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Isolation, Identification, and Antimicrobial Profiling of Bacteria from Aquaculture Fishes in Pond Water of Bangladesh

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ABSTRACT

Bacterial diseases are widespread and can be of particular importance in fish farming of Bangladesh. This investigation was done to assess and compare the bacteria diversities and population in local fresh water pond fishes. Out of 95 samples, 54(56.9%) were Shing (*Heteropneustes fossilis*), 14(14.7%) were Pangas (*Pangasius pangasius*), 9(9.5%) were Pabda (*Ompok spp*), 7(7.3%) were Thai Koi, (*Anabas testudineus*), and 11(11.6%) were others infected fishes such as Shol (*Channa striata*), Magur (*Clarias spp*), Tilapia (*Oreochromis niloticus*), and Tengara (*Mystus cavasius*) fishes. Among 95 infected fishes 84(88.4%) were infected with pathogenic bacteria and 11(11.6%) were normal flora. There were eight types of different isolated spp with frequency of occurrence were 36 (42.9%) *Aeromonas spp*, 15 (17.9%) *Pseudomonas spp*, 7(8.3%) *Vibrio spp*, 9 (10.7%) *Staphylococcus spp*, 7(8.3%) *Flavobacterium spp*, 7(8.3%) *Edwardsiella spp*, others were 3(3.6%) *Citobacter spp*, and *Enterobacter spp* respectively. All of the isolated pathogenic bacteria showed 84/84(100%) resistant to Amoxicillin and 18/84(64.3%) resistant to Erythromycin. All the strains showed sensitive to Ciprofloxacin, Cotrimoxazole, Enorfloxacin, Doxyciline, Clotetracycline, and Colistin with the frequency of occurrence were 78/84(92.8%), 76/84 (90.5%), 73/84(86.9%), 67/84(67.9%), 53/84(63.1%) and 52/84(61.9%) respectively. The physiochemical characteristics of 95 pond water samples were analyzed; the average water temperature, p^H, and ammonia were 27.3⁰C, 7.6 and 0.87mg/L respectively. The significant variation in the physiochemical parameters like water temperature, p^H, ammonia were observed within these five types of ponds water. Hence, it is important to detect fish diseases, responsible pathogens and other agents for protection of our water resources.

Keywords: Fresh water fishes, Pathogenic microorganisms, antibiotic sensitivity, physiochemical parameters.

1. INTRODUCTION

The fresh water fishes are well known and highly valuable fish species in Bangladesh. Among them, Shing (*H. fossilis*), Pangas (*P. pangasius*), Thai Koi

(*A. testudineus*), Pabda (*Ompok spp*), Shol (*C. striata*), Tengara (*M. cavasius*), Magur (*Clarias spp*), and Tilapia (*O. niloticus*) are very popular in Asian region. A wide range of bacterial pathogens were associated

with fishes specially, with the aquaculture fishes in ponds. The naturally living in the fish's habitat is reservoir of the indigenous bacterial pathogens such as *Aeromonas spp* and *Vibrio spp*. There are some factors that induce bacteria to be pathogens such as poor water quality and physiologically uneven fish which permit opportunistic bacterial diseases to win (Austin, 2011; Rahman *et al.*, 2019).

A number of pathogenic bacteria are related with fish and shellfish for instance *Streptococcus spp*, *Aeromonas spp*, *Vibrio spp*, *Flavobacterium spp* and others pathogens to cause diseases. The economic losses because of fish mortality are mainly caused by various pathogenic bacteria and other microorganisms in Bangladesh (Md *et al.*, 2014). In the fishes there are two types of bacteria like indigenous bacteria and non-indigenous microbes. *Staphylococcus aureus*, *Escherichia coli*, *Clostridium botulinum*, *Listeria monocytogenes* are some examples of non-indigenous microorganisms and then again, indigenous microbes incorporate *Aeromonas spp.*, *Vibrio spp.*, *Salmonella spp*, and *Pseudomonas spp*. In Bangladesh, generally fresh water fishes are found in little rivers, swamp and canal. Now a day's commercial fish cultivating in lake is exceptionally well known. The dangerous microbes such as *Pseudomonas spp*, *Aeromonas spp*, *Staphylococcus spp*, *Flavobacterium spp*, *Citobacter spp* *Edwardsiella spp*, and *Vibrio spp* that live in every pond causing perilous bacterial diseases, for example, ulcer, blade decay and tail spoil of fishes (Uddin *et al.*, 2017).

The poor water quality is also a great reason that consumes off the sludge coat or stresses the fishes making them progressively powerless to bacterial infection. Therefore, this study was designed with a view to assess the occurrence of bacterial infection of ponds cultivated fishes and their respective ponds water quality assessment and antibiotic susceptibility analysis against bacterial pathogens of fishes.

