Isolation and Identification of the Spoilage Causing Microorganisms in Fishes Collected from Local Markets of District Charsadda Khyber Pakhtunkhwa, Pakistan

Muhammad Idrees[*], Danish Ahmad[†], Muhammad Sohail[‡], Basit Ali[§], Salman Khan[**]

Abstract

In current study microorganism causing the spoilage was isolated from two important fish species, available commercially in local market of District Charsadda, Khyber Pakhtunkhwa, Pakistan. A total 50 samples of fish were collected for analysis, consisting 25 samples of Clupisoma naziri and 25 samples of Cyprinus carpio. In these 50 samples 30 were cooked, 10 were raw and 10 were frozen fish. The bacterial isolates from these samples were identified microscopically and biochemically. Kirby-Bauer disk diffusion method was performed to check the antibiotic sensitivity. The antibiotic resistance test showed different resistance rate to different antibiotics. Among all antibiotic the highest resistance was recorded to commonly used antibiotics including penicillin, ampicillin, erythromycin and cephalosporin. The highest resistance was recorded for penicillin (40%) with sensitivity of 60%, while a moderate resistance was recorded for chloramphenicol (30%), ampicillin (30%), erythromycin (30%) and neomycin (25%) respectively. The antibiotic tetracyclin, gentamycin, amoxillin and streptomycin recorded highest sensitivity of 90%, 90%, 85% and 80%. Different preservation techniques were evaluated in this study that effect the microorganism's, resulting in reduction of microbial load by up to 95% in both species by cooling technique. Similarly, the drying and freezing techniques were also resulted in reduction of microorganism development. These findings highlighted different preservations techniques and antibiotic resistance in fish products to handle the fish products and enhance food safety worldwide.

Keywords: *Clupisoma naziri*, *Cyprinus carpio*, Antibiotic Susceptibility, Preservation Techniques, Bacterial Isolation, Fungal Identification, Disk Diffusion Method.

Introduction

Fish plays an important role in the world food supply, providing high source of protein worldwide. Additionally, it's significant to note that in many developing countries around 17% of their protein intake comes

[*]Department of Biotechnology, University of Swabi, Swabi 23561, Pakistan, midress@uoswabi.edu.pk

[†]Department of Health and Biosciences, Abasyn University, Peshawar 25000, Pakistan, danishookhan999@gmail.com

[‡]Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan, msohail818283@gmail.com

[§]Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan, basitali75543@gmail.com

[**]Corresponding Author: Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan, salmank.biotec@awkum.edu.pk

from fish, making it a vital source of nutrition for those populations. However, fish can rot quickly if it is not handled and stored correctly because of its very perishable nature. Fish rotting is a serious issue that puts public health at risk by causing food waste and an increase in foodborne infections (Adedeji et al., 2012).

The largest group in the kingdom of animal is made of fish, with a population of more than 30,000 known species which is used for the production as animal-based foods. Commercially about 700 species used for the production of different food products. Nowadays to identify the spoilage causing organism, different molecular techniques like next-generation sequencing, 16S ribosomal RNA (rRNA) sequencing and Polymerase Chain Reaction (PCR) are used (Migaou et al., 2024).

Previously researchers have identified different microbial communities that cause spoilage of fish from infected fish samples. The bacterial communities were identified through 16S rRNA that is conserved region of bacteria. Different fish samples were collected from local markets of Pakistan and bacteria responsible for causing spoilage to fish were identified. The results showed that Vibrio caused spoilage to fish at room temperature, while *Pseudomona*s and *Shewanella* were reported to cause spoilage at low temperature (Chen et al., 2023). Different microorganisms were identified in different species of fish including *Shigella dynteriae*, *Clostridium botulinum* and *Salmonella*. The highly pathogenic bacteria to fish include *Vibrio spp*., *Mycobacteium*, and *Aeromonas spp*., (Zin Eldin et al., 2023).

It is important to identify the spoilage causing microorganism to take better steps towards controlling the spoilage of foods. In current era different quick methods like RNA sequencing and PCR are key methods to identify these microorganisms precisely. By identifying such bacterial species causing fish spoiling different control strategies can be develop for food safety (Nawaz et al., 2022).

Different environmental factors affect the rotting of fish such as humidity, temperature and oxygen availability. According to research conducted in the area of Charsadda, the rising temperature causes the shortage of fish shelf life and degrade more quickly. The oxygen availability is also an important factor for rotting of fish. Under normal air condition the rotting of fish worsen due to the growth of aerobic bacteria such as *Pseudomonas spp*., due to the high oxygen. While in low oxygen such as vacuum packed the anaerobic bacteria grow in fish products. The microbial community of fish highly dependent on the storage techniques and the packing material (Luqman et al., 2024).

