



Enumeration of Emergent Bacterial Pathogen Isolated from Small Indigenous Fish Species: Pabda (*Ompok Spp*) and Gulsha (*Mystuscavasiuous spp*)

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Research Article

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Abstract

The present study attempted to determine the prevalence of pathogenic bacteria within two most popular small indigenous fish species of Bangladesh. A total of 51 infected fish samples were collected from different places in Bangladesh between April 2019 to December 2020. Among 51 infected fish samples (26 Pabda, & 25 Gulsha), 47(92.16%) were infected with pathogenic bacteria and 4(7.84%) were normal flora. From the total of 51 isolated bacterial strains, the highest number was 16(31.37%) for *Vibrio spp.*, the second highest for *Aeromonas spp.*, was 12(23.52%), and the next was 5(9.80%) for *Streptococcus spp.* On the other hand, *Pseudomonas spp.*, only 4(7.84%) and the rest 3(5.88%) *Flavobacterium spp.*, *Citobacter spp.*, and *Edwardsiella spp.*, *Enterobacter spp.*, and parasite 1(1.96%). In our study, among the isolated pathogenic bacteria, 39(76.47%) were resistant to Amoxicillin and 28(54.90%) were resistant to Erythromycin. Whereas maximum sensitivity was found for Ciprofloxacin 35(68.63%). The isolation of emergent bacterial pathogens that impose a threat to fish is the main objective of this article.

Keywords: Emergent; Small Indigenous Fish; Pathogenic Bacteria; Sensitivity

Introduction

Fish and fish products have been one of the main food ingredients for humans and animals from the ancient period. Approximately, 60% of animal proteins come from fish in Bangladesh [1]. In Bangladesh, freshwater aquaculture plays a major role in improving the economic condition of fish farmers. Most of the fish production Bangladesh come from rural freshwater aquaculture [2]. From Bangladesh, a total of

253 freshwater fish species have been recorded [3], of these, over 150 species have been classified as 'small indigenous species' [4]. This 'small indigenous species' group of fish is an important source of protein, calcium and minerals in the daily diet and has been identified as an important source of income for small-scale fishermen [5].

Ompok spp (pabda) locally known as Modhupabda (commonly known as Butter catfish), is a small indigenous

catfish that belongs to the family Siluridae of the order Siluriformes [6]. This fish species is considered to be one of the most nutritious, tasty and demanding fish of the people of Bangladesh. *Mystuscavasiuous* locally known as 'Gulsha' has been attracting the attention among fish farmers in Bangladesh due to its profitable farming, high market value, and good taste [7]. For this reason, farmers of the greater Mymensingh region are cultivating these fish to a large extent. Pabda is omnivorous in nature, feeding on fishes, algae, crustaceans, insects, and protozoans, parts of plants and debris [8], while Gulsha is a carnivore, eating insect larvae, and small fish [9]. Pabda and Gulsha are usually found in low-lying areas, beels and canals. During the monsoon months (May to July) the young and adult fish of these species gain access to these waters from flooded rivers where they breed.

Unfortunately, due to the growth and activity of pathogenic microorganisms a large amount of fish in the country deteriorates every year. The export market of Bangladesh is threatened by poor quality processed foods that may be infected by pathogenic bacteria such as coliform, faecal coliform, *Vibrio cholerae*, *Salmonella spp.*, *Listeria monocytogenes*, *Clostridium botulinum*, *Shigella spp.*, *Streptococci* and *Staphylococcus aureus*. Such pathogenic flora of living fish depends on the microbial content of the aquatic habitat [10,11]. Soft tissue and the condition of the aquatic habitats make fish susceptible to microbial contamination which deteriorates the quality of fish [12]. However, it is reported that improper handling, poor icing and bad trading practices are the factors that have a negative impact on the fish quality [13].

Aquatic environmental conditions which are undoubtedly important for the persistence of bacterial pathogens contribute to long-term survival resulting in disease outbreaks [14]. The extensive misuse of antibiotics has led to serious resistance problems and limiting the effectiveness of antibiotics in eliminating bacterial infections [15,16]. In Bangladesh, a study showed that more than 70% of infectious pathogens were resistant to at least one of the commonly used antibiotics [17]. Therefore, this study attempted to enumerate the bacterial load in Pabda and Gulsha fishes and to isolate the newly emerged pathogens.

