piles of fishes may cause structural stress and proliferation of various microbes cause the contamination in fishes. Contamination in retailer markets may occur due to the transportation of low quality/old strock of fishes [18]. While super-stores (site C) showed less

bacteria in comparison to whole sale (site A) and retailer market (site B) because of less cross-contamination, better sanitary conditions, proper handling, timely transportation and single line arrangements. Low level of cross-contamination and the use of cooling facilities promotes reduction of bacterial load of fishes in the super stores [19]. Super stores may also gain attention by consumers due to good display in tray, preserved with clean ice, clean surface and better processing. Shewan [20] findings agreed with present study who reported that freezing and cold storage reduced the bacterial load on fishes.

A significantly high bacterial load in *L. rohita* was recorded as compared to *W. attu.* The major reason of high bacterial load in *L. rohita* might be due to having scales which harbors the bacteria while in comparison to scale less fish, *W. attu*. Dash *et al.* [21] supported the present results where *W. attu* having less bacterial count in comparison to *L. rohita* and it could be due to lack of having scaly skin but it secreted more substantial amount of mucus than scaled fish, that enables it to inhibit the colonization of bacteria in skin. The mucus secreted by skin especially in scale less fishes acts as primary immunological defensive barrier against the entry of bacteria [22],[23]. Slippery nature of mucus also helps in preventing the adherence and stable colonization of bacteria [24].

In present study, higher bacterial load was noted in fish skin and the gills samples as compared to muscles. Skin and gills of fish usually covered with mucus lining that mark as interface between its body and environment and considered as adhesive in nature which provide successful attachment of substrate and bacteria [25]. It also has ability to trap and immobilize the bacteria and other pathogens on their entry [26]. Moreover, it acts as barrier against site of interaction between the fish meat and bacteria hence, play a vital role in the defense mechanism in fishes by acting as a biological barrier [27],[28]. Similarly, high bacterial loads on the skin of Tilapia (*Oreochromis niloticus*) was recorded when compared with the bacterial load on intestines and gills [29]. Adebayo–Tayo *et al.* [30] also reported similar observations. The highest bacterial load in the outer layer of skin occurs due to the direct contamination during capturing or transportation and storage [31]. The findings of present study supported by another study [29] in which gills were found in lowest bacterial count as compared to skin and intestine. The microbes present on the exterior surfaces of fish such as slime of skin and gills are considered to be potentially spoiler. The spoiler microbes are preventing away from proliferation and invasion in to the fish through its natural defense mechanism. Just after the capturing or death of fish, its defense mechanism breaks down and microbes proliferate in the tissue of fish to destruct its nutritional properties [32]. Normally, fish flesh is considered to be sterile or free of microbes, when alive. The contamination of fish flesh may result from rupturing of fish intestine during poor processing or inadequate washing are the causative agent for invasion of bacteria in muscles [33], [34].

In present study, genus recovered on TSA signifies ($P<0.001$) the highest bacterial load of *Streptococcus* in skin of *L. rohita* from site A (whole sale market/mandi) followed by *Shigella*, *Aeromonas*, *Staphylococcus*, *Bacillus*, *Moraxella* and least with *Yersinia*. While on Maconkey agar, *Klebssiella* was found significantly higher in the skin of *L. rohita* collected from site A as compared to *Enterobacter*. Similarly, the predominant genera *Staphylococcus* and *Streptococcus* were isolated from tissues of fish captured from different locations of Nigeria river [35]. The *Staphylococci* and *Streptococcus* were also isolated from Cuttlefish muscles and skin [36]. Similar results were also reported by other researchers [37], [38]. *Staphylococcus* and *Streptococcus* is normal flora of humans but their presence in fish organs indicates the contamination through un-hygiene handlings [39]. In present study, genus *Aeromonas* was isolated from all fish organs (skin, gills and muscles). The highest mean CFU/g ($66.0\pm5.2 \times 10^4$) of *Aeromonas* were recorded in skin of *L. rohita* and minimum ($1.6\pm0.8 \times 10^4$ CFU/g) *Aeromonas* were recorded in muscles of *W. attu*. Sami *et al.* [36] also reported incidence of *Aeromonas* in fish gills, muscles and skin. Some species of *Aeromonas* became emerging pathogens not only at the ambient temperature but also under the cold storage conditions [40]. *Aeromonas* is not only fish pathogen but also an opportunistic pathogen which cause diseases of gastroenteritis, septicemia, and traumatic and wound infections in human [41]. *Aeromonas* also cause food spoilage by producing lipases and proteases [42].

