Spoilage Causing Fish Skin Bacterial Flora

Zaigham Hasan[*], Urooj Syed[†], Muneer Ahmad Khan[‡]

Abstract

To check for the presence of skin-related bacteria that could lead to fish spoiling, fish samples are taken from Peshawar's Ghanta-ghar market. Blood agar medium, MacConkey agar, Pseudomonas medium, nutrient agar medium, and Manitol salt agar medium are among the media used to cultivate bacteria. A variety of biochemical tests, including those using coagulase, citrate utilisation, oxidase, hemolysis, indole, catalase, and gramme staining, are performed to identify and characterise the morphological features of the microbial isolate. Fish skin is home to five distinct human bacterial infections, including Escherichia coli (E. coli), Pseudomonas, Klebsiella, Staphylococcus aureus, and Klebsiella epidermidis. Staphylococcus aureus, Cyprinus carpio, Hypothalmychthys molitrix, Tor putitora (T. putitora), and Wallago attu (W. attu) are isolated from the skin of Labeo rohita (L. rohita). Staphylococcus epidermidis is also discovered on W. attu and L. rohita's skin there. Spp.Pseudomonas, E. coli, and Bacillus-like Klebsiella are recovered from the skin of T. putitora, Hypothalmichthyes molitrix, W. attu, Cyprinus carpio, and L. rohita. It is believed that these bacteria are the source of fish spoiling at Peshawar's Ghanta-ghar fish market.In addition to causing fish to decay, the bacteria on fish skin also causes a number of serious illnesses in humans.

Keywords: Spoilage; Microbial; Ghantaghar; Pathogens; Causative Agent.

Introduction

Products from the fishing industry are very important and play a major role in the nation's economy (Hasan & Khan, 2011).The first step in preventing fish from quickly spoiling and maximising its benefits is to boost the demand for fish eating in line with the world's population growth. A metabolic process known as spoilage results in food that is no longer suitable for human consumption because of differences in its nutritional and sensory qualities (Doyle, 2007).

Ali et al. (1992) have conducted research showing that *Aeromonas*, a significant spoiling organism, is found on the skin and gills of *L. rohita* and carp. They stated that bacterial pathogens such as *Staphylococcus*, *Acinetobacter*, *Micrococcus*, *Enterobacteriaceae*, *Aeromonas, Pseudomonas, and Moraxella* are present in the micro flora

[*]Corresponding Author: Department of Zoology, University of Peshawar, Peshawar 25120, Pakistan, zaigham@uop.edu.pk

[†] College of Home Economics, University of Peshawar, Peshawar 25120, Pakistan, uroojsyed@uop.edu.pk

[‡] Department of Zoology, Govt. College Sabir Abad, Karak 27000, Pakistan, muneerzoology@yahoo.com

of recently collected freshwater fish. According to Ghaly et al. (2010) the three fundamental mechanisms via which fish deteriorates: Microbial growth, oxidation and enzymatic breakdown.

Fishes in our nation are treated carelessly both in the marketplace and in transit. Fish are farmed, harvested, and sold for food, but there are a lot of issues in this whole process. Fish spoils and loses value due to bacterial and other reasons, which also result in financial losses.Sanaa & Yaqoub (2009) recovered *Enterobacteriaceae* from the skin, gills, intestine, and muscles of 83 (55%) of the 150 randomly picked fish. *Klebsiella spp., E. coli, Enteriobacter spp*., and *Citrobacter spp*. are the most common isolates. Discoveries also included the highly pathogenic *Enterobacteriaceae,* which include *Shigella pp., Alklegens species, Salmonella species,* and *Proteus species.* Randomly, *pseudomonas spp.* is isolated from 62% of the fish that are gathered. We examined the percentages and quantities of the isolated microorganisms by season. All of the sample portions that we collected are used to estimate the coliform count, total bacterial count, and *E coli* count. We also talked about how the presence of *Pseudomonas* species and *Enterobacteriaceae* in fish could be harmful to public health because of their tendency to cause fish to spoil more quickly.