Materials and Methods

Collection and Transportation of Samples

A total of 26 infected Pabda (*Ompok spp*) and 25 infected Gulsha (*Mystuscavasiuous spp*) fish samples were taken from different Upazila (Fulbaria, Gowripur, Ishwargonj, Maskanda, Netrokona, Tarakanda, Trishal, Muktagacha, Purbodala, Mukkurpur, Kishorgonj, Kendua, Mowna) in greater Mymensingh, and Sherpur districts in Bangladesh

between April 2019 to December 2020. Aseptic measures were maintained during the collection of fish samples to avoid touch and cold chains were maintained by using an ice box. The samples have then brought to the Quality Aqua Laboratory of Quality Feeds Limited, Mymensingh.

Sample Processing and Enrichment of Bacteria

During the sampling process, aseptic measures were strictly maintained to prevent sample contamination. For the microbiological test, three types of specimens including skin, intestine and gills of infected fishes were collected (Figures 1&2). The samples were taken on a sterile cutting board then chopped them appropriately and grinded together. Ten (10) gm of samples is diluted with 90 milliliters (ml) of freshly prepared 0.1% peptone water. After that for enrichment of bacterial isolates, 0.1 ml of homogenized sample was then inoculated on to selective media, for example: *Pseudomonas* Base agar (for *Pseudomonas spp*), Rimler-Shotts Medium Base agar (for *Aeromonas spp.*), Thiosulfate citrate bile salt sucrose (TCBS) agar (for *Vibrio spp.*), Brain Heart Infusion (BHI) Agar (for fastidious organisms), Tryptic Soy Agar (TSA) and incubated at 37^o C for 24 hours.



Figure1: Pabda (*Ompok spp*) with pop eye and reddish discoloration around the eye and mouth.

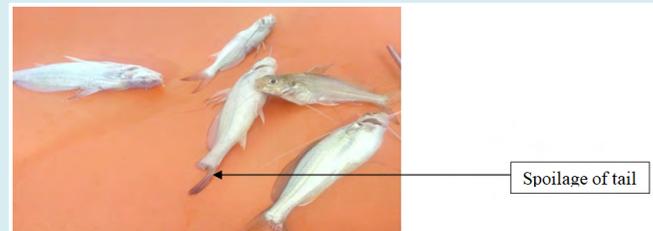


Figure2: Infected tail of Gulsha fishes.

Identification of Bacterial Pathogens

From different culture plates, suspected bacterial colonies were isolated and then streaked on TSA slants, Simon citrate agar slant, and MIU medium followed by overnight

incubation at 37°C. Bacterial cell morphology, alkaline and acidic reaction, H₂S (hydrogen sulfide production) and gas production, motility test, indole production, urease test, oxidase test, catalase test, Methyl Red (MR) test, and Voges-Praskaure (VP) test were performed for identification of pure isolates. Gram positive and Gram negative bacteria were identified by performing Gram staining techniques. According to Bergey's Bacteriological Classification Manual [18], biochemical tests were performed to identify pathogens.

Biochemical Tests for Bacterial Identification

The pure bacterial isolates were identified by performing a group of biochemical tests including alkaline reaction, acidic reaction, indole production, H₂S (hydrogen sulfide production) gas production, urea hydrolysis, oxidase test, catalase test, Methyl-Red (MR) test, Voges-Praskaure (VP) test, motility test. Among the isolated microbes, only one microbe was found as Gram positive Cocci and the remaining 7 isolates were found as Gram negative bacteria.