Beside the financial losses the spoiling of fish also causes a major risk to public health. Most of the fish market like Charsadda don’t have

refrigeration facilities leading to severe spoiling of fish for the buyers and sellers. Due to rapid spoilage of fish cause by microbes leads to significant loses of the markets. The food infection also causes severe gastrointestinal problem to the people consuming such food. Therefore, it is highly demanded to apply proper methods to control the spoilage of fishes and safeguarded the public health (Rashid et al., 2025).

Different conventual techniques like salting, smoking and dry are still applied by people to preserve the fishes. These methods are simple and don't require any high and expensive equipment's. The salting technique increases the osmotic pressure and makes the environment unfavourable for microorganism to grow, but this technique may encourage the salt tolerance bacteria like *Aspergillus* and *Penicillium* to grow and produce mycotoxins (Tarroum et al., 2022). The aim of the current research work is to investigate the spoilage causing microorganisms in different types of fish collected from local market of Charsadda and to evaluate drug susceptibility of isolated microorganism from *Cyprinus carpio* and *Clupisoma naziri*, as well as to compare different techniques for fish preservation.

Methodology

Study Area

The study was carried in the district Charsadda, Pakistan (Figure 1). Local fish markets in this area are well-known for offering a wide selection of both processed and fresh fish. This area was selected because of its wide variety of fish products and the possibility of significant microbiological contamination as a result of different preservation techniques.

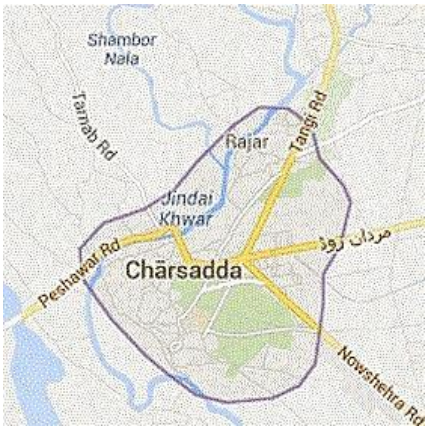

Figure 1: Map of District Charsadda KP, Pakistan.

Fish Species and Sample Collection

The study was conducted in the District Charsadda, Pakistan. All processes were carried out in compliance with the ethical standards for both human and animal subjects in research. The appropriate consent was acquired before any samples were collected, and all procedures followed ethical and safety guidelines. Fish species i.e. *Cyprinus carpio* and *Clupisoma naziri* (Fresh, freeze and cooked fish) collected from local markets were included in the study (Figures 2 and 3). Other fish species were excluded in this research study. Fish sample preserved for more than week were excluded from the study. A total of fifty fish samples were collected from local markets in District Charsadda, consisting of twenty-five samples each of *Clupisoma naziri* and *Cyprinus carpio*. The samples included thirty cooked, ten frozen, and ten raw fish. Table 1 shows the distribution of samples by fish type and condition, indicating a balanced representation of both species.

Table 1: Summary of fish samples collected from local markets.

Fish Type	Sample Condition	Number of Samples
Clupisoma naziri	Cooked	15
	Frozen	5
	Raw	5
Cyprinus carpio	Cooked	15
	Frozen	5
	Raw	5

Figure 2: Clupisoma naziri.

Figure 3: Cyprinus carpio.

Sample Processing

To avoid contamination, samples were processed in an aseptic manner in the laboratory. Petri dishes were used to hold individual fish samples. To isolate bacteria, samples were streaked over a variety of

substrates using sterile stick swabs. This process adhered to the guidelines set out by (Svendsen, 2021).

Inclusion and Exclusion Criteria

Fish species i.e. *Cyprinus carpio* and *Clupisoma naziri* (Fresh, freeze and cooked fish) collected from local markets were included in the study. Other fish species were excluded in this research study. The prevalence of bacteria and fungi were evaluated while viruses and parasites are excluded from the study. Fish sample preserved for more than week were excluded from the study.

