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Identification and antimicrobial sensitivity profiling of bacteria isolated from cultured catfish shing *(Heteropneustes fossilis)*

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Abstract: The cultivated species of catfish *(Heteropneustes fossilis)*, also known as "Shing" has a delectable flavor, a high market value, a high nutritional and therapeutic value, and a high iron and calcium content. In Bangladesh, the shing *(H. fossilis)* fish is one of the most sought-after, popular, and extremely valuable indigenous species. The purpose of this research was to isolate bacterial pathogens from infected shing *(H. fossilis)* fish and determine their antibiotic sensitivity. In total, 76 medically infected shing fish were collected by the cultivators from their own ponds in the Jashore district, in southern Bangladesh. Disease-causing bacteria were found in 62 (81.5%) of the infected fish, while normal flora were found in 14 (18.5%) of the 76 samples. There were 62 pathogenic bacterial isolates found, and *Aeromona* spp., *Pseudomonas* spp., *Streptococcus* spp., *Flavobacterium* spp. and *Vibrio* spp. were the main causes of disease in the sick fish. Among the species of bacteria that were isolated, the prevalence of the largest pathogens, *Aeromonas* spp. was 34 (51.4%) and the prevalence of the second largest, *Pseudomonas* spp. was 13 (20.3%). The other isolates were comprised of the following species: *Streptococcus* spp. 9 (9.4%), *Flavobacterium* spp. 4 (5.4%) and *Vibrio* spp. 2 (4.1%). The farming of shing *(H. fossilis)* fish has been increased rapidly all around the country. Therefore, bacterial infections may reduce the production of fish in ponds. In this research, bacterial strains showed a high degree of sensitivity to ciprofloxacin (77%) and levofloxacin (54%). Significantly, oxytetracycline (52%) enrofloxacin (45%) and erythromycin (45%) were the intermediate/moderate sensitive antibiotics. All the strains were found to be resistant to amoxicillin 57/62 (92%) and neomycin 42/62 (68%). The findings of this research will be beneficial to fish farmers in the treatment of

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bacterial infections in fish, as these diseases are caused by microorganisms. The PCR method might be studied further as a means of identifying all bacterial cultures, which would require additional research.

Keywords: Catfish, *H. fossilis*, Bacteria, Diseases, Antibiotics, Sensitivity

1 Introduction

Bangladesh's fishing sector has a significant impact on the country's economy, as well as on rural employment and poverty reduction. Fish make up over 60% of the country's animal protein requirements. More and more people are getting involved in the aquaculture industry in Bangladesh thanks to the government's efforts to disseminate adaptable and new technology and to provide need-based extension services. Bangladesh is the exception to the global trend of aquaculture's rapid expansion. Aquaculture, especially pond aquaculture, needs to find ways to boost fish production in order to feed the world's growing population (FAO, 2010). Most Asian countries, including Bangladesh, rely heavily on fish as an animal protein source. As a source of essential macronutrients and micronutrients, it aids in human growth. Nutrition, employment, GDP growth, and foreign exchange gains are all boosted by the fishing industry. It is one of our country's most important sources of revenue. In Bangladesh, freshwater fish are well-known and highly prized. There are a number of popular Asian species, including Shing, Pangas, Thai koi, Pabda, Shol, Tengra, Magur, and Tilapia, all of which are native to the region. The farmed fish in ponds were shown to have a broad range of bacterial infections.

