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Comparative Analysis of Bacterial Flora in Different External Body Parts of Fishes Collected from Kabul River and Nowshera Fish Market, Pakistan

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ABSTRACT

Abstract

Fish is a major source of protein with a balanced nutrition ratio of essential components necessary for the human body. The main purpose of this study was to analyze the bacterial flora associated with three economically important fish species including *Hypophthalmichthys molitrix* (Silver carp), *Wallago attu* (Malhi), and *Labeo rohita* (Rohu), collected from the Kabul River and a fish market in District Nowshera, Pakistan. Comprehensive microbial analysis and biochemical tests identified several bacteria belonging to genus *Enterobacteriaceae*, including *Streptococcus*, *Salmonella*, *Staphylococcus*, *Klebsiella*, and *Escherichia*, in the sampled fish specimens. It identified that the river samples have a higher incidence of bacteria than the market samples. The study highlights the health risks associated with consuming river fish due to higher bacterial loads, which are most likely caused by inadequate handling, lack of sanitation and environmental contamination. Recommendations include improved water quality monitoring, increased regulation, better handling practices and public awareness campaigns, to reduce these risks. These finding highlight the need for

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improved management practices to ensure fish safety for human consumption.

Keywords: *Hypophthalmichthys molitrix*, *Wallago attu*, *Labeo rohita*, pathogenic bacteria, serial dilution, microbial analysis, biochemical tests.

INTRODUCTION

Fish are cold-blooded animal adapted to live in water, breathes through gills, and has paired appendages in the form of fins and scales for locomotion (Ali, 2018). It is a key source of protein, with a balanced nutrition ratio of essential elements required by the human body (Li *et al.*, 2022). Fish plays an important role in many aspects of human life, contributing to the world's food security, nutrition, and economic well-being. Fish is a valuable source of proteins, vitamins, fats and many other products such as fish oil, fish glue, fish meal, etc (Ahmad *et al.*, 2021). Fish also play an essential role in maintaining the equilibrium of aquatic habitats, contributing to biodiversity, controlling populations of other species, and acting as indicators of environmental health because of its lower cost and higher nutritional value, it is a significant source of foods derived from animals (Jothi *et al.*, 2018).

Exploring Pakistan's fish not only reveals the remarkable diversity found in its water bodies but also the necessity for long-term management and conservation activities to ensure their long-term existence. A wide range of aquatic species can be found in Pakistan's coastal and inland waters. Various species of fish are the part of aquatic diversity (Gondal *et al.*, 2012).

The global demand for fish reflects its vital role as a key source of nutrition, which contributes significantly to the global economy. With a growing worldwide population, the demand for fish has increased, due to its status as high-quality protein source, rich in omega-3 fatty acids and essential nutrients. It is one of the most important sources of animal-derived food products because of its lower cost and higher nutritious value (Jothi *et al.*, 2018). Over the last five decades, the increase in the global fish supply for human consumption has doubled to 3.2% as compared to population growth i.e. 1.11% (Giri, 2018). In the near future, seafood will become a vital source of protein in diets as it is important for the general well-being of humans, that aquaculture products are safe for their consumption (Jothi *et al.*, 2018).

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Fish spoilage is a significant concern leading to economic losses and health risks. Spoilage is defined as food degradation, making it tasteless, stinky and a bearer of numerous diseases for other organisms (Jan *et al.*, 2014). Increase in temperature can accelerate the growth of bacteria, which result in rapid degradation. Poor handling procedures at several stages i.e. from catch to processing, can accelerate the spoilage of fish. The off-odour and off-taste of the products produced by lipid oxidation and certain other metabolites may have a substantial influence on consumer acceptance while causing a range of diseases following the consumption of microbiologically damaged fish (Moini *et al.*, 2009). During post-harvesting of fish, approximately one out of every fourteen kilograms of fish spoils and is discarded as a result of inappropriate handling (Eyo, 1997). This is a major economic loss, and there is an urgent need to find the responsible bacteria and improve fish handling practices to reduce the loss of this vital food source caused by spoilage (Jan *et al.*, 2014).