Isolation of Bacteria

Samples were streaked onto Nutrient Agar (NA) plates in order to isolate microorganisms. A large variety of bacteria may grow on nutrient agar, facilitating the first stages of isolation. After being incubated for 24 to 48 hours at 37°C, isolated colonies were then sub cultured into specific media (Cheung et al., 2016). MacConkey agar is a selective and differential medium used primarily to isolate and differentiate Gram-negative bacteria, especially enteric bacteria. The crystal violet and bile salts of this inhibit Gram-positive bacteria. It also contains lactose and a pH indicator, neutral red, to differentiate lactose fermenters (pink/red colonies) from non-fermenters (colourless colonies) (da Silva Araújo, 2022). For the isolation of staphylococci especially staphylococcus mannitol salt agar media was used (Hardy et al., 2020). For the isolation of Gram-negative bacteria Eosin Methylene Blue agar was used. The Gram-positive bacteria growth was inhibited by methylene blue and dye eosin. The dark purple colour produced due to lactose fermentation while colourless colonies indicate no fermentation (Subhi et al., 2017). For the growth of wide range of bacteria Blood agar media was used that is rich media for all bacteria. This media contains red blood cell and is used to detect bacteria that are breaking down the red blood cells (Ribault et al., 2005).

Isolation of Fungi

For the isolation of fungus like *Aspergillus* and *Penicillium* from rotted fish samples potato Dextrose media was used. The plates were stored at 28°C for seven days (Hashem, 2011).

Morphological and Biochemical Identification

The morphological examination of bacteria was performed by gram staining. Based on the cell walls gram negative and gram-positive

bacteria were identified, while other biochemical examination was performed by following the protocol (Alhadrami et al., 2021).

Catalase Test

The bacteria producing enzyme catalase that break down hydrogen peroxide into water and oxygen was performed by a biochemical test. When bacterial culture exposed to hydrogen peroxide the bubbles were appeared that is due to the release of oxygen by the presence of catalase. This test was used to differentiate the catalase negative and catalase positive bacteria (Akpeji et al., 2025).

The Oxidase Test

For the indication of bacteria producing oxidase the oxidase test was performed, quantifying an enzyme cytochrome c oxidase. The bacterial culture on addition with reagent (usually tetramethyl-p-phenylenediamine) produced purple or blue colour that was the indication of oxidase (Jurtshuk & McQuitty, 1976).

Coagulase Test

An enzyme coagulase was detected by coagulase test which cause clotting of blood. The clot formation indicated positive while no clotting indicates negative (Hasan et al., 2014).

Antibiotic Susceptibility Testing

Disk Diffusion Method

The disk-diffusion method was used for the antibiotic sensitivity testing. A total of eight different types of antibiotics (Table 2) was used on Mueller Hinton agar (MHA) media. All the cultures were streaked on to sterile MHA plates and was incubated for 24 hours at 37˚C using Clinical Laboratory Standard Institute guidelines (2020).

Table 2: List of antibiotics used in the study against fungal, bacterial pathogens.

S.No.	Antibiotic Disc	Concentration
1	Ampicillin	(10µg)
2	Streptomycin	(10µg)
3	Tetracycline	(10µg)
4	Erythromycin	(15µg)
5	Amoxicillin	(30µg)
6	Cephalosporin	(10µg)
7	Penicillin	(10µg)
8	Neomycin	(30 µg)
9	Gentamycin	(10ug)
10	Chloramphenicol	(15ug)

Fish Preservation Techniques

For short term preservation the fish sample were covered with ice, reducing the growth of microorganisms (Ghaly et al., 2010). The fish sample were frozen at -40°C so that the water content in fish was solidified and the microbial growth were inhibited (El-Dengawy et al., 2012). The fish sample were dried when exposed to sunlight by evaporating the water. This is an old method to avoid contamination (Mansur et al., 2013).

Statistical Analysis

All the data was subjected to a software SPSS foe statistical analysis by performing one way Anova (Clark et al., 2012). A significant level of $p < 0.05$ was established.

Results

Sample Collection and Processing

A total of 50 fish samples were collected from local markets in District Charsadda, consisting of 25 samples each of *Clupisoma naziri* and *Cyprinus carpio*. The samples included 30 cooked, 10 frozen, and 10 raw fish as shown in Table 3. All samples were processed immediately upon arrival at the laboratory to maintain their integrity and minimize microbiological changes.

Table 3: Summary of fish samples collected from local markets.