One of Bangladesh's native air-bredingg catfishes, the stinging catfish *(H. fossilis)* has been nicknamed "Shing" often known as shing, is a well-known and high-valued fish in the country. It is not simply appreciated for its delicious taste and market value, but also regarded as a beneficial and restorative supplement. It is grown in fields with high stocking densities because of the high market and economic price. The fact that Bangladesh's shing *(H. fossilis)* farming has enormous potential, the high mortality rate of shing during cultivation means that diverse ailments cause real financial hardships. *Aeromonas* spp. infects a wide variety of Bangladeshi aquaculture and freshwater fish species (Sarker et al., 2000). Another distinction was made between *A. hydrophila* and the epizootic ulcerative syndrome (EUS) in *H. fossilis.* This once abundant resource has been depleted by human exploitation and environmental changes in Bangladesh's vast waterways during the last century. An obscure disease of shing fish, is still causing significant losses to the industry despite the development of innovative techniques for raising fry and brood stock of shing fish in recent years. This fish is good for aquaculture because it can handle pressure, fights off disease, grows quickly, reproduces, and looks good (Anyanwu et al., 2014).

Disease is one of the main reasons for the decline in fish production in both aquaculture and the wild. A lot of fish die in ponds because of environmental stress, parasite invasion, and infections from bacteria, fungi, protozoa, and monogeneans (Hossain et al., 2011). There are only a very few clinical labs or technical support in Bangladesh, but all of these research labs are

located in academic institutions or government research institutions, which are far from remote fish farms. Farmers fail to report disease outbreaks because they are unable to recognize the warning signs of illness. There was a marked decrease in the detection of disease clinical symptoms and signs in the coastline and rural aquaculture sector. Through laboratory analysis, pathogens and diseases can be detected. Only a small number of samples were examined by the government fisheries officers, making their diagnosis inadequate for the country as a whole. Bangladesh is blessed with a wide range of fishing resources, including inland capture, marine capture, and aquaculture.

Rather than focusing on aquaculture, many resources and international assistance are devoted to ensure the safety and quality of fish exported from artisanal fisheries. More attention has been paid to Bangladesh's captured fisheries because of the impact on global trade, but little attention has been paid to the bacteria, water quality, and feed used to raise aquaculture fish in this country. Aquaculture fish is supposed to be contaminant-free because of this perception. At this time, there is no information available on the frequency of microbial strains that might be present in Bangladesh's aquaculture industry. Anti-microbial agents, commonly used in farmed animals and horticulture in the country, are not known to be effective against these bacteria. Accordingly, if fish is to play an important role in both offering much-needed protein and being able to contribute to the national economy, recent data on such aspects as fish-borne diseases is required. In addition, if aquaculture is going to reduce the supply gap, it is essential to formulate the microflora of aquaculture species, pond water, and soil particles in the bodies of water and dam sediment.

The goal of this study was to find out which fish farms and dams have the most different kinds of bacteria by isolating and identifying the bacteria from sick stinging catfish *(H. fossilis).* The results of this study will help decide which antimicrobials to use in aquaculture systems in the future, as well as how to control fish farms and determine the proper use of antibiotics in the treatment of diseases of shing *(H. fossilis).*

2 Materials and Methods

2. 1. Sample collection and transportation

In total, 76 contaminated shing fish were directly collected from various regions of Jashore district in southern Bangladesh. During the process of collecting fish samples, safety precautions were taken to prevent any contact with the fish, and an ice box was utilized to keep the chain of cold temperatures intact. After that, the samples were taken to the laboratory of the Department of Fisheries and Marine Bioscience (FMB), Jashore University of Science and Technology.

2. 2. Sample processing and bacterial enrichment

For the microbiological test, fish liver, skin, and gills were used as specimens to be examined. It was necessary to slice and grind these samples on a sterile cutting board. A total of 10 g of samples were homogenized in 90 ml of freshly prepared 0.1% peptone water before inoculating 0.1 ml of the sampling distribution onto selective media such as Rimler Shotts

Medium Base Agar (for *Aeromonas* spp.), Pseudomonas Base Agar (for *Pseudomonas* spp.) and Thiosulfate Citrate Bile Salt Sucrose (for *Vibrio* spp.). Bacterial isolates were grown in TSA and Brain Heart Infusion Agar (BHI) and then they were kept at 37 ºC for 24 h.