In present study, genus *Bacillus* ranged between 46.0 ± 0.4 to 02.8 ± 1.2 was recorded in different organs of both fish species collected from different sampling sites. Shinkafi and Ukwaja [29] reported the *Bacillus pumilius* from skin and gills of *Oreochromis niloticus* sold at central market Sokoto. Latha and Ramachandra [43] isolated bacterial genera (*Pseudomonas, Acienitobacter, Lactobacillus, Aeromonas, Bacillus, Enterobacteriaceae*, and *Micrococcus* from fish organs (gills and skin) of the fish *Glossogobius giuris*. The presence of family *Enterobacteriaceae* includes *Yersinia*, *Klebsiella*, *Shegilla* and *Enterobacter* in the fish and fish organs might be due to fecal contamination or contamination from aquatic environments during processing, handling and transportation where they survive for a long duration (months) and cause gastro-intestinal diseases [44]. Toxins produce by *Moraxella*, *Bacillus*, *Aeromonas*, and *Micrococcus* spp in the fish cause the spoilage of food and infections in human [45].

V.CONCLUSION

Higher bacterial count in both fish species (*Labeo rohita* and *Wallago attu*) collected from whole sale market (site A) were recorded in comparison to fish retailer market (site B) and super stores (site C). The dominated bacterial genera were *Klebsiella*, *Shigella*, *Enterobacter*, *Yersinia*, *Aeromonass*, *Bacillus*, *Streptococcus* and *Staphylococcus* isolated from both species may associated with fish spoilage and human illness. Among the fishes, scaleless fishes are less prone to bacterial susceptibility because fish scales harbors and facilitate the bacterial invasion.

ACKNOWLEDGEMENTS:

Thanks to University of the Punjab, Lahore, for the financial support under Punjab University Research Project under financial year 2021-22.

REFERENCES

[1]. A. Mahmud, B. Abraha and M. Samuel, "Fish preservation: a multi-dimensional approach", *MOJ Food Processing and Technology*, vol. 6, no. 3, pp. 303–310, 2018.

[2]. A. Kawser, M. Y. Abu-Hera, Y. Sabina, A. Nazmul, R. Mujibur and I. Monirul, "Prevalence of Microbial load in Shrimp, *Penaeus monodon* and Prawn, *Macrobrachium rosenbergii* from Bangladesh", *World Journal of Agricultural Sciences*, vol. 4, no. 3, pp. 852–855, 2008.