S.No.	Fish Type	Sample Condition	Number of Samples
1	*Clupisoma naziri*	Cooked	15
		Frozen	5
		Raw	5
2	*Cyprinus carpio*	Cooked	15
		Frozen	5
		Raw	5

Bacterial Isolates

The study identified various bacterial isolates, with the high genera being *Pseudomonas*, *Escherichia coli*, and *Staphylococcus aureus*. The raw fish sample results in higher bacterial counts. *Clupisoma naziri* showed the presence of *E. coli* in cooked, frozen, and raw sample, *Pseudomonas* and *Salmonella* were isolated frozen and raw samples. *Cyprinus carpio* also has *E. coli* in raw samples, with *Staphylococcus aureus* was detected in cooked fish and *Pseudomonas* in frozen fish sample as shown in Table 4.

Table 4: Isolated Bacteria.

S.No.	Fish Type	Sample Condition	Isolated Bacteria
1	*Clupisoma naziri*	Cooked	*E. coli*
		Frozen	*Pseudomonas, E. coli*
		Raw	*Salmonella, E. coli*
2	*Cyprinus carpio*	Cooked	*Staphylococcus aureus*
		Frozen	*Pseudomonas*
		Raw	*E. coli,*

Fungal Isolates

Fungal microbes isolated were primarily identified as *Aspergillus* and *Penicillium*, with higher occurrences noted in raw and frozen samples compared to cooked fish. In *Clupisoma naziri*, *Penicillium* was found in cooked samples, while both *Penicillium* and *Aspergillus* were isolated from raw and frozen fish. In *Cyprinus carpio*, *Penicillium* was found in cooked, frozen, and raw samples, while *Aspergillus* was only present in frozen and raw fish samples as shown in Table 5.

Table 5: Isolated Fungi.

S.No.	Fish Type	Sample Condition	Isolated Fungi
1	*Clupisoma naziri*	Cooked	*Penicillium*
		Frozen	*Aspergillus*
		Raw	*Aspergillus, Penicillium*
2	*Cyprinus carpio*	Cooked	*Penicillium*
		Frozen	*Aspergillus*
		Raw	*Penicillium, Aspergillus*

Bacterial Identification

Bacterial isolates were identified using Gram staining and biochemical testing. Identification of bacterial isolates based on Gram staining and biochemical tests. The identification results, indicating the presence of both Gram-negative and Gram-positive bacteria were shown in Table 6.

Table 6: Bacterial Identification.

S.No.	Bacterial Isolate	Gram Staining	Biochemical Test Results
1	*Pseudomonas spp.*	Negative	Catalase positive and coagulase negative
2	*Staphylococcus aureus*	Positive	Coagulase positive and oxidase negative
3	*Escherichia coli*	Negative	Catalase positive and Oxidase negative
4	*Salmonella spp.*	Negative	Catalase positive and coagulase negative

Fungal Identification

Fungal species were identified through microscopy and culture characteristics. *Aspergillus spp*. and *Penicillium spp.* were identified on basis of microscopy and the characteristics of fungal culture as shown in Table 7. *Aspergillus* formed green black colonies on solid PDA media and under microscope septate hyphae were observed with round sporangia, while bluish green colonies appeared for *Penicillium* on PDA media and

brush like *conidia* were observed under microscope. These characteristics help identify these fungi, which are common contaminants in fish samples.

Table 7: Fungal Identification.

S.No.	Fungal Isolate	Microscopic Characteristics	Culture Characteristics
1	*Aspergillus spp.*	Septate hyphae	Green-black colonies on PDA
2	*Penicillium spp.*	Brush-like conidia arrangement	Bluish-green colonies on PDA

Antibiotic Susceptibility Testing

To assess the antibiotic susceptibility disk diffusion method was employed for the bacteria isolated from different samples. The results showed different resistivity and sensitivity levels between isolates from *Clupisoma naziri* and *Cyprinus carpio*.

Resistance Patterns

Patterns of antibiotic resistance were assessed to understand the efficiency of treatment options against spoilage-causing bacteria. Antibiotic susceptibility for isolated bacteria was also observed. The sensitivity of isolated bacteria to different antibiotics was assessed (Figure 4). The findings illustrate the varying degrees of susceptibility and resistance of isolated bacteria strains to the tested antibiotics. Different antibiotics have different sensitivity and resistance pattern which is shown in Table 8. According to the result obtained Penicillin showed highest resistant (40%) against isolated bacteria with least sensitivity 60%. The resistance toward Ampicillin, Chloramphenicol, Neomycin and Erythromycin resulted 30% 30%, 25% and 25% respectively. The highest sensitivity observed toward Gentamycin (90%), Tetracycline (90%), Amoxicillin (85%), and Streptomycin (80%) as summarized in Table 8 and Figure 4.