2. 3. Bacterial culture medium and reagents

2. 3. 1. Preparation of nutrient agar medium

The preparation of nutritional agar medium consisted of dissolving 28 g of nutrient agar powder in 1 L of distilled water in accordance with conventional procedures. After sterilizing the medium, it was continued to pour into sterile petri dishes (5 ml for each petri dish) and allowed to solidify. It was then incubated at 37 °C for an entire night to ensure that it was sterile. After that, it was either put to use based on culture characteristics or refrigerated at 4 °C for later use.

2. 3. 2. Chemical reagents

There were a variety of chemicals and reagents utilized for this investigation, including phosphate buffer solution (PBS), staining chemicals (crystal violet, iodine gram's acetone alcohol, safranin), hydrogen peroxide (3%) and methylene blue (3%). Standard procedures were used to create methyl red (MR), potassium hydroxide (KOH), gram's iodine solution, as well as bromothymol blue or gentian violet (also known as methyl violet 10B). The stain was made by mixing crystal violet and ammonium oxalate monohydrate solutions. It was done using standard methods to prepare the acetone-ethanol solution, counter stain (the safranin solution), and normal and physiological saline solutions (PSS).

2. 4. Bacterial isolation and identification

Gram's staining reaction, colony attributes, physiochemical reaction, catalase test, and motility test were used to isolate and identify bacteria from the aforementioned samples. In order to encourage the growth of a specific type of bacterium, the presumed colony from all these media was sub-cultured in agar media and nutrient broth. Finally, the selective media yielded a pure culture. A variety of tests, including gram's staining, were carried out. During the course of the study, strict aseptic procedures were observed. Striking on various types of solid agar was carried out in a laminar flow environment. After these tests, the results were analyzed and the bacteria found in the samples were named.

After being isolated from various culture plates, bacterial colonies were streaked on slant stands for TSA, MIU and Simon citrate agar mediums, and incubated at 37 °C. In order to characterize the pure isolates, we used several commonly used tests such as morphological characteristics, alkaline and acidic reactions, hydrogen sulfide (H_2S) as well as gas production, motility testing (indole and urease), enzyme assays (oxidase and catalase), and the methyl red (MR) and voges praskaure (VP) tests. These tests were used to characterize the pure isolates. Microbiologists used gram staining to distinguish between gram-positive and gram-negative bacteria. Biochemical tests were used to find out what the pathogens were (John et al., 1998). These tests were based on Bergey's Manual of Bacteriological Classification.

2. 5. Antimicrobial sensitivity test

When it came to determining which bacteria were sensitive to which antibiotics, Antibiotic Sensitivity Testing (AST) was used. The disc diffusion test is the standard method for determining antimicrobial activity. After a period of incubation, all inhibition zones around every disc were analyzed. We measured the zone radius from the antibiotic disc's center out to the clear zone's outer edge, which is exactly where microbes were seen growing. All of the bacterial pathogen isolates were subjected to in-vitro antibiotic susceptibility tests using the Kirby-Bauer disc diffusion procedures (Table 1), as recommended by the CLSI guidelines (CLSI, 2015). The *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., *Vibrio* spp. and *Flavobacterium* spp. strains were used as quality controls for the culture and antimicrobial sensitivity testing throughout the whole research project. Sterile PBS water was used to prepare the suspected bacteria colonies, which were subsequently adjusted to meet the 0.5 McFarland turbidity threshold. Incubation took place at 37 ºC for 24 hours using antibiotic-impregnated discs (Himedia, India) on Mueller–Hinton agar (Himedia, India). Antibiotic inhibition zones conform around the discs, with a diameter of around a millimeter (mm). The anti-microbial plate's focus point was scaled as far as practicable to a suitable range where microscopic organisms may be observed to grow. The diameter of the antibiogram was measured to the nearest millimeter (mm) and put into one of three groups: sensitive, moderate, or resistant.

Table 1. Interpretation standards for disc diffusion susceptibility testing (Kirby-Bauer technique).