In-Vitro Antimicrobial Sensitivity Test

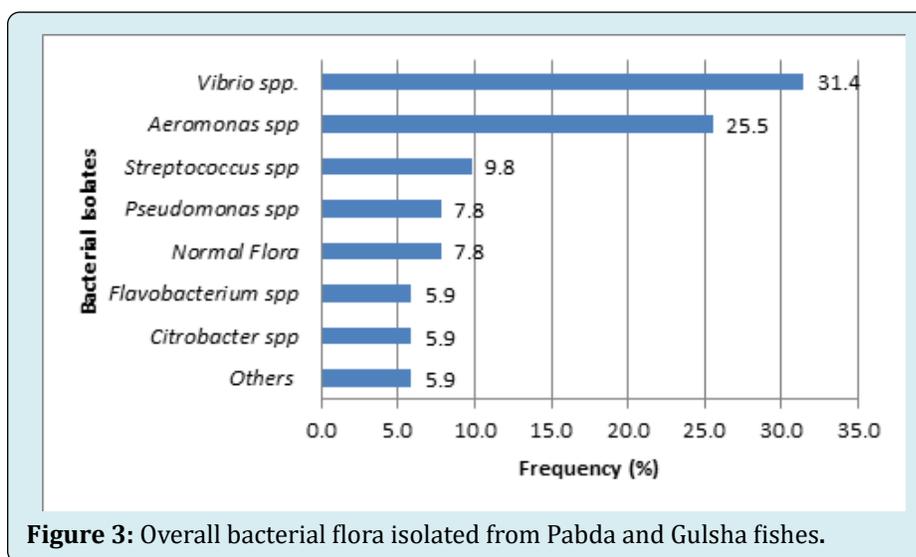
According to the CLSI guidelines [19], the Kirby-Bauer disc diffusion methods were used for *in-vitro* antimicrobial susceptibility tests of all the pathogenic bacterial isolates. In this experiment, the antibiotics used were namely Colistin (25µg), Doxycycline (30µg), Ciprofloxacin (5µg), Amoxicillin (10µg), Erythromycin (15µg), Clotetracyclin (30µg), Cotrimoxazole (25µg). Throughout the study *Vibrio cholerae* (ATCC 14035), *Aeromonas hydrophila* (ATCC 7966),

Pseudomonas aeruginosa (ATCC 27853), and *Flavobacterium columnare* (ATCC 23463) were used as quality control. The suspected isolated bacterial colonies were taken in sterile PBS (phosphate buffered saline) water and then adjusted to 0.5 McFarland's turbidity standard. The bacterial suspension was spread onto Mueller-Hinton agar (Hi-media, India) and afterwards impregnated antibiotic discs (Himedia, India) were placed and incubated at 37°C for 24 hours. According to the manufacturer's guidelines, the interpretation of the antimicrobial spectrum is measured in millimeters (mm) in terms of diameter as sensitive, medium and resistant.

Results

Fifty-one (51) bacterial strains were isolated from the investigated samples of Pabda and Gulsha fishes, where 47 were pathogenic and 4 were normal flora. The bacterial pathogens isolated from infected fish are shown in Figure 3. Among the infected fish samples, 47(92.2%) were infected with pathogenic bacteria and 4(7.8%) were normal flora. Out of the culture positive samples, 26(51.0%) were Pabda (*Ompok spp*), 25(49.0%) were Gulsha fishes.

The highest number were 16(31.4%) for *Vibrio spp.*, the second highest for *Aeromonas spp.*, was 13(25.5%), and the next was 5(9.8%) for *Streptococcus spp.*, 5(9.8%) for *Pseudomonas spp.*, and 3(5.9%) for *Flavobacterium spp.*, and 3(5.9%) for *Citrobacter spp.*, and the rest 3(5.9%) were *Edwardsiella spp.*, *Enterobacter spp.* and parasite and each of this was only 1(1.9%).



Bacteria Isolated from Pabda (*Ompok Spp*)

The bacteria isolates and others flora were identified from 26 infected of Pabda (*Ompok spp*) fish samples which

were confirmed through morphological characteristics, Gram staining, and different biochemical tests. The isolated bacterial pathogens were founded in the infected fishes of pabda are shown in Figure 4. Out of 26 infected fishes

samples, 22(84.6%) were infected with pathogenic bacteria and 4(15.4%) were normal flora. Among the total isolation of 26 bacterial strains, the highest number was 10(38.5%) for *Vibrio spp.*, the second highest for *Aeromonas spp.*, was 6(23.1%), and the next was 2(7.7%) for *Pseudomonas spp.* On the other hand, only 3(11.5%) of *Flavobacterium spp.*, *Edwardsiella spp.*, and *Streptococcus spp.* and the rest of 1(3.8%) was parasite.