Effectiveness of preservation techniques

The effectiveness of various fish preservation techniques was evaluated based on microbial load reduction.

Table 8: Antibiotic resistance patterns among isolated bacteria.

S.No.	Antibiotic	Resistance (%)	Sensitive (%)
1	Ampicillin	30	70
2	Streptomycin	20	80
3	Tetracycline	10	90
4	Erythromycin	25	75
5	Amoxicillin	15	85
6	Cephalosporin	20	80
7	Penicillin	40	60
8	Neomycin	25	75
9	Gentamycin	10	90
10	Chloramphenicol	30	70

Cooling

Cooling technique was highly effective for both species resulted in 95% reduction in microbes. The large level reduction of microbial load significantly lowers the spoilage and contamination risk, improving the quality and shelf life of fish. Cooling technique was equally effective for both *Clupisoma naziri* and *Cyprinus carpio*, as shown in Figure 5 and Table 9.

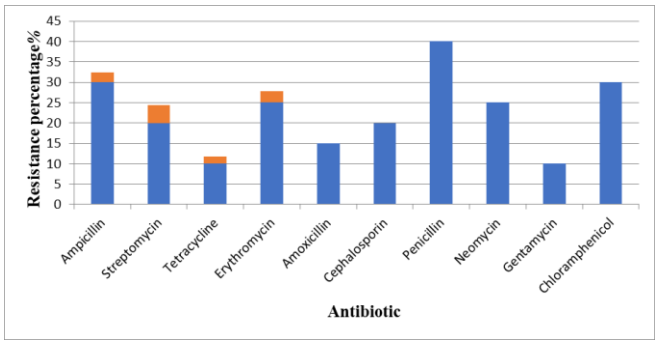

Figure 4: Antibiotic resistance patterns among isolated bacteria

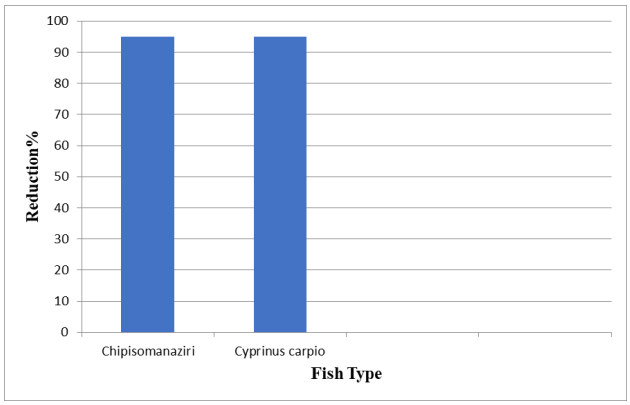

Figure 5: Effect of cooling on microbial counts in fish.

Table 9: Effect of cooling on microbial counts in fish.

S.No.	Fish Type	Initial Count (CFU/g)	Count After Cooling (CFU/g)	Reduction (%)
1	*Clupisoma naziri*	2.0 x 10^5	1.0 x 10^4	95
2	*Cyprinus carpio*	1.8 x 10^5	0.9 x 10^3	95

Freezing

Freezing showed a reduction in microbial load, with a 20% for *Clupisoma naziri* and a 17% for *Cyprinus carpio*. The reduction was higher in *Clupisoma naziri* compared to *Cyprinus carpio*, though the

difference is minimal. Freezing is less effective in reducing microbial loads compared to cooling as shown in Figure 6 and Table 10, where microbial reduction reached 95%.

Drying

Drying caused a moderate reduction in microbial counts, with reductions of 50% for *Clupisoma naziri* and 56% for *Cyprinus carpio*. Drying was slightly more effective for *Cyprinus carpio* than for *Clupisoma naziri*, as indicated by the higher percentage reduction. While drying was effective, it was less efficient than cooling (95% reduction) but more effective than freezing (20% and 17% reduction) (see Figure 7, Table 11 for details).

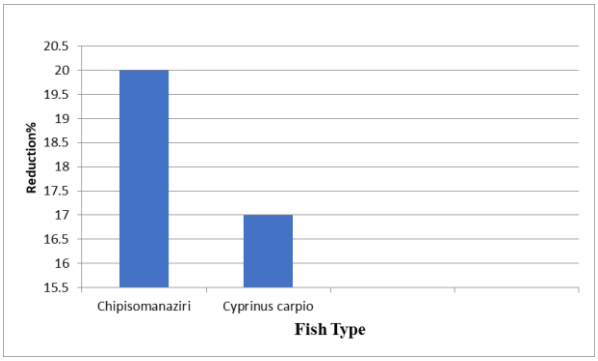

Figure 6: Effect of freezing on microbial counts in fish.