Antibiotics disc	Interpretation of results (zone in diameter in mm)		
	Resistant (R)	Medium susceptible (M)	Susceptible (S)
Amoxicillin	≤13	14-17	≥18
Ciprofloxacin	≤15	16-20	≥21
Colistin	≤8	9-10	≥11
Clotetracycline	≤14	15-18	≥19
Doxycycline	≤12	13-15	≥16
Erythromycin	≤13	14-22	≥23
Levofloxacin	≤13	14-16	≥17
Enrofloxacin	≤14	15-17	≥18
Trimethoprim	≤10	11-15	≥16
Neomycin	≤12	13-16	≥17

2. 6. Statistical analysis

The data was analyzed using SPSS 20 and Excel 2013. Analyses of descriptive statistics and chi-square values were carried out to ensure the accuracy of the data. *P*-values below 0.5 were used to detect statistically significant differences.

3 Results and Discussions

3. 1. Clinical signs and postmortem findings

After performing a clinical examination on diseased fish, a number of serious damages

were found, including equilibrium damage, rectal protrusion, dropsy, hemorrhagic ulcerative lesion, skin and tail erosion, dark red discoloration around the eye and mouth, skin lesions on the body surface, and profuse mucous secretion. Body part enlargement and congestion within the internal organs of diseased fish were also observed during postmortem examinations.

3. 2. Identification of bacterial colonies

The bacteria were identified based on their cultural traits, as well as the results of biochemical and gram staining tests.

3. 2. 1. Cultural characteristics of bacterial isolates

The color, shape, and transparency of grown bacterial colonies were observed in order to examine the cultural properties of *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., *Flavobacterium* spp., and *Vibrio* spp. (Table 2).

Table 2. Colony characteristics of bacterial isolates observed during the study.

Sl. No.	Colony characteristics	Bacterial isolates
1	Colony of a bright yellow color with a round shape and dense density	*Aeromonas* spp.
2	Colony of creamy white, round and transparent spheres	*Pseudomonas* spp.
3	Colonies that are either mucous or smooth	*Streptococcus* spp.
4	A yellowish-grey and irregularly transparent colony	*Flavobacterium* spp.
5	Colony of darkish yellow color	*Vibrio* spp.

3. 2. 2. Biochemical tests of the isolated bacterial species from infected fishes

The results of the identification of different species of bacteria from the infected fishes based on their detailed biochemical parameters during the present study are summarized in Table 3.

Table 3. Biochemical tests result of the isolated bacterial species from infected fishes.

Sl. No.	Results of tests						Bacterial isolates
	Oxidase	Catalase	Motility	MR Test	VP Test	Indole	
1	+	+	+	+	+	+	*Aeromonas* spp.
2	+	+	+	-	-	-	*Pseudomonas* spp.
3	-	-	-	+	+	-	*Streptococcus* spp.
4	+	+	+	+	-	+	*Flavobacterium* spp.
5	+	+	+	-	+	+	*Vibrio* spp.

The different bacterial isolates identified from the infected fishes on the basis of gram staining tests during the present study are presented in (Table 4).

Table 4. Morphology and Gram's staining properties of the isolated bacterial species from infected fishes.

Shape	Gram's staining reaction	Identified Bacteria
Rounded ends on straight rods	Gram negative	*Aeromonas* spp.
Small rods	Gram negative	*Pseudomonas* spp.
Cocci	Gram positive	*Streptococcus* spp.
Rhizoidally edged small rods	Gram negative	*Flavobacterium* spp.
Curved-rod (Comma)	Gram negative	*Vibrio* spp.

3. 3. Isolated bacteria from infected shing fishes

Isolation of bacteria is accomplished through the use of various morphological and cultural criteria. Five different bacterial species such as *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., *Flavobacterium* spp., and *Vibrio* spp. were found in the samples of both the sick and healthy shing fish *(H. fossilis)*. A total of 62 (81.5%) pathogenic bacteria were isolated from 76 samples of infected shing *(H. fossilis)* fishes, while only 14 (18.5%) normal flora isolates were found in the samples (Figure 1).