Bacteria Isolated from Gulsha (*Mystuscavasiuous*)

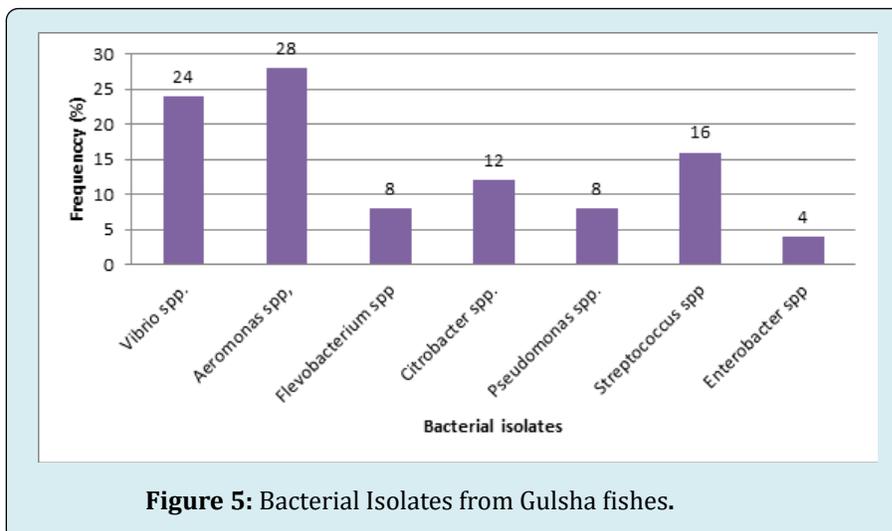
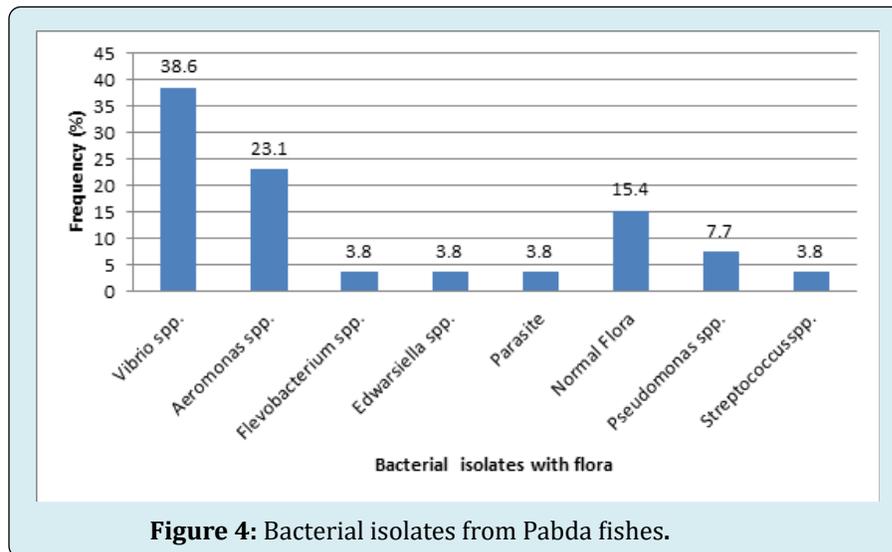
A total of 25 infected Gulsha fishes (*Mystuscavasiuous spp.*) were used to isolates of enumeration of total viable bacterial count and antimicrobial susceptibility test. A 7(28.0%) of *Aeromonas spp.*, were the most predominant bacterial isolates of Gulsha fishes and then *Vibrio spp.*, 6(24%) were the second dominant species among the isolates. The remaining 4 (16%) were *Streptococcus spp.*, 3(12%) *Citrobacter spp.*, 2(8%)

Flavobacterium spp., 2(8%) *Pseudomonas spp.*, and 1(4%) *Enterobacter spp.* were shown in Figure 5.

Antimicrobial Susceptibility

In this study, all the pathogenic isolated bacteria from Pabda fishes were 21(100%) resistant to Amoxicillin and 8(38.09%) resistant to Erythromycin and Coletetracycline. On the other hand, 21 (100%) isolates showed sensitive to Ciprofloxacin, 17(80.95%) to Doxycycline, 15(71.42%) to Cotrimoxazole, and Colistin (Table 1).

In present study, among the pathogenic bacteria isolated from Gulsha fish, 20(80%) and 18(72%) showed resistant to Erythromycin, Amoxicillin respectively. On the other hand, 14(56%) showed sensitive to Ciprofloxacin, 13(52%) to Colistin, Doxycycline, and Cotrimaxazole (Table 2).