Table 10: Effect of freezing on microbial counts in fish.

S.No.	Fish Type	Initial Count (CFU/g)	Count After Freezing (CFU/g)	Reduction (%)
1	*Clupisoma naziri*	2.0 x 10^5	1.6 x 10^5	20
2	*Cyprinus carpio*	1.8 x 10^5	1.5 x 10^5	17

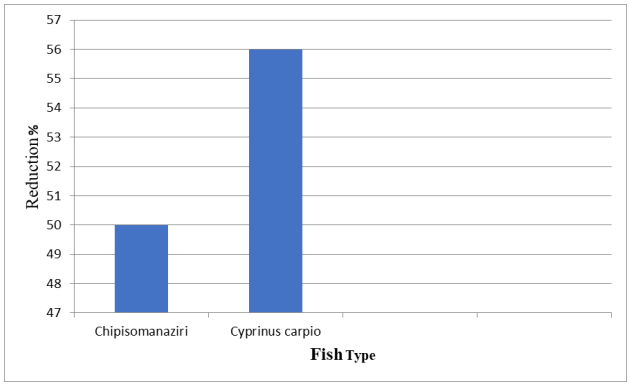

Figure 7: Effect of drying on microbial counts in fish

Table 11: Effect of drying on microbial counts in fish.

S.No.	Fish Type	Initial Count (CFU/g)	Count After Drying (CFU/g)	Reduction (%)
1	*Clupisoma naziri*	2.0 x 10^5	1.0 x 10^5	50
2	*Cyprinus carpio*	1.8 x 10^5	0.8 x 10^4	56

Discussion

This study was designed to isolate and identify those microorganisms that cause spoilage from two commercially available and important species of fish *Clupisoma naziri* and *Cyprinus carpio*, that were collected from District Charsadda local markets, Pakistan. This study reported different microorganism, fungus and bacteria that cause spoilage and different antimicrobial treatments and important preservation methods were analysed. The screening of microorganism from these fish showed high level of bacteria such as *Pseudomonas spp.*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella spp.*, that cause spoilage. The raw samples recorded highest microbial load as compare to other samples. This result of our study aligned to previous studies in which *Pseudomonas* was reported as major spoilage causing agent under aerobic conditions, highly recorded in raw while moderate in processed samples of fish. This high level in raw sample a compared to cooked sample suggest that the heat treatment reduce the level of microorganism in fish. The presence of *E. coli* in both raw and cooked sample indicates the contamination during handling in local markets (Mumbo et al., 2023).

The fugal isolates *Aspergillus* and *Penicillium* were present in both raw and frozen samples, that is consistent with previous reports that fungal growth occurs in frozen conditions but with slow rate. The presence of these fungus cause safety concerns of food due to mycotoxins produced by this fungus. The antibiotic susceptibility test resulted that among all the isolated bacteria *Pseudomonas spp.*, showed resistance to ampicillin while susceptibility to tetracycline. This study aligns to previous study *Pseudomonas* was reported as resistant to beta lactam antibiotics due to the production of lactamase enzyme that degrade beta lactam. Other bacteria showed resistance to different antibiotic like *Staphylococcus aureus* to erythromycin while susceptibility to tetracycline (Glen and Lamont, 2021).

The presence of bacteria showing resistance to multiple antibiotics in this study highlights a growing concern regarding antibiotic resistance in local fish markets. For instance, some isolates exhibited resistance to penicillin, ampicillin, and erythromycin, suggesting potential multi-drug resistance patterns.

The resistance showed by this microorganism is concerning as these strains are already present in local markets of fish. The *E. coli* was

found resistant to the commonly use antibiotic chloramphenicol that is consumed by both human and animals. The fish contain such microorganism consumed by human is a potential risk to health and these resistant bacteria can be transmitted through food chain. These results highlight the Stricker control of antibiotic in aquaculture and better strategies to be applied to control antibiotic resistance bacteria (Yuan et al., 2023).