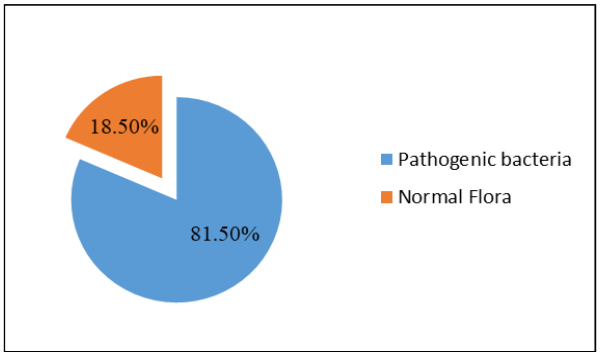

Figure 1: Bacterial infection in the shing fish *(H. fossilis)*.

3. 4. Occurrence of bacteria from infected shing (*H. fossilis)*

Among the 62 different strains isolated, *Aeromonas* spp. was the highest number with 34 (54.8%), followed by *Pseudomonas* spp. with 13. (20.9%), *Streptococcus* spp. with 9 (14.5%), *Flavobacterium* spp. with 4 (6.4%) and *Vibrio* spp. with 2 (3.2 %) in this order (Figure 2).

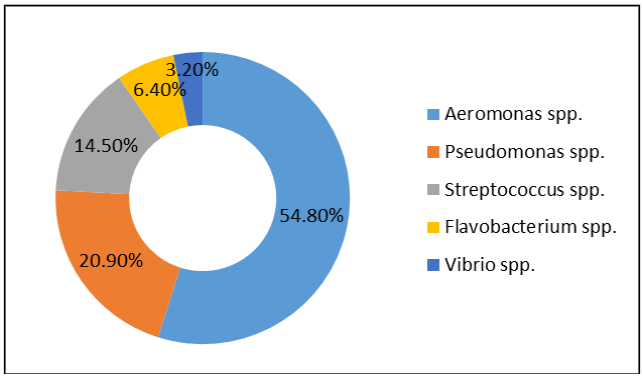

Figure 2: Prevalence rate (%) of the isolated bacteria from infected *H. fossilis.*

3. 5. Antibiotic sensitivity pattern of *Aeromonas* spp.

Aeromonas spp. showed the maximum sensitivity to ciprofloxacin and levofloxacin, and the most intermediate/moderate reaction to oxytetracycline and enrofloxacin, while no sensitivity was detected against amoxicillin and neomycin (Table 5).

Table 5. Antibiotic sensitivity patterns of *Aeromonas* spp.

Antibiotics	Sensitive	Moderate	Resistant
Amoxicillin	0	0	34 (100%)
Erythromycin	14 (41%)	11 (32%)	9 (27%)
Ciprofloxacin	30 (88%)	4 (12%)	0
Levofloxacin	23 (68%)	9 (26%)	2 (6%)
Oxytetracycline	8 (34%)	17 (50%)	9 (16%)
Enrofloxacin	10 (29%)	16 (47%)	8 (24%)
Colistin	12 (35%)	9 (26%)	13 (39%)
Neomycin	0	12 (35%)	22 (75%)

Number of *Aeromonas* spp. infected isolates (*n* = 34).

3. 6. Antibiotic sensitivity pattern of *Pseudomonas* spp.

The antibiotics amoxicillin, erythromycin and neomycin were ineffective against the *Pseudomonas* spp. tested, while antibiotics with the of names ciprofloxacin and enrofloxacin were found to be the most effective against *Pseudomonas* spp. (Table 6). Like *Aeromonas* spp., *Pseudomonas* spp. exhibited a reaction that was intermediate when exposed to oxytetracycline.