Fish spoilage is mainly caused by the poikilothermic nature of fish, characterized by their dependency on external environmental temperatures to control their body temperature. Fish, unlike warm-blooded creatures, do not have a constant internal body temperature, making them highly susceptible to bacterial and enzymatic activity that accelerates spoilage (Gram and Huss, 1996).

Contaminants are increasingly recognized as a potential constraint on aquaculture farming and trade, creating health hazards in humans and massive financial loss through fish mortality or inferior meat quality, resulting in lower profit margins (Tanekhy, 2015). Beyond environmental pollution, the fish handling has a significant impact on their safety for consumption. Unhygienic handling and processing of fish most definitely introduces secondary bacterial contamination (Binta *et al.*, 1982). Contamination can occur at any stage, during capture, transport, storage, and even at markets (Begum *et al.*, 2010).

Unhygienic fish handling disturbs the delicate balance of its natural microflora, creating a pathway for contamination. Even naturally occurring microflora can become reactive by improper handling, which cause diseases in fish internal body organs that results in further contamination (Jan *et al.*, 2014). Overall, market conditions characterized by profit-driven targets and insufficient quality control procedures greatly increase the chance of fish contamination, posing

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considerable dangers to public health and strict actions are required to ensure food safety (Begum *et al.*, 2010).

Pathogenic organisms, including viruses, bacteria, fungus, and protozoa can cause these diseases (Darak and Barde, 2017). Fish spoilage on the large extent is caused by bacteria. About 70 species of bacteria have been identified as potentially harmful to humans (Yagoub, 2009). Bacterial pathogens are microscopic microorganism that cause fatal fish diseases. Their pathogenicity is associated with specific fish hosts (Darak and Barde., 2014). Although most people believe that newly caught fish are free of the contamination, bacteria were found in the muscles of freshly caught fish (Binta *et al.*, 1982). Bacteria are the most common fish pathogens, posing serious risks to the global aquaculture and fisheries business. These Infected fish ultimately end up in human food, causing a major threat to public health (Darak and Barde., 2014). Bacterial disease is of significant concern in all animal production systems for growth, and development of the aquaculture industry (Eleonor *et al.*, 2001). Interaction with unhealthy fish can not only expose people to germs but it can also spread bacteria that infect humans with diseases (Li *et al*, 2022).

The fisheries industry is an important source of income for Pakistan's coastal inhabitants. Aquaculture business is playing a key role in providing the increasing need for good-quality animal protein. Fish, an important source of protein, have a balanced nutrition ratio of essential components required by the human body (Li *et al*, 2022). It is a good source of protein, polyunsaturated fatty acids (particularly omega-3), calcium, zinc, and iron and economic importance highlights its diverse role in supporting livelihoods, promoting trade, and enhancing global food security and nutrition (Jothi *et al.*, 2018).

MATERIAL AND METHOD

Three fish species including, *Hypophthalmichthys molitrix* (Silver carp), *Wallago attu* (Malhi), and *Labeo rohita* (Rohu) were collected from both the Kabul River and the local fish market in Nowshera. Study was carried out at Kabul River which flows through district Nowshera in the Khyber Pakhtunkhwa province of Pakistan, situated approximately 27 miles (43 km) East of the provincial capital, Peshawar.

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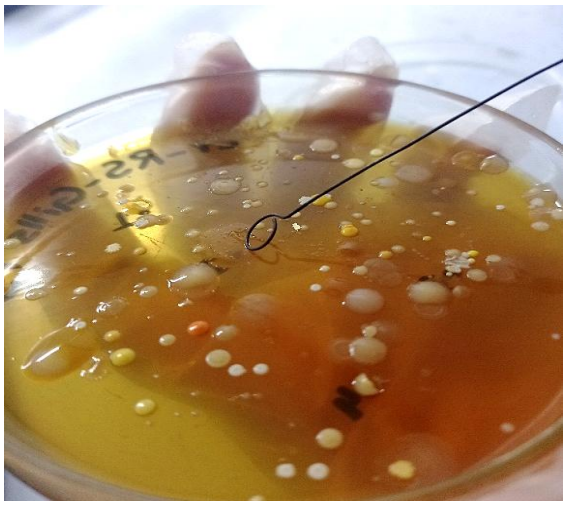
River fish samples were collected from Pir Sabaq, Kabul River, at depths ranging from 5 to 10 feet. Samples were collected by the current method using a wire net. The temperature was 26℃ during collection.