Dhanya & Mathew (2017) examined how fish microbiological deterioration is caused by both Gramme positive and Gramme negative bacteria, such as *Micrococcus*, and bacteria such as *Moraciella, Flavobacterium, Vibrio, Acinetobacter, Pseudomonas, Shwenella, and Photobacterium.Bacillus, Lactobacillus, and Clastridium.*Fish can contain variable amounts of coryneforms, which cause spoiling. Micro flora of marketed *Rohu* are studied by Sinha et al. (1991). *Aeromonas, pseudomonas, E. coli, Salmonella, Staphylococcus, Micrococcus, Proteus, Streptococcus, moulds, and Klebsiell*a are among the microorganisms he observe on the fish surface. Therefore, the goal of the current investigation is to pinpoint the germs that harm our fish for sale and provide a health risk to consumers who consume them.

Materials And Methods

A study is conducted in the Ghanta Ghar fish market in Peshawar, which is situated on a city road about ten kilometres away from the Pakistan Council of Scientific & Industrial Research (PCSIR) Peshawar laboratory. The fish store is chosen next to the roadside, where the fish are not in optimal condition to be kept for a long time before being sold. Additionally, many stores lacked a suitable cold storage system. The Ghanta Ghar fish market is a busy, crowded, dirty, and unhealthful area,

especially in the winter when the temperature starts to drop and there is a greater risk of coming into contact with contaminated fish.

Every week, fish are collected in the morning or evening from the designated study area *(Ghanta ghar)*. Market temperatures vary during the study periods, ranging from 15°C to 21°C. Two different kinds of fish samples are gathered; some looked to be fresh and showed no signs of rotting, while others are showing signs of early decomposition, such as blue gills and rough skin, among other symptoms.

After being gathered from *Ghanta ghar*, samples are transported in polythene bags to the PCSIR (Pakistan Council of Scientific & Industrial Research) laboratory in Peshawar within 20 minutes by cab in order to identify the bacterial flora on their skin. The fish are taken to the University of Peshawar's Department of Zoology for identification after being initially collected for germs in the PCSIR Laboratory. There, the morphometric and meristic features are examined, and with the aid of various standard books and fish keys, the fish are isolated up to the species level. Assays for biochemical processes such as *hemolysis, citrate utilisation, indole, oxidase,* and *catalase.* To identify and examine the morphological features of the microbial isolate, Gramme staining is used. A nutrient agar medium is utilised for the cultivation of mixed bacterial pathogens. For the purpose of identifying the microflora on fish skin, selective media such as Agar medium, *MacConkey, Manitol salt agar*, *Pseudomonas agar medium, Blood agar EMB medium*, and *Simmon citrate medium* are also employed. Fish samples that are chosen are brought to the lab and kept fresh in an agar dish. Subsequently, little fragments of fish itegument skin, about 1-3 cm in size, are removed from the head, tail, or abdomen and stored in a fumigated petri dish in a sterile Laminar Air Flow environment.

Initially, bacteria are isolated using a cotton swabbing method, although contamination made this approach unsuccessful. After that, the chosen fish are carried straight to the laboratory, where they are stripped using an aseptic cotton swab on the prepared substrate. Laminar air flow is utilised to achieve the streaking after separating. To disinfect the Laminar Air flow, 70% alcohol is sprayed on it initially. A sterile stick swab is used to aid with the streaking operation. After being fully rubbed with the sample, the stick swab is immersed in peptone solution. Following that, it is streaked over the prepared media and subjected to nurture the bacteria for 24 hours at 35°C in an incubator.

Results And Discussion

In order to discover if bacteria are ubiquitous on the skin of certain fish species, 15 fish samples, namely *T. putitora, L. rohita, W. attu*

Hypothalmychthys molitrix, and *Cyprinus carpio*, are obtained from the *Ghanta gha*r vend site. There have been reports of five distinct bacterial species, including *E. coli, Pseudomonas, Klebsiella, Staphylococcus aureus, and Bacillus species.* Fish samples are obtained from their heads, abdomens, and tails. When the samples are taken in December of 2014, the temperature ranged from 15 to 21°C, while the humidity stayed between 52 and 70%.