Table 6. Antibiotic Sensitivity Pattern of *Pseudomonas* spp.

Antibiotic	Sensitive	Moderate	Resistant
Amoxicillin	0	4 (30%)	9 (70%)
Erythromycin	2 (15%)	6 (46%)	5 (39%)
Ciprofloxacin	11 (85%)	2 (15%)	0
Levofloxacin	5 (39%)	7 (54%)	1 (7%)
Oxytetracycline	5 (39%)	8 (61%)	0
Enrofloxacin	7 (54%)	6 (46%)	0
Colistin	3 (24%)	6 (46%)	4 (30%)
Neomycin	0	0	13 (100%)

Number of *Pseudomonaas* spp. infected isolates (*n* = 13).

3. 7. Antibiotic sensitivity pattern of *Streptococcus* spp.

Streptococcus spp. showed the maximum sensitivity to enrofloxacin and ciprofloxacin, and the most intermediate/moderate reaction to oxytetracycline and erythromycin, while no sensitivity was detected against amoxicillin (Table 7).

Table 7. Antibiotic sensitivity patterns of *Steptococcus* spp.

Antibiotic	Sensitive	Moderate	Resistant
Amoxicillin	0	0	9 (100%)
Erythromycin	2 (22%)	7 (78%)	0
Ciprofloxacin	5 (56%)	3 (32%)	1 (12%)
Oxytetracycline	2 (22%)	7 (78%)	0
Doxycycline	4 (46%)	3 (32%)	2 (22%)
Enrofloxacin	7 (78%)	2 (22%)	0
Colistin	5 (56%)	3 (32%)	0
Neomycin	2 (22%)	5 (56%)	2 (22%)

Number of *Streptococcus* spp. infected isolates (*n* = 9).

3. 8. Antibiotic sensitivity pattern of *Flavobacterium* spp.

Colistin and levofloxacin were the most sensitive to *Flavobacterium* spp., and doxycycline and enrofloxacin had the most intermediate or moderate response, while amoxicilin and neomycin had no detectable sensitivity (Table 8).

Table 8. Antibiotic Sensitivity Pattern of *Flavobacterium* spp.

Antibiotic	Sensitive	Moderate	Resistant
Amoxicillin	0	1 (25%)	3 (75%)
Erythromycin	1 (25%)	2 (50%)	1 (25%)
Ciprofloxacin	2 (50%)	2 (50%)	0
Levofloxacin	3 (75%)	1 (25%)	0
Doxycycline	0	4 (100%)	0
Enrofloxacin	1 (25%)	4 (100%)	0
Colistin	4 (100%)	0	0
Neomycin	0	1 (25%)	3 (75%)

Number of *Flavobacterium* spp. infected isolates (*n* = 4).

3. 9. Comparative analysis among antibiotics based on antimicrobial responsiveness exerted by the isolated bacterial pathogens

In this study, bacterial pathogens demonstrated a high degree of sensitivity to ciprofloxacin (77%) and levofloxacin (54%). Oxytetracycline (52%) enrofloxacin (45%) and erythromycin (45%) were the intermediate/moderate sensitive antibiotics and all of the bacteria showed resistance to 57/62 (92%) of the amoxicillin and 42/62 (68%) of the neomycin (Table 9 and Figure 3).

Table 9. Comparative analysis among antibiotics sensitivity pattern of isolated bacterial pathogens.

Antibiotics	Sensitive	Moderate	Resistance
Amoxicillin	0	5 (8%)	57 (92%)
Erythromycin	19 (31%)	28 (45%)	15 (24%)
Ciprofloxacin	48 (77%)	13 (21%)	1 (2%)
Levofloxacin	33 (54%)	26 (41%)	3 (5%)
Oxytetracycline	19 (31%)	32 (52%)	11 (17%)
Enrofloxacin	27 (44%)	28 (45%)	8 (11%)
Colistin	25 (40%)	19 (31%)	17 (29%)
Neomycin	2 (3%)	18 (29%)	42 (68%)

Number of total infected pathogenic bacterial isolates (*n* = 62).