[3]. T. P. R. A. Legrand, S. R. Catalano, M. L. Wos-Oxley, F. Stephens, M. Landos and M. S. Bansemer, "The inner workings of the outer surface: skin and gill microbiota as indicators of changing gut health in yellowtail kingfish", *Frontiers in Microbiology*, vol. 8, pp. 17, 2018.
[4]. S. I. Ikape, "Fish spoilage in the tropics: A review", *Octa Journal of Biosciences*, vol. 5, no. 2, pp. 34–37, 2017.
[5]. H. D. Rida, A. Fareeha, A. S. Ali, M. Munazzah, K. Aleena I. Kiran and I. R. Muhammad, "Effects of Storage Temperature on the Microbiological Quality of Fish Meat from Two Different Managemental Systems", *Pakistan Journal of Zoology*, vol. 53, no. 4, pp. 1–4, 2021.
[6]. F. Yeni, S. Acar, O. Polat, Y. Soyer and H. Alpas, "Rapid and standardized methods for detection of foodborne pathogens and mycotoxins on fresh produce", *Food Control*, vol. 40, pp. 359–367, 2014.
[7]. A. Bermejo, Mondaca, M. A. Roeckel and M. C. Marti, "Growth and characterization of the histamine-forming bacteria of jack mackerel (*Trachurus symmetricus*)", *Journal of Food Processing Preservation*, vol. 26, no. 6, pp. 401–414, 2003.
[8]. A. I. Doulgeraki, D. Ercolini, F. Villani and G. J. E. Nychas, "Spoilage microbiota associated to the storage of raw meat in different conditions", *International Journal of Food Microbiology*, vol. 157, no. 2, pp. 130–141, 2012.
[9]. H. Ahmed, Al-Harbi and M. Naim-Uddin, "Bacterial Populations of African Catfish, *Clarias gariepinus* (Burchell 1822) Cultured in Earthen Pond", *Journal of Applied Aquaculture*, vol. 22, pp. 187–193, 2010.
[10]. C. C. Ezemba, S. O. Eze and E. J. Archibong, "Bacteriological Analysis of Iced Fish Retailed at Eke-Awka Market, Anambra State", *International Journal of Pharmacy and Biology*, vol. 4, no. 8, pp. 1–9, 2017.
[11]. J. Arif, H. Zaigham, S. Hussain, U. Rooh, A. Iftikhar and Y. Muhammad, An Investigation of the Bacterial Flora Causing Spoilage of Fishes at Board Fish Market, Peshawar, Pakistan. *Pakistan Journal of Zoology*, vol. 46, no. 5, pp. 1371–1375, 2014.
[12]. D. K. Sinha, S. B. Choudhary and K. G. Narayan, "Microbiological characteristics of marketed rohu *(Labeo rohita)*", *Indian Journal of Fisheries*, vol. 38, pp. 69–71, 1991.
[13]. C. M. Shankar, M. Hassan, M. S. Rhman, M. H. Manik, H. M. Zahid and M. D. Siraj-ul-Islam, "Spectral, optical and cytotoxicity studies on 2-isonicotinoyl-N-phenylhydrazine-1 carboxamide(H_3L) and some of its metal complexes", *World Journal of Fish and Marine Sciences*, vol. 3, pp. 160–166, 2009.
[14]. M. M. Hossain and A. A. Barman, "Post-harvest quality loss of small indigenous fish species in sylhet region: ensure quality up to consumer level", *Journal of Asiatic Society of Bangladesh, Science*, vol. 42, no. 1, pp. 115–125, 2016.
[15]. M. Mhango, S. F. Mpuchane and B. A. Mpuchanem, "Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish", *African Journal of Food, Agriculture, Nutrition and Development*, vol. 10, pp. 4202–4218, 2010.
[16]. A. K. M. N. Alam, "Participatory Training of Trainers: A new approach applied in fish processing", *Bangladesh: Bangladesh Fisheries Research Forum*, pp. 328, 2007.
[17]. M. Eltholth, K. Fornace, D. Grace, J. Rushton and B. Häsler, "Assessing the chemical and microbiological quality of farmed tilapia in Egyptian fresh fish markets", *Global food security*, vol. 17, pp. 14–20, 2018.
[18]. G. P. Jeyasekaran, R. Ganesan, J. Anandaraj, R. Shakila and D. Sukumar, "Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaeus indicus*) stored in dry ice". *Journal of Food Microbiology*, vol. 23, no. 6, pp. 526–533, 2006.
[19]. N. A. Thampuran, Surendraraj and P. K. Surendran, "Prevalence and characterization of typical and atypical Escherichia coli from fish sold at retail in Cochin", *Indian Journal of Food Protection*, vol. 68, no. 10, pp. 2208–2211, 2005.
[20]. J. M. Shewan, "The microbiology of seawater fish. In G. Borgstrom (editor), Fish as food", vol. I: Acad. Press Inc., N.Y, pp. 487, 1961.
[21]. S. Dash, S. K. Das, J. Samal and H. N. Thatoi, "Epidermal mucus, a major determinant in fish health: a review", *Iranian Journal of Veterinary Research*, vol. 19, no. 2, pp. 72, 2018.
[22]. M. Reverter, N. Tapissier-Bontemps, D. Lecchini, B. Banaigs and P. Sasal, "Biological and ecological roles of external fish mucus: a review", *Fishes*, vol. 3, no. 4, pp. 41, 2018.
[23]. G. A. C. Lirio, J. A. A. De Leon and A. G. Villafuert, "Antimicrobial activity of epidermal mucus from top aquaculture fish species against medically-important pathogens", *Walailak Journal of Science and Technology*, vol. 16, no. 5, pp. 329–340, 2019.
[24]. S. Rakers, L. Niklasson, D. Steinhagen, C. Kruse, J. Schauber, K. Sundell and R. Paus, "Antimicrobial peptides (AMPs) from fish epidermis: perspectives for investigative dermatology", *Journal of Investigative Dermatology*, vol. 133, no. 5, pp. 1140–1149, 2013.
[25]. L. A. Thomas and C. O. Hermans, "Adhesive interactions between the tube feet of a starfish, *Leptasterias hexactis*, and Substrata", *Biology Bulletin*, vol. 169, pp. 675–688, 1985.
[26]. R. A. Cone, "Barrier properties of mucus. Journal of Advanced Drug Delivery Review" vol. 61, pp. 75–85, 2009.
[27]. V. S. Raj, G. Fournier and K. Rakus, "Skin mucus of *Cyprinus carpio* inhibits cyprinid herpesvirus 3 binding to epidermal cells", *Veterinary Research*, vol. 42, pp. 92, 2011.
[28]. M. A. Esteban, "An overview of the immunological defenses in fish skin", *ISRN* Immunology, vol. 29, 2012.
[29]. S. A. Shinkafi and V. C. Ukwaja, "Bacteria Associated with Fresh Tilapia Fish (*Oreochromis niloticus*) Sold at Sokoto Central Market in Sokoto, Nigeria", *Nigerian Journal of Basic and Applied Sciences*, vol. 18, no. 2, pp. 217–221, 2010.
[30]. B. C. Adebayo-Tayo, N. N. Odu, L. M. Anyamele, N. J. Igwiloh and I. O. Okonko, "Microbial quality of frozen fish sold in Uyo metropolis", *African journal of Biotechnology*, vol. 8, no. 13, pp. 3068–3071, 2012.
[31]. I. D. Zmyslowka, Lewandowska and J. Guziur, "Microbiological study of ide (*Leuciscus idus L.*) from ponds of different trophy" *Archives of Polish Fisheries*, vol. 8, pp. 259–269, 2000a.
[32]. I. J. Clucas and A. R. ward, "Post-Harvest Fisheries Development. A guide to Handling, Preservation, Processing and Quality. Chatham Maritime, Kent ME4TB", United Kingdom, pp. 665, 1996.
[33]. S. Kaneko, "Microbiological study of fresh fish new