The bacteria are found in fish skin that has been processed improperly or stored in unsanitary settings. It is discovered on *W. attu, Ctenopharangodon idella, Hypopthalmychthys molitrix, T. putitora, and L. rohita.* These fish become filthy as a result of it. On *T. putitora, W. attu, Hypopthalmychthys molitrix, Cyprinus carpio, and L. rohita, E. coli* is identified.

Table1. ***Different modes used for recognization of unlike bacterial strain on fish's skin.***

S.No.	Species	Media used for Culturing of Bacterial Species				Spps Names
		EMB	Mac Conkey	Pseudomonas	Manitol	
1.	*L. rohita*	+ve	+ve	-ve	+ve	*S. aureus, E. coli, pseudo*
2.	*T. putitora*	+ve	+ve	+ve	-ve	*E. coli, S. epidermidis, Klebsiella*
3.	*L. rohita*	+ve	+ve	+ve	-ve	*Klebsiella, E. coli, S.epidermidis*
4.	*W. attu*	+ve	+ve	+ve	-ve	*Klebsiella, S.epidermidi, E. coli*
5.	*L. rohita*	+ve	+ve	-ve	-ve	*E.coli, S.aureus*
6.	*H. molitrix*	+ve	+ve	-ve	+ve	*Pseudo, S.epidermidis, E..coli*
7.	*C. carpio*	+ve	+ve	+ve	-ve	*S.aureus, Klebsiella, E..coli*
8.	*W. attu*	+ve	+ve	+ve	-ve	*Klebsiella, E. coli, S.aureus*
9.	*T. putitora*	+ve	+ve	-ve	+ve	*Pseudo spp, S.aureus, E..coli*
10.	*L. rohita*	+ve	+ve	-ve	+ve	*S.aureus, Pseudo, E. coli*
11.	*T. putitora*	+ve	+ve	+ve	-ve	*S.aureus, E. coli, Klebsiella*
12.	*L. rohita*	+ve	+ve	-ve	+ve	*S.epidermis, Pseudo spp, E..coli*
13.	*H. molitrix*	+ve	+ve	-ve	+ve	*S.aureus, Pseudomonas,E. coli*
14.	*C. carpio*	+ve	+ve	+ve	-ve	*S.aureus, Klebsiella, E. coli*
15.	*W. attu*	+ve	-ve	+ve	-ve	*Klebsiellsa, S.aureus*

Table 2:*Prevalance of bacteria on different fish species.*

S.No.	Bacterial spp	*L.rohita (5)*	*H.molitrix (2)*	*C. carpio (2)*	*T. putitora (3)*	*W. attu (3)*	% prevalence
1.	*E. coli*	5	2	2	3	2	100.00
2.	*Klebsiella*	1	0	2	2	3	53.13
3.	*Pseudo spp*	3	2	0	1	0	40.00
4.	*S.aureus*	3	1	2	2	2	66.66
5.	*S.epidermidis*	2	1	0	1	1	33.33

Shagufta and Jafri conducted the same investigation in 1988. *S. aureus and S. epidermidis* are the germs they found on the skin of *L. rohita* and *Cirrhinus murgala*. The same germs are found on *L. rohita's* skin in the current investigation.

A comparable study on the microbial diversity and alterations to the deteriorating microflora of Ice Ilaska Pink Salmon is conducted by Morey et al. (2014) who documented the presence of aerobic bacterial populations, including *Shewanella putrefaciens, Pseudomonas fluorescens, P. putida, Moraxella, Psychrobacter, Acidovorax, and Brevundimonas. Here, P. putida, Pseudomonas* fluorescens formed a major and *Shewanella putrefaciens* incorporate the minor percentage of the spoilage flora, while the *revundimonas* and *Acidovorax* species are observed as a portion of the fresh fish microflora. *Psychrobacter, Moraxella, and Acientobacter* are only related with fish gills and skin.