Market fish samples were collected from Nowshera main fish market during daytime at 8:15 am. Fishes were freshly brought to the market that morning. Market condition was good but no proper cold storage system was available in these shops. The temperature of market was 27.7℃ during collection.

Preparation of Sample

Fish samples were transported to the microbiology laboratory (PCSIR) under ice-cold conditions and stored in a refrigerator at 4℃. Fish external body parts like skin and gills were targeted. Streaking was performed under aseptic conditions using a sterilized stick swab through 2 methods: Plate swabbing method and serial dilution method. Serial dilution was preferred to use for sampling because in plate swabbing method, bacterial colonies were too numerous to count (TNTC) i.e. >400 CFU (colony forming unit). These streaked plates were placed in incubator at 37℃ for 24 hours to facilitate bacterial growth.



Number of colonies were counted through colony counter. Morphological parameters, including shape, elevation, margin and colour was examined manually under aseptic conditions of laminar flow hood (Sousa *et al.*, 2013).

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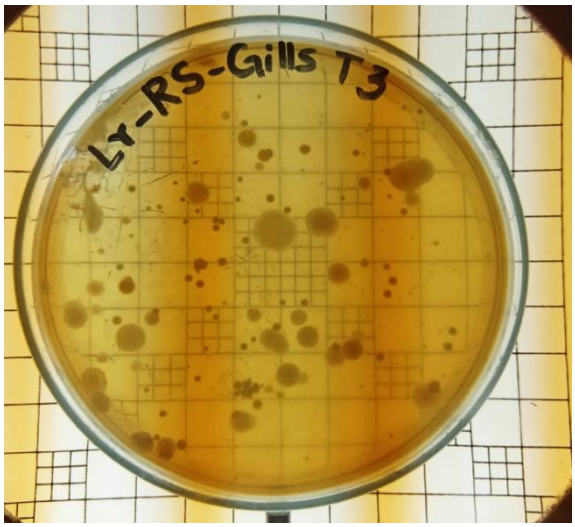

Culture Media

Mueller-Hinton agar was used for the culturing and identification of bacterial pathogens (Otajevwo and Osawaru, 2020).

Confirmation of Bacterial species by different tests

IMVIC test series of four biochemical tests such as the indole ring test, methyl red (MR) test, Voges-Proskauer (VP) test, catalase test, citrate utilization test was performed for the identification of bacteria. Gram staining was performed to confirm the identification of bacterial isolates.

RESULT

In present study,18 samples of fishes were collected including three individuals of each species from two localities: river and market. Through comprehensive microbial analysis and biochemical tests, several bacteria belonging to genus *Enterobacteriaceae* were identified, including *Streptococcus*, *Salmonella*, *Staphylococcus*, *Klebsiella*, and *Escherichia*, in the sampled fish specimens. The outcomes will be discussed concerning the various fish species studied.

Hypophthalmichthys Molitrix

According to CFU measurement, number of bacteria in *Hypophthalmichthys molitrix* was greater in gills of river sample (5.8×10^5). Through comprehensive microbial analysis and biochemical tests, several genera of bacteria were identified, including *Streptococcus*, *Salmonella*, *Staphylococcus*, *Klebsiella*, and *Escherichia*, in the sampled fish specimens. This study indicates that fish collected from markets have yielded more

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isolates than fresh fish pond samples.

Wallago Attu

As per CFU measurement, the number of bacteria in *Wallago attu* was greater in the skin of the market sample (15.5 x 10^6). Several bacterial genera, including *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Salmonella*, and *Escherichia*, were identified in the sampled fish specimens after extensive microbial analysis and biochemical testing.