Bacterial isolates	Sensitivity patterns	Antibiotics susceptibility patterns (%)						
		CL	DO	ERY	CIP	AMX	COT	CTE
<i>Vibrio spp.</i> , (n=10)	S	80	90	50	100	0	90	40
	M	20	10	40	0	0	0	30
	R	0	0	10	0	100	10	30
<i>Aeromonasspp.</i> , (n=6)	S	66.7	66.7	33.3	100	0	33.3	50
	M	33.3	33.3	16.7	0	0	33.3	0
	R	0	0	50	0	100	33.4	50
<i>Pseudomonasspp.</i> , (n=2)	S	100	100	0	100	0	100	0
	M	0	0	50	0	0	0	50
	R	0	0	50	0	100	0	50
<i>Flavobacterium spp.</i> , (n=1)	S	100	0	100	100	0	0	0
	M	0	100	0	0	0	0	0
	R	0	0	0	0	100	100	100
<i>Edwardsiellasp.</i> , (n=1)	S	0	100	0	100	0	100	100
	M	100	0	0	0	0	0	0
	R	0	0	100	0	100	0	0
<i>Streptococcus spp.</i> , (n=1)	S	0	100	0	100	0	100	0
	M	100	0	0	0	0	0	100
	R	0	0	100	0	100	0	0

Note: R=Resistant, S=Sensitive, CL-Colistin, DO-Doxycycline, ERY- Erythromycin, CIP- Ciprofloxacin, Amx- Amoxicilin, COT- Cotrimoxazole, CTE-Coletetracycline.

Table 1: Frequency (%) of antibiogram profiling of identified bacterial isolates from Pabda fishes.

Bacterial isolates	Sensitivity pattern	Antibiotics susceptibility patterns (%)						
		CL	DO	ERY	CIP	AMX	COT	CTE
<i>Aeromonas spp.</i> , (n=7)	S	0	43	0	100	0	14	42.8
	M	0	43	0	0	0	43	28.6
	R	100	14	100	0	100	43	28.6
<i>Vibrio spp.</i> , (n=6)	S	17	33	17	67	0	33	16.7
	M	83	67	33	33	33.3	50	33.3
	R	0	0	50	0	66.7	17	50
<i>Streptococcus spp.</i> , (n=4)	S	50	100	0	50	0	50	25
	M	50	0	25	50	0	50	25
	R	0	0	75	0	100	0	50
<i>Citrobacter spp.</i> , (n=3)	S	67	0	0	33	0	33	0
	M	33	100	0	33	66.7	0	100
	R	0	0	100	33	33.3	67	0
<i>Flavobacterium spp.</i> , (n=2)	S	100	0	0	0	0	0	0
	M	0	100	100	100	100	0	100
	R	0	0	0	0	0	100	0
<i>Pseudomonas spp.</i> , (n=2)	S	0	0	0	0	0	0	0
	M	100	100	50	100	50	0	0
	R	0	0	50	0	50	100	100
<i>Enterobacter spp.</i> , (n=1)	S	100	100	0	100	0	0	100
	M	0	0	0	0	0	0	0
	R	0	0	100	0	100	100	0

Note: R=Resistant, S=Sensitive, CL-Colistin, DO-Doxycycline, ERY- Erythromycin, CIP- Ciprofloxacin, Amx- Amoxicilin, COT- Cotrimoxazole, CTE-Coletetracycline.

Table 2: Frequency (%) of antibiogram profiling of identified bacterial isolates from Gulsha fishes.