2. MATERIALS AND METHODS

2.1. Collection and transportation of samples - A total of 95 infected fishes including Shing (*H. fossilis*), Pangas (*P. pangasius*), Thai Koi (*A. testudineus*), pabda (*Ompok spp*) and others like Shol (*Channa striata*), Magur (*Clarias spp*), Tilapia

(*Oreochromis niloticus*), and Tengara (*M. cavasius*) and with their water samples were taken from different 18 Upazila in greater Mymensingh, Netrokona, and Sherpur districts in Bangladesh between April 2019 to December 2019. During the collection of fish samples and water precautionary measures were maintained to avoid touch and ice box were used to maintain cool chain. The samples were then brought to the laboratory of the Quality Aqua Laboratory, Quality feeds Limited, Mymensingh.

2.2. Sample processing and enrichment of bacteria

Aseptic measures were undertaken during the sampling procedure to prevent contamination of the samples. Three types of specimens such as intestine, skin and gill of infected fishes were collected for microbiological test. These specimens were taken in a sterile chopping board and then minced properly and grinded together. Ten (10) gm of samples were homogenized with 90 milliliters(ml) of freshly prepared 0.1% peptone water and 0.1 ml of homogenized sample was inoculated according to standard methods on to selective media such as: Rimler Shotts Medium Base agar (for *Aeromonas spp.*), *Pseudomonas* Base agar (for *Pseudomonas spp*), Thiosulfate citrate bile salt sucrose (TCBS) agar (for *Vibrio spp.*), Tryptic Soy Agar (TSA) for enrichment of bacterial isolates, Brain Heart Infusion (BHI) Agar (for fastidious organisms) and finally incubated at 37°C for 24 hours.

2.3. Identification of bacterial pathogens

- Suspected bacterial colonies obtained from different culture plates were isolated and then streaked on TSA slants, MIU medium, Simon citrate agar stant and incubated overnight at 37°C. The pure isolates were characterized by 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.

The Gram staining techniques were performed to identify Gram positive and Gram negative bacteria. The biochemical tests were carried out to identify the pathogens following Bergey's manual of Bacteriological classification (John *et al.*, 1998).

2.4 In-vitro antimicrobial sensitivity test - According to the CLSI guidelines (CLSI, 2015), the Kirby- Bauer disc diffusion methods were used to in-vitro antimicrobial susceptibility tests of all the pathogenic bacteria isolates. The commonly used antibiotics were: Amoxicillin (10µg), Ciprofloxacin (5µg), Colistin (25µg), Clotetracyclin (30µg), Doxyciline (30µg), Erythromycin (15µg), Cotrimoxazole (25µg), and Enrofloxacin (5µg). *Aeromonas hydrophila* (ATCC 7966), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Vibrio cholerae* (ATCC 14035), and *Flavobacterium columnare* (ATCC 23463) were used as quality control throughout the study for culture and antimicrobial susceptibility testing. 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 (Himedia, India) and then impregnated antibiotic discs (Himedia, India) were placed and incubated at 37⁰C for 24 hours. Around the discs, the antibiotic zones of inhibition conformed were estimated in diameter of millimeter (mm). The zone span was really scaled from the focal point of the anti-microbial plate as far as possible of the reasonable zone where microscopic organisms could be seen developing. The interpretation of antibiogram was measured in millimeter (mm) of diameters as sensitive, intermediate and resistant as per the producer's guidelines.

2.5 Water physio-chemical parameters - Ponds water temperature and pH were analyzed by the digital thermometer and digital pH meter (Hanna instruments, USA) respectively. The ideal value of temperature and pH was 30 - 32⁰ C and 7.0 - 8.5 separately. Ammonia, nitrate, and nitrite were determined by colorimetric method by using Rapid Hanna Test kit (Hanna instruments, USA), with a special smallest increment range of 0.5 mg/L NH₃-N, <10mg/L NO₃-N, and < 0.2mg/L NO₂-N respectively. The total hardness and alkalinity were measured by EDTA, phenolphthalein, and bromophenol titration by using Rapid Hanna Test Kit (USA) with a special ideal value of 20-100 mg/L and 50-160 mg/L separately.

2.6 Statistical analysis of experimental data - Data obtained were analyzed by SPSS version 20 and Excel

2016. Descriptive statistics and chi-square tests were done to check the statistical evaluation. The p-value that considered as significant was <0.5.

3. RESULTS:

3.1. Clinical Signs and post mortem findings - After clinical examination of diseased fishes, several severe damages were observed such as equilibrium loss, hemorrhagic ulcerative lesion, rectal protrusion, dropsy, body and tail erosion, reddish discoloration around the eye and mouth, skin lesions on body surface, and profuse mucous secretion (**Fig 1**). In postmortem examination of diseased fishes, organ enlargement and congestion in internal organs were also appeared.