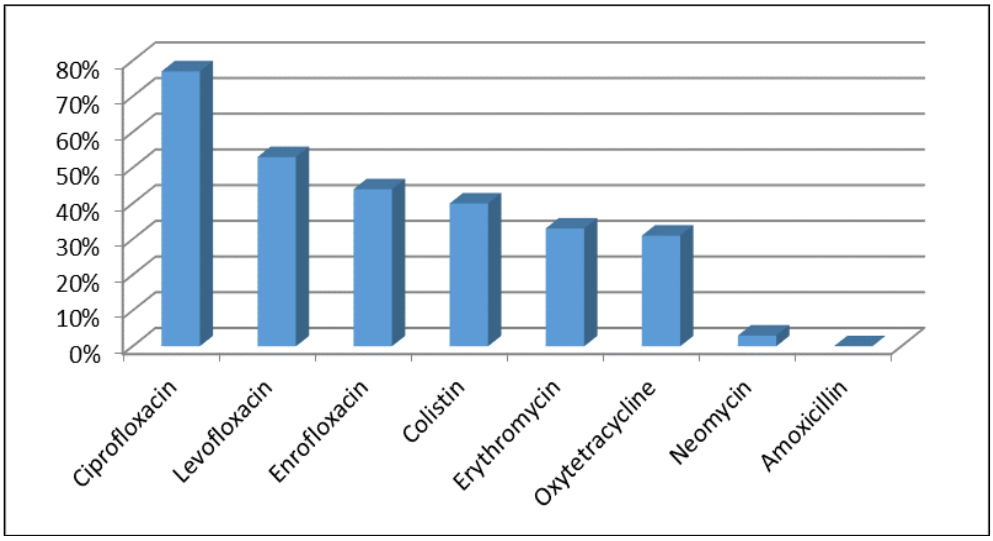

Figure 3: Antibiotics sensitivity profiling of infected shing fish *(H. fossilis)*.

The results revealed that ciprofloxacin and levofloxacin were the most sensitive antibiotics, while amoxicillin and neomycin were the resistant antibiotics against common bacterial diseases of shing fish in Jashore district, the southern Bangladesh.

Increasingly, our native fish are in high demand on the world market due to their higher nutritional content, and this is not just true in Bangladesh. These fish have been harmed for a variety of reasons, including the spread of bacterial, viral, and fungal infections in pond water (Sharif et al., 2019). Matured shing, pangas, Thai koi, pabda, and other native fishes, such as shol, magur, tilapia, and tengra, all died at higher rates in Bangladeshi freshwater culture ponds. *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp., *Staphylococcus* spp., *Flavobacterium* spp., *Edwardsiella* spp., *Citobacter* spp., and *Enterobacter* spp. are among the pathogenic bacterial species that affect fish (Shayo et al., 2012; Anshary et al., 2014). Although shing *(H. fossilis)* is one of Bangladesh's most prized and hardy fish species, a number of factors have led to a decline in its production in cultured ponds and farms in the greater Jashore region, including diseases caused by viral, bacterial, and fungal pathogens. Other studies have found that the clinical symptoms include loss of equilibrium, skin lesions, mucous secretion, hematopoiesis, body and tail erosion, congestion, and enlargement with hemorrhage of the internal organs such as the

body cavity and abdomen (Khalil et al., 2010; Goni et al., 2020). Other researchers found a swollen liver and other internal organs in the sick fish (Ahmed and Shoreit, 2001).

Despite being a hardy fish, the shing *(H. fossilis)* has been affected in freshwater ponds by different bacterial, viral, and parasitic pathogens, even though they can adapt many different water parameters. The bacterial infections of shing fishes and their antibiotic sensitivity and resistance patterns were identified by collecting infected shing fishes from different regions of Jashore district, in Southern Bangladesh. In addition to *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., *Flavobacterium* spp., *Edwardsiella* spp. and *Citobacter* spp., a small number of pathogenic bacteria have been identified (Abedin et al., 2020; Anshary et al., 2014).