Different preservation techniques were evaluated and their effectiveness were elucidated. Among all the preservation techniques Cooling method was found highly effective resulting over 95% reduction in microbes in both fish species. These finding aligns with previous study that cooling reduce the metabolic activity of bacteria that cause spoilage resulting in extension of shelf life of fish products (Yuan et al., 2023). The freezing technique also found fruitful with moderate reduction in microbes but some bacteria like *Pseudomonas* can survive at low temperature and resume its growth when exposed to normal conditions. Drying technique showed 50-56% decreased the microbes 50-56%, that was the least reduction among three techniques. The spoilage causing microorganisms especially fungi like *Aspergillus* adapt and survive at low water content (Abdullah et al., 2000).

Conclusion

The current study focused on the isolation and identification of different microorganism responsible to the spoilage in two fish *Clupisoma naziri* and *Cyprinus carpio* that were collected from local fish market in Charsadda district, Pakistan. The study revolved different microorganism as causative agent of fish spoilage. Different bacterial isolates like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas spp*., and *Salmonella* spp., as well as fungal species like *Aspergillus* and *Penicillium* were isolated from fish samples. These microorganisms were found in high quantity in raw fish as compared to processed sample of fish. This study revealed different bacteria resistant to antibiotic and best preservation techniques for storing the fish were that are safe for the human health.

References

Abdullah, N., Nawawi, A. & Othman, I. 2000. Fungal Spoilage Of Starch-Based Foods In Relation To Its Water Activity (Aw). *Journal of Stored Products Research,* 36**,** 47-54.

Adedeji, O., Okerentugba, P., Innocent-Adiele, H., Okonko, I., Ojeniyi, S., Adejoro, S. & Mohamed, S. 2012. Benefits, Public Health

Hazards And Risks Associated With Fish Consumption. *New York Science Journal,* 5**,** 33-61.

Akpeji, C., Ewetan, D., Bosah, B., Otali, C. & Ossai, C. 2025. Microbiological And Nutritional Characteristics Of Locally Fermented Fufu Commonly Sold In Amai, Delta State, Nigeria. *Journal Of Applied Sciences And Environmental Management,* 29**,** 477-482.

Alhadrami, H. A., Orfali, R., Hamed, A. A., Ghoneim, M. M., Hassan, H. M., Hassane, A. S., Rateb, M. E., Sayed, A. M. & Gamaleldin, N. M. 2021. Flavonoid-Coated Gold Nanoparticles As Efficient Antibiotics Against Gram-Negative Bacteria—Evidence From In Silico-Supported In Vitro Studies. *Antibiotics,* 10**,** 968.

Chen, Y., Ma, F., Wu, Y., Tan, S., Niu, A., Qiu, W. & Wang, G. 2023. Biosurfactant From Pseudomonas Fragi Enhances The Competitive Advantage Of Pseudomonas But Reduces The Overall Spoilage Ability Of The Microbial Community In Chilled Meat. *Food Microbiology,* 115**,** 104311.

Clark, R. A., Shoaib, M., Hewitt, K. N., Stanford, S. C. & Bate, S. T. 2012. A Comparison Of Invivostat With Other Statistical Software Packages For Analysis Of Data Generated From Animal Experiments. *Journal Of Psychopharmacology,* 26**,** 1136-1142.

Da Silva Araújo, L. P. 2022. *Optimization Of Culture Medium Composition Using Agro-Industrial By-Products For The Production Of Silk-Elastin-Like Proteins By Escherichia Coli.* Universidade Do Minho (Portugal).

El-Dengawy, R., El-Shehawy, S., Kassem, A., El-Kadi, S. & Farag, Z. S. 2012. Chemical And Microbiological Evaluation Of Some Fish Products Samples. *Journal Of Agricultural Chemistry And Biotechnology,* 3**,** 247-259.

Ghaly, A. E., Dave, D., Budge, S. & Brooks, M. 2010. Fish Spoilage Mechanisms And Preservation Techniques. *American Journal Of Applied Sciences,* 7**,** 859.

Glen, K. A. & Lamont, I. L. 2021. B-Lactam Resistance In Pseudomonas Aeruginosa: Current Status, Future Prospects. *Pathogens,* 10**,** 1638.

Hardy, B. L., Bansal, G., Hewlett, K. H., Arora, A., Schaffer, S. D., Kamau, E., Bennett, J. W. & Merrell, D. S. 2020. Antimicrobial Activity Of Clinically Isolated Bacterial Species Against Staphylococcus Aureus. *Frontiers In Microbiology,* 10**,** 2977.

Hasan, A. A., Hassawi, D. S., Al-Daghistani, H. I. & Hawari, A. D. 2014. Molecular And Biochemical Identification Of Coagulase Positive Staphylococcus Species Isolated From Human And Animal

Sources In Jordan. *International Journal Of Medicine And Medical Sciences,* 47**,** 1491-1507.