Labeo Rohita

According to CFU measurements, a higher bacterial count (15.8 x 10^6) was observed on the skin of *Labeo rohita* from the market sample. Extensive microbial analysis and biochemical testing revealed that the sampled fish specimens included several bacterial genera, including *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Salmonella*, and *Escherichia*.

Biochemical test helped to identify suspected bacteria by comparing the results with an identification chart (Arefin 2014). In case of *Escherichia*, positive results were found in indole and MR reactions, whereas VP reaction and citrate utilization gave negative results. In case of *Klebsiella*, VP reaction showed positive result while MR reaction showed negative results and in case of *Salmonella*, citrate utilization showed positive result while indole showed negative results.

The Most prevalent bacteria found was *Streptococcus* whose occurrence was 16 in the river sample and 11 in the market sample. *Streptococcus* was followed in terms of incidence by *Escherichia* having an occurrence of 14 in river sample and 11 in market sample. The least abundant bacteria were *Staphylococcus*, whose total occurrence was 7 in both river and market samples.

Table 1: ***Comparison between Market Sample and River Sample***

S.No	Isolated Bacteria	Market Sample		River Sample	
		Frequency	Percentage	Frequency	Percentage
1.	*Staphylococcus*	7	14.5%	7	11.3%
2.	*Streptococcus*	11	22.9%	16	25.8%
3.	*Escherichia*	11	22.9%	14	22.5%
4.	*Klebsiella*	10	20.8%	11	17.8%

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5.	*Salmonella*	9	18.7%	14	22.5%
		∑=48		∑=62	

Table 1 shows that ***Staphylococcus*** was isolated from 7 out of 48 cultured plates of the market sample, representing the Relative frequency of 14.5%. In the river sample it was isolated from 7 out of 62 cultured plates, representing the frequency of 11.3% of total isolated bacterial flora.

Streptococcus was isolated from 11 out of 48 cultured plates of the market sample, representing the Relative frequency of 22.9%. In the river sample it was isolated from 16 out of 62 cultured plates, representing the frequency of 25.8% of total isolated bacterial flora.

Escherichia was isolated from 11 out of 48 cultured plates of the market sample, representing the Relative frequency of 22.9%. In the river sample it was isolated from 14 out of 62 cultured plates, representing the frequency of 22.5% of total isolated bacterial flora.

Klebsiella was isolated from 10 out of 48 cultured plates of the market sample, representing the Relative frequency of 20.8%. In the river sample it was isolated from 11 out of 62 cultured plates, representing the frequency of 17.8% of total isolated bacterial flora.

Salmonella was isolated from 9 out of 48 cultured plates of the market sample, representing the Relative frequency of 18.7%. In the river sample it was isolated from 14 out of 62 cultured plates, representing the frequency of 22.5% of total isolated bacterial flora.

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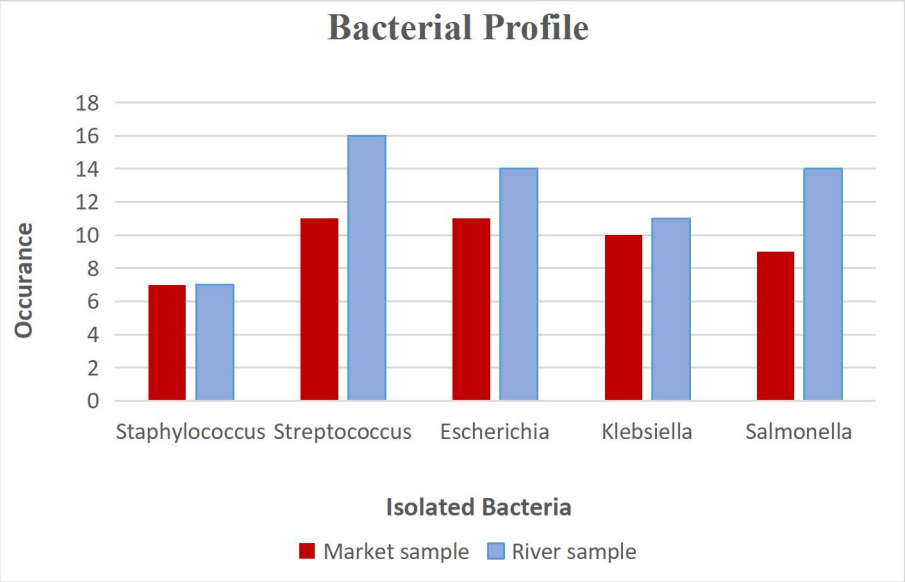
Statistical Analysis