However, this study found that, *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., *Flavobacterium* spp., and *Vibrio* spp. were the most common pathogens isolated. Ahmed and his colleagues observed these results and isolated the bacteria (Ahmed and Shoreit, 2001). Autotrophic *Aeromonas* species have been found to be the most common cause of infections in catfish in Southeast Asia and other parts of the world (Anyanwu et al., 2014). Similar outcomes were found in our research, which had a success rate of (54.80%). *Aeromonas hydrophila* was identified by Sarkar and Rashid (2012) from a variety of fish species with epizootic ulcerative disorder. It has been found that gram-negative bacteria in freshwater fish are responsible for the skin sores of the shing fish (Yanong, 2011), and the bacteria have been isolated from infected fish of a variety of water bodies (Hossain et al., 2011). Ahmed and colleagues isolated *Aeromonas* spp. and *Pseudomonas spp*. from infected shing fish (Ahmed and Shoreit, 2001).

There have been reports of *Flavobacterium columnare* in symptomatic catfish *(Clarius batrachus)* in the Himalayan and Sub-Himalayan regions of the world. Diseased fish were found to be infected with the pathogenic bacteria *Pseudomonas* and *Flavobacterium*. *Carassius auratus* and *Cirrhinus mrigala* specimens were found to be infected with *Flavobacterium columnare* (Nayak et al., 2014). Isolated *Flavobacterium columnare* from carp and goldfish exhibited similar biochemical characteristics (Nayak et al., 2014). Gram-negative bacteria are the most common cause of skin lesions in freshwater fish. Bacteria from freshwater fish have been isolated by numerous researchers. Each and every one of the infections was found to be a mixture of bacteria. *Aeromonas* spp. and *Pseudomonas* spp. mixed bacterial infections have also been reported (Ahmed and Shoreit, 2001).

Eight antibiotics were tested on isolates of *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., *Flavobacterium* spp., and *Vibrio* spp. While ciprofloxacin was completely effective against *Aeromonas* and *Pseudomonas* bacteria, levofloxacin was completely effective against *Streptococcus, Flavobacterium,* and *Vibrio* bacteria. *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., were found to be highly effective against ciprofloxacin (77%) and levofloxacin (54%). Amoxicilin (92%) was the only antibiotic that was resistant to other bacterial species. All *Pseudomonas* spp. isolates tested positive for ciprofloxacin and neomycin resistance in the antibiogram. Abedin et al. (2020) found the isolates of *Aeromonas* spp. to be susceptible to cotrimoxazole and ciprofloxacin.

4 Conclusion

The bacterial pathogens in shing *(H. fossilis)* and their sensitivity to generally used antibiotics were the focus of this study. According to the findings of this study, bacterial infections of shing *(H. fossilis)* could be a key contributor to the significant economic loss that local fish farmers in Bangladesh experience. A variety of bacterial species, such as *Aeromonas* spp., *Pseudomonas* spp., *Streptococcus* spp., and *Flavobacterium* spp., appear to be the primary pathogens and key causes of bacterial infections that shing species are susceptible to. The careless administration of these antibiotics in shing fish farming ponds results in the development of multidrug resistance in a variety of bacterial species. In order to be successful antimicrobial treatment, it is important to first isolate the disease-causing agent, identify it, and then determine the antibacterial profile of the bacteria that are linked with the infection. There should be cautious consideration before prescribing an antibiotic in order to avoid resistance from developing. Farmers that cultivate catfish for the purpose of identifying and controlling disease by administering specific antibiotics will benefit from this study's findings. All bacterial isolates could be identified by utilizing PCR techniques in future studies.

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Conflict of interests

The authors declare that they have no conflict of interests.

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