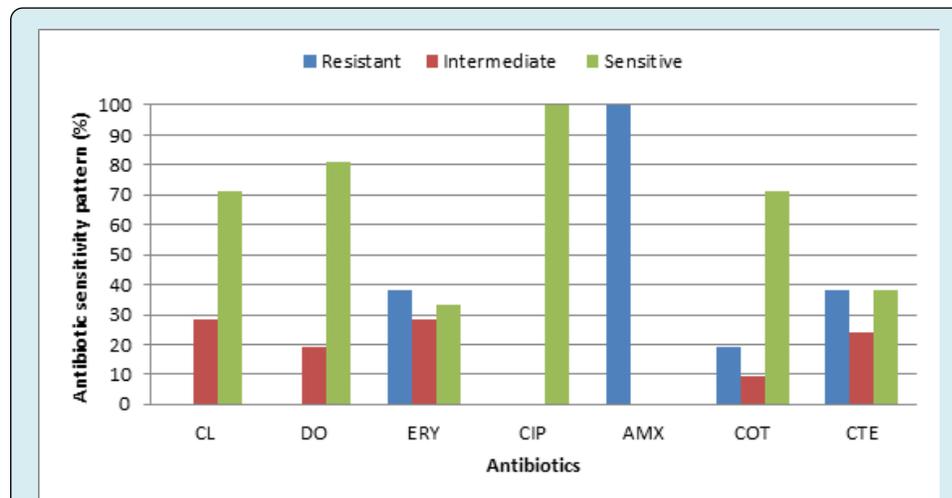


Figure 6: Overall antibiotic sensitivity of bacterial isolates of Pabda fish.

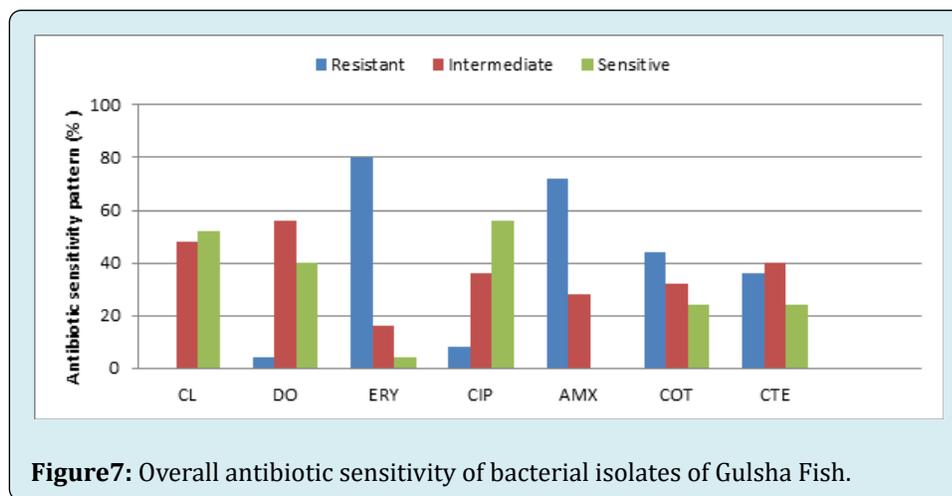


Figure 7: Overall antibiotic sensitivity of bacterial isolates of Gulsha Fish.

The overall sensitivity pattern of the isolates among the total isolates tested is shown in Table 1&2. Prevalence of their resistance patterns to the antibacterial agents tested is shown in Figure 6&7. In case of Pabda fish, the prevalence of resistance was highest to Amoxicillin followed by Erythromycin, Coletetracycline and the lowest to Cotrimoxazole. On the other hand, when it comes to Gulsha fish, the prevalence of resistance was the highest to Erythromycin followed by Amoxicillin after that Cotrimoxazole and lowest observed in isolates of Gulsha fish.

Discussion

Since 1988, Bangladesh has been experiencing outbreaks of various diseases in farmed and wild fish, and this disease has been a limiting factor in fish production. The study of fish diseases in farmed fish within the framework of this study shows that the incidence of fish diseases in Bangladesh is very high every year. A few species of bacteria are pathogenic to fish, including *Aeromonas spp*, *Pseudomonas spp*, *Vibrio*

spp, *Staphylococcus spp*, *Flavobacterium spp*, *Edwardsiella spp*, *Citobacter spp*, and *Enterobacter spp* [20,21,29-31].