Cross Tabulation

Source of Sample * Cross tabulation

			Isolated Bacteria					
			Staphylococcus	***Streptococcus***	***Escherichia***	***Klebsiella***	***Salmonella***	**Total**
Source of Sample	**Market**	**Count**	7	11	11	10	9	**48**
		Percentage %	50.0%	40.7%	44.0%	47.6%	39.1%	**43.6%**
	River	**Count**	7	16	14	11	14	**62**
		Percentage %	50.0%	59.3%	56.0%	52.4%	60.9%	**56.4%**
Total		**Count**	14	27	25	21	23	**110**
		Percentage %	100.0%	100.0%	100.0%	100.0%	100.0%	**100.0%**

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Chi Square Test

Chi-Square Test

	df	P Value
Pearson Chi-Square	4	.957
Likelihood Ratio	4	.957

Interpretation

As P-value is greater than Alpha= .05. We cannot reject H0 so we conclude that there is no association between the two variables and the type of bacteria is independent of source of fish. There is no difference, either the data is taken from Market or River.

DISCUSSION

A comprehensive microbial analysis and biochemical tests on the fish's skin and gills procured from the river and the market revealed the presence of bacteria from the Enterobacteriaceae family, including *Streptococcus*, *Salmonella*, *Staphylococcus*, *Klebsiella*, and *Escherichia*. Results are quite close to the findings of Jan et al., 2014 which indicated the presence of *S. aureus*, *S. epidermidis*, *E. coli*, *Pseudomonas* and *Klebsiella spp.*

The statistical analysis shows no association between the bacteria identified from a river or a market, meaning that both localities have the same bacterial abundance. However, the river samples have notably higher incidence of bacteria than the market samples. *Escherichia* and *Staphylococcus* were the most commonly identified pathogens, followed by *Klebsiella* and *Streptococcus*, with *Salmonella* were also being detected, primarily from the Enterobacteriaceae family.

It is recommended to implement regular and comprehensive river water quality monitoring to identify and address sources of bacterial contamination. Fishermen and handlers should be educated on the best practices for fish handling and storage to reduce bacterial contamination. This includes proper sanitation, the use of clean equipment, and keeping temperatures appropriate during storage and transportation.

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CONCLUSION

A comprehensive microbial analysis and biochemical tests on the fish's skin and gills procured from the river and the market revealed the presence of bacteria from the Enterobacteriaceae family, including *Streptococcus*, *Salmonella*, *Staphylococcus*, *Klebsiella*, and *Escherichia*.

The statistical analysis shows no association between the bacteria identified from a river or a market, meaning that both localities have the same bacterial abundance. However, the river samples have notably higher incidence of bacteria than the market samples. *Escherichia* and *Staphylococcus* were the most commonly identified pathogens, followed by *Klebsiella* and *Streptococcus*, with *Salmonella* were also being detected, primarily from the Enterobacteriaceae family.

The reason behind this might be that the river fish are more viable to bacteria as compared to market where conditions are well maintained. There might be some flaws during collection because market fish were collected in the morning time when fishes were freshly brought to the market. So, less bacterial isolates in market fish shows that there might be chances that the fish was not exposed to the environment of market. Another reason might be the poor sanitation standards or poor sewage disposal methods within river which expose the fishes in the river with more bacterial burden a described by Donde *et al.* (2014) as compared to market fish which are raised in maintained conditions of hatchery.

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