Hashem, M. 2011. Isolation Of Mycotoxin-Producing Fungi From Fishes Growing In Aquacultures. *Research Journal Of Microbiology,* 6**,** 862.

Jurtshuk Jr, P. & Mcquitty, D. N. 1976. Use Of A Quantitative Oxidase Test For Characterizing Oxidative Metabolism In Bacteria. *Applied And Environmental Microbiology,* 31**,** 668-679.

Luqman, M., Hassan, H. U., Ghaffar, R., Bilal, M., Kanwal, R., Raza, M., Kabir, M., Fadladdin, Y., Ali, A. & Rafiq, N. 2024. Post-Harvest Bacterial Contamination Of Fish, Their Assessment And Control Strategies. *Brazilian Journal Of Biology,* 84**,** E282002.

Mansur, M. A., Rahman, S., Khan, M. N. A., Reza, M. S. & Uga, S. 2013. Study On The Quality And Safety Aspect Of Three Sun-Dried Fish. *African Journal Of Agricultural Research,* 8**,** 5149-5155.

Migaou, M., Macé, S., Maalej, H., Marchand, L., Bonnetot, S., Noël, C., Sinquin, C., Jérôme, M., Zykwinska, A. & Colliec-Jouault, S. 2024. Exploring The Exopolysaccharide Production Potential Of Bacterial Strains Isolated From Tunisian Blue Crab Portunus Segnis Microbiota. *Molecules,* 29**,** 774.

Mumbo, M. T., Nyaboga, E. N., Kinyua, J. K., Muge, E. K., Mathenge, S. G., Rotich, H., Muriira, G., Njiraini, B. & Njiru, J. M. 2023. Antimicrobial Resistance Profiles Of Salmonella Spp. And Escherichia Coli Isolated From Fresh Nile Tilapia (Oreochromis Niloticus) Fish Marketed For Human Consumption. *Bmc Microbiology,* 23**,** 306.

Nawaz, A., Hussain, S. & Shafique, M. 2022. Sajid Ali, Muhammad Akbar Anjum, Shaghef Ejaz, Mahmood Ul Hasan. *Shelf Life And Food Safety***,** 227.

Rashid, S. A., Noh, N. A. M., Zarkasi, K. Z., Salikin, N. H., Jalil, M. T. M., Zakaria, N. A. & Tong, W. Y. 2025. Pollution By Microorganisms And Its Impact On The Food Safety. *Controlling Environmental Pollution: Practical Solutions***,** 47-72.

Ribault, S., Faucon, A., Grave, L., Nannini, P. & Faure, I. B. 2005. Detection Of Bacteria In Red Blood Cell Concentrates By The Scansystem Method. *Journal Of Clinical Microbiology,* 43**,** 2251-2255.

Subhi, S. A., Risan, M. H. & Al-Wattar, W. M. 2017. Collection, Isolation And Identification Of Pathogenic Bacteria From Blood Clinical Specimens In Baghdad. *European Journal Of Biomedical And Pharmaceutical Sciences,* 4**,** 1-8.

Svendsen, F. S. M. 2021. *A Pilot Study Of Lumpfish (Cyclopterus Lumpus) Skin Health, Reared With Three Different Treatments In Land-Based Facilities And Commercial Net-Pens.* The University Of Bergen.

Tarroum, M., Romdhane, W. B., Al-Qurainy, F., Ali, A. A. M., Al-Doss, A., Fki, L. & Hassairi, A. 2022. A Novel Pgpf Penicillium Olsonii Isolated From The Rhizosphere Of Aeluropus Littoralis Promotes Plant Growth, Enhances Salt Stress Tolerance, And Reduces Chemical Fertilizers Inputs In Hydroponic System. *Frontiers In Microbiology,* 13**,** 996054.

Yuan, X., Lv, Z., Zhang, Z., Han, Y., Liu, Z. & Zhang, H. 2023. A Review Of Antibiotics, Antibiotic Resistant Bacteria, And Resistance Genes In Aquaculture: Occurrence, Contamination, And Transmission. *Toxics,* 11**,** 420.

Zin Eldin, A. I. A., Salman, M. B., Salah Eldin, D. A. & Eissa, N. 2023. An Overview On: Bacterial Fish Zoonoses. *Journal Of Current Veterinary Research,* 5**,** 196-215.