food industries", *Journal of Molecular Biology*, vol. 13, pp. 176–180, 1971.
[34]. H. Sugita, M. Tsunohara, T. Ohkoshi and Deguchi, "The establishment of an intestinal microflora in developing goldfish (*Carassius auratus*) of culture ponds", *Microbiology Ecology*, vol. 15, pp. 333–344, 1988.
[35]. M. G. Abu-Onisokyetu and U. Uwadirioha, "Comparative Study on Bacterial Load in Intestine, Gills and Skin of Cultured African Catfish (*Clarias gariepinus)* from Different Locations in Rivers State Nigeria", *International Journal of Innovative Studies in Aquatic Biology and Fisheries*, vol. 2, no. 3, pp. 21–29, 2016.
[36]. S. Sami, M. A. Al-Shabeeb, I. Mohamed and H. A. R. Ghamri, "A Comparative Microbial Quality Assessment among Fishes, Prawns and Cuttlefishes collected from Dammam Fish Market", *International journal of current Microbiology and Applied sciences*, vol. 5, no. 9, pp. 405–418, 2016.
[37]. F. C. Herrera, J. A. Santos, A. Otero and M. L. Garcia-Lopez, "Occurrence of Food borne pathogenic bacteria in retails prepackage portions of marine fish in Spain", *Journal of Applied Microbiology*, vol. 100, no. 3, pp. 527–36, 2006.
[38]. I. T. D. Evelyn, N. G. P. Lawana, M. F. Hamilton, H. M. S. Luiza and A. C. R. Manoel, "Microbiota of two species of commercially important fish in the Amazon region (Belem-Para-Gayil) Butterfly Peacock Bass (*Cichla ocellaris*) and Piramutaba (*Brachyplatystoma vailantii*)", *African Journal of Microbiology Research*, vol. 9, no. 9, pp. 572–580, 2015.
[39]. B. Austin and D. A. Austin, "Bacterial Fish Pathogens: Disease of Farmed and Wild fish", 4th ed. Springer-Praxis, London, England, 2007.
[40]. K. Neyts, G. Huys, M. Uyttendaele, J. Swingsm and J. Debevere, "Incidence and Identification of mesophilic *Aeromonas spp*. From retail foods", *Letters in Applied Microbiology*, vol. 31, no. 5, pp. 359–363, 2000.
[41]. H. Daskalov, "The importance of *Aeromonas hydrophila* in food safety. Food Control" vol. 17 no. 6, pp. 474–483, 2006.
[42]. H. Farag, "Incidence of Haemolysin producing Motile *Aeromonas* in some shell fish and their public health significance in Port-Said City", *Journal of Applied Sciences and Research*, vol. 2, pp. 972–979, 2006.
[43]. N. Latha and M. M Ramachandra, "The Bacterial Microflora in the Fish Organs-A Public Health Aspect", *Indian Journal of Advance Chemical Sciences*, vol. 1, no. 3, pp. 139–143, 2013.
[44]. S. O. Yagoub and T. M. Ahmed, "Pathogenic microorganisms in freshwater samples collected from Khartoum Central Market, Sudan", *Journal of Veterinary Sciences and Animal Husbandry*, vol. 43, no. 1-2, pp. 32–37, 2009
[45]. B. Wysok, U. Jan and G. P. Malgorzata, "Toxins occurring in fish, crustacea and shellfish-a review", *Polish Journal of Food and Nutrition Sciences*, vol. 57, pp. 1, 2007.

AUTHORS

First Author – Sibgha Farooq, PhD scholar,
Institute of Zoology, University of the Punjab, Lahore

Second Author/Corresponding author – Hafiz Abdullah Shakir,
PhD, Associate Professor (Tenured), Institute of Zoology,
University of the Punjab, Lahore.