In another noteworthy study, *Klebsiella spp*. are isolated in the hands of Akinyemi & Buoro (2011). *Proteus* species. *Staphylococcus aureus, Citrobacter species,* etc. *Streptococcus pyogenes, Micrococcus species,* and *Salmonella species. Shigella species, Pseudomonas aerugenosa, Alcaligenes species, Bacillus licheniformis, Enterobacter aerogenes, E. coli*, and *Serratia species*. Similar to this, the bacteria that is recovered in current investigation included *E. coli, Pseudomonas species, Klebsiella species, Staphylococcus aureus, and Staphylococcus epidermidis.*

In addition to causing fish to deteriorate, the bacterial flora on fish skins also causes serious illnesses in humans, such as respiratory and digestive system damage and other skin conditions. Our study matched with Jan et al. (2014) who investigated fish skin germs in Peshawar's Board Fish Market and identified five different bacterial species: *E. coli, Klebsiella species, Pseudomonas species, Staphylococcus aureus, and Klebsiella epidermidis.* Maullet et al. (2012) furthermore described the related bacterial species, including the *Pseudomonas, Shewanella, Bacillus, Aeromonas, Myroides,* and *Enterobacter species*. They are found to have had a part in the spoiling of the catfish.

Another notable work Bacterial Micro flora of Fish, Revised is done by Austin (2006) in Scotland and *Aeromonads* (especially *Aeromonashydrophila, A. bestiarum, Acinetobacter johnsonii, A. caviae, A. jandaei, A. schubertii, and A. veroniibiovarsobria*), *Alcaligenespiechaudii, Flexibacter spp, E. coli, Flavobacterium, Vibrio fluvialis, Micrococcus luteus, Enterobacteraerogenes Moraxella spp, Psychrobacters,* and *Pseudomonas fluorescens* have been reported to harbouring a variety of fish surface bacterial species. Only two of the

indicated bacterial species—*Pseudomonas* and *E. coli*—are included in our current survey.

A similar study is also conducted by Al-Harbi & NaimUddin (2003) have studied the bacterial flora of hybrid *tilapia* (*Oreochromisniloticus & O. aureus*) grown in earthen ponds in Saudi Arabia, have reported 15 bacterial genera and 18 species, including *Pseudomonas* species and *E. coli* species that are studied in this study. Their findings have been reported both quantitatively and qualitatively. Commensal bacteria, including , *E. coli*, are found in the digestive system, according to the findings of the bacteria isolation procedure. Conversely, *S. aureus* originates from external sources. because there's a chance the isolated bacterium may contaminate seafood and result in foodborne disease. In this case, eating fresh fish or partially cooked fish should be prohibited in order to eradicate zoonotic infections from fish, especially in this location. Thus, the goal of this study is to provide fundamental knowledge about these bacteria that might result in food-borne disease when they are present in fish that is ready to eat.

Table 3: Comparison of bacterial pathogens with others studies.

Sample	Shagufta [1988]	Sadaf [2012]	Sehrish [2012]	Present Study [2014]
L.rohita	*S.aureus, Aerobacter*	*S.aureus, Klebsiella, E..coli, S.epidermidis, Pseudo*	*Klebsiella, S.aureus, S.epidermidis, E. coli*	*S.aureus, Klebsiella, S.epidermidis, Pseudo, E. coli*
W.attu	*S.aureus, Aerobacte-r, Salmonella*	*S.aureus, Klebsiella, E. coli, S.epidermidis, Pseudo*	*S.epidermidis, S.aureus, Klebsiella Pseudo, E. coli*	*S.aureus, Klebsiella, S.epidermidis, Pseudo, E..coli*
Cyprinusc arpio	_	*S.aureus, Klebsiella, E..coli*	*S.epidermidis, S.aureu, Klebsiella, E..coli*	*S.aureus, Klebsiella, S.epidermidis, Pseudo, E. coli*
Hypopthal michthysm olitrix	_	*S.aureus, E..coli, Klebsiella, Pseudo*	*Klebsiella S.aureus.*	*S.aureus, Klebsiella, Pseudo, E. coli, S.epidermidis*
T. putitora	_	*Klebsiella, Pseudo, E..coli, S.aureus*	*S.aureus, S.epidermidis, Klebsiella, Pseudo, E. coli*	*Klebsiella, S.epidermidis, E..coli, S.aureus Pseudo*