A total of 47(92.16%) pathogenic bacteria and 4(7.84%) non- pathogenic bacteria were isolated. Among the pathogenic bacteria, Gram positive cocci and Gram negative bacilli were 5(9.80%) and 45 (88.23%) respectively. In this work, a variety of bacterial species have been recovered from Pabda and Gulsha fishes including *Vibrio spp*, *Aeromonas spp*, *Streptococcus spp*, *Flavobacterium spp*, *Pseudomonas spp*, *Citobacter spp*, with a frequency of 16(31.4%), 13(25.5%), 5(9.8%), 3(5.9%), 5(9.8), and 3(5.9%) respectively, and the remaining 3(5.9%) were *Edwardsiella spp.*, *Enterobacter spp.* and parasites which each of this was only 1(1.9%). Similar findings have also been reported from other districts of Bangladesh [22-24,26]. From aquaculture pond fishes, *Pseudomonas* strains, *Aeromonas spp* and other isolates were identified by Hossian, et al. [25]; Abedin MZ, et al. [29-31]. Pathogenic bacteria are quite dominant on normal flora and among those pathogens; the curve of gram negative strains

is at peak.

In our work, *Vibrio spp* 16(31.3%) were the most predominant Gram negative bacteria and *Aeromonas spp* 13(25.49%) were the second common isolated bacteria found in Pabda fish (*Ompok spp*) and Gulsha fish [26]. In this study, among the bacterial isolates, 87.5% *Vibriospp* were resistant to Amoxicilin. *Aeromonas spp* was 100% resistant to Amoxicilin, *Flavobacterium spp* was 100% sensitive to Colistin, and 66.7% showed intermediate sensitivity to Amoxycilin, Erythromycin, Ciprofloxacin, and Clotetracyclin. *Vibrio spp* were highly sensitive (87.5%) to Ciprofloxacin, 68.75% to Coletetracyclin and Doxycycline, 56.25% to Colistin. 100% *Streptococcus spp.*, in our study, was found sensitive to Doxycycline and 60% showed sensitivity to Ciprofloxacin and Cotrimoxazole. On the other hand, 100% *Streptococcus spp.*, showed resistance to Amoxycilin and 80% showed resistance to Erythromycin. Among the 8 strains, *Edwarrseilla spp.* were not found in Gulsha and *Citrobacter spp.* and *Enterobacter spp.* are limited to Gulsha fish. These three strains are uncommon whereas the other strains are found in both fish samples.

Pseudomonas spp., showed maximum resistance of 75% to Amoxicillin and Coletetracycline 50% of it showed sensitivity to Colistin, Doxycycline and Ciprofloxacin. *Citrobacter spp.*, showed the highest 100% resistance to Erythromycin and 100% of it was found intermediately sensitive to Doxycycline and Coletetracycline. These findings were not similar to a different study led by Simu, et al. [27].

Surprisingly, many isolates were found to be resistant to the common antibacterial agents tested. The rate of resistance to Amoxicillin and Erythromycin was found to be very high. Many of these resistant isolates possessed multiple patterns of resistance to the drug tested. The reasons for these resistance properties have not been studied, but it is presumed that resistance was developed due to the illegal use of antibacterial drugs in fishponds.

According to Begum, et al. [28], the uncontrolled and irregular use of antibacterial agents in aquaculture systems is one of the reasons for the emergence of multiple antibiotic resistances [28]. We ourselves should practice rational use of antimicrobial agents in fish ponds as well as the fish farmers of the community. The government might play a great role in educating people by arranging seminar or training which in turn will be paid off as through this the people for example fish consumers will be benefitted directly.

Conclusion

This study is intended to ascertain the existing situation of aquaculture infection and drug resistance among small

indigenous fish species particularly in Pabda and Gulsha fish. The prevalence of pathogenic bacteria in fish sample indicates a significant risk to public health. From the data of prevalence and resistance, we should be cautious in using antibiotics in fish ponds. The conclusion drawn from this study is that *Vibrio spp.*, and *Aeromonas spp.*, were the predominant aquaculture infections followed by *Streptococcus spp.*, *Flavobacterium spp* and *Pseudomonas spp.*, In the current study, Ciprofloxacin, Doxycycline and Cotrimoxazole were found as a reliable therapeutic choice for the identified pathogens due to their broad-spectrum activity. In addition, the types of drug resistance traits of the pathogen predicted in the study, can greatly contribute to the treatment of fish-borne diseases during outbreaks. Antibiotic selection should be guided by culture and susceptibility testing, and empirical drug status should be determined based on the recent spectrum of antibiotics in a specific geographic area

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Conflict of Interests

The authors declare no conflicts of interests.

Ethical Statement

Institutional ethical clearance was taken from the ethical committee of Khwaja Yunus Ali University before conducting the research.

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