Conclusion

The present study's findings showed that the market's inadequate sewer infrastructure, unchecked pollution, and tainted fish-spraying water all delayed the development of the bacteria found in fish skins. Vehicle smoke, dust, etc. are the cause of the pollution. The primary causes of contamination, according to the study, are similarly poor storage facilities after collection and filthy circumstances all through processing. The present study also confirms that human pathogenic bacteria occur in fish populations in both marine and mountainous ecosystems as well as in their natural habitat and open market. To stop germs from growing, fresh fish must be cautiously kept at low temperatures.To reduce the risk of bacterial illnesses, fish from the local market must be harvested and handled properly.Fish skin should not be sprayed with contaminated water. Pollution in the designated area can be reduced to stop the spread of these microbes. If the fisheries department intends to reduce the unhygienic practices and stern handling linked to container operations, it must collaborate with processing businesses to produce a proper design and regulation for insulated containers. As a result, the enormous fish holds in the insulated containers won't crush as many fish or handle them harshly.

References

Akinyemi, A.A. and Buoro, O.O. 2011. Occurrence of Bacteria Found in Gills, Skin, Buccal Cavity of *Lutjanus agennes*, *Pseudotolithus elongatus* and *Sphyraena barracuda* from Lagos Lagoon, Nigeria. *Journal of Fisheries and Aquatic Science*, 6,555-562.

Al-Harbi and NaimUddin. 2003. Quantitative and Qualitative studies of bacterial flora of hybrid tilapia (*Oreochromis niloticus* & *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture Research* 34, 43-48.

Alisa and Krunasagar, I. 1992. Bacteriological changes during iced storage of the tropical freshwater carp. *L. rohita*. *Fisheries Research*.,13, 189-197.

Austin, B. 2006. The Bacterial Microflora of Fish, Revised, *Scientific World J.* 11(6), 931-945 (ISSN 1537-744X; DOI 10.1100/tsw.181).

Dhanya, P.R., and Saleena, M. 2017. Microbial Spoilage in Fish. *Imperial journal of Interdisciplinary Research* 3(4), ISSN: 2454-1362, http://www.onlinejournal.in

Doyle, E. M. 2007.Microbial Food spoilage- Losses and Control Strategies. Food Research Institute, University of Wisconsin - Madison, WI 53706

Ghaly, A.E., Dave, D., Budge, S. and Brooks, M.S. 2010. *American Journal of Applied Sciences* 7(7): 859-877

Jan, A., Hasan, Z., Shah, H., RoohUllah, Ahmad, I. and Younas, M. 2014. An Investigation of the Bacterial Flora Causing Spoilage of Fishes at Board Fish Market, Peshawar, Pakistan *Pak. J. Zool.*, 46(5), 1371-1375.

Javed, S. 2012. Isolation and identification of Bacterial pathogens on the skin of fishes, causing spoilage, A Case Study of Selected Markets of Hayatabad, Peshawar. M.Sc. thesis. SMA Seminar Library, Department of Zoology, University of Peshawar.

Khan, M.A. and Hasan, Z. 2011. A Preliminary survey of fish fauna of Changhoz Dam, Karak, K.P.K. Pakistan. *World Journal of Fish and Marine Sciences.* 3(5), 376-378.

Maull, K.D., Hickey, M.E., Lee, J.l. 2012. The Study and Identification of Bacterial Spoilage Species Isolated from Catfish during Refrigerated Storage. *J Food Process Technol* 11(3), 1-5 (doi: 10.4172/2157-7110.S11-003)

Morey, A., Himelbloom, H. B, and Oliveira, C.M.A. 2014. Bacterial Diversity and Changes towards Spoilage Microflora of Iced Alaska Pink Salmon. *J Nutr Health Food Eng* 1(1), 25-29.

Shagufta, K. and Jaafri, R. H. 1988. Biologia. Vol, 34 (2): 329-334.

Yagoub, S.O. 2009. Isolation of *Enterobacteriaceae* and *Pseudomonas spp.* from raw fish sold in fish market in Khartoum state. *Journal of Bacteriology Research*, 1(7), 085-088.

Sinha, D. K., Choudhary, S. B. and Narayan, K. G. 1991.Microbiological characteristics of marketed rohu (*L. rohita*). *Ind*