3.2. Bacteria isolated from different diseased fishes

- The isolated bacterial pathogens were founded from different infected fishes are presented in **Table 1**. Out of 95 infected fishes samples, 84(88.4%) were infected with pathogenic bacteria and 11(11.6%) were normal flora. Out of 84 infected fishes spp, 47(49.5%) were Shing (*H. fossilis*), 13(13.7%) were Pangas, 6(6.3%) were pabda (*Ompok spp*), 7(7.3%) were Thai Koi (*A. testudineus*), and other 11(11.6%) infected fishes were Shol, Gulsha, Magur, Tilapia, and Tengra fishes. Among the total isolation of 84 bacterial strains, the highest number was 36(42.9%) for *Aeromonas spp*, the second highest for *Pseudomonas spp*, was 15(17.9%), and the next was 9(10.7%) for *Staphylococcus spp*. On the other hand, only 7(8.3%) spp of *Vibrio spp.*, *Flavobacterium spp.*, *Edwardsiella spp* and the rest of 3(3.7%) *Citobacter spp*, and *Enterobacter spp*.

3.3. Biochemical tests for bacterial identification

- The identification of the pure bacterial isolates were performed by biochemical parameters included alkaline reaction, acidic reaction, H₂S (hydrogen sulfide production) gas production, motility test, indole production, urea hydrolysis, catalase test, oxidase test, Methyl-Red (MR) test, Voges-Praskaure (VP) test are presented in **Table 2**.

In all of the isolated strains it was observed that only one strain was found as Gram positive Cocci and the rest 7 isolates were found as Gram negative bacteria. The biochemical analysis revealed that all of the strains were catalase negative in our experiment.



Fig 1: Infected fish samples from farmer’s ponds. (a) Shing (*H. fossilis*) with hemorrhagic ulcerative lesion, (b) Pangas (*P. pangasius*) with dropsy, (c) Pangas (*P. pangasius*) with rectal protrusion, (d) Pabda (*Ompok spp*) with pop eye and reddish discoloration around the eye and mouth, (e) Koi (*A. testudineus*) with deep haemorrhagic ulcerative lesion on tail region.

Table 1: Bacteria isolated from different infected fishes.

Fish pathogens	Name of fishes: N=95 (%)					
	Shing	Pangas	Pabda	Koi	Other fishes	Total isolated bacterial pathogens
<i>Aeromonas spp</i>	23	2	1	4	6	36 (42.9%)
<i>Pseudomonas spp</i>	13	0	0	1	1	15 (17.9%)
<i>Vibrio spp.</i>	1	0	5	0	1	7 (8.3%)
<i>Staphylococcus spp</i>	5	1	0	2	1	9 (10.7%)
<i>Flavobacterium spp</i>	2	5	0	0	0	7 (8.3%)
<i>Citobacter spp,</i>	2	0	0	0	0	2 (2.4%)
<i>Edwardsella spp</i>	1	5	0	0	1	7 (8.3%)
<i>Enterobacter spp</i>	0	0	0	0	1	1 (1.2%)
Total isolated bacterial pathogens fishes (%)	47 (49.5%)	13 (13.7%)	6 (6.3%)	7 (7.3%)	11 (11.6%)	84 (88.4%)
Normal flora	7	1	3	0	0	11 (11.6%)
Total isolates	54 (56.9%)	14 (14.7%)	9 (9.5%)	7 (7.3%)	11 (11.6%)	95 (100%)

Out of eight (8) isolates only four strains namely *Aeromonas spp.*, *Vibrio spp.*, *Citobacter spp.*, *Edwardsiella spp* could ferment only glucose and peptone catabolized and showed alkaline (red color) and acidic (yellow) butt. Only *Pseudomonas spp* was unable to ferment glucose and expressed all stant and butt were red in color. There was only one strain of Gram positive cocci that was able to ferment glucose, lactose and sucrose and produced yellow color stant and butt. *Flavobacterium spp* exhibited little bit different pattern and that was acidic reaction in slant and weak positive butt. The isolated bacteria *Aeromonas spp* showed alkaline reaction in stant, acidic reaction in butt, gas production, motility, indole production, oxidase test, MR-VP, Simon citrate test, catalase test were positive whereas H₂S production,

urea hydrolysis test were negative. Various strains of *Pseudomonas spp* showed motile in motility test, Simon citrate and catalase were positive. The carbohydrate utilization presented alkaline reaction in both in stant and butt and both were red in color. These strains also showed only MR-VP test, H₂S and gas production were negative. The strains of *Vibrio spp* showed positive motile, indole, oxidase, and Simon citrate test whereas their gas, H₂S, urea production and MR test were negative. Out of the 7 isolated strains it was found that H₂S production by *Citobacter spp*, urea production by *Pseudomonas spp* and *Citobacter spp*, Simon citrate test by *Aeromonas spp* and VP tests by *Aeromonas spp* and *Vibrio spp* were variable respectively.

Table 2: Results of biochemical tests of the isolated bacterial species from infected fishes.

Bacterial isolates	Gram reaction	KIA				MIU medium			Oxidase	S. citrate	MR	VP	Cat
		Stant	Butt	Gas	H ₂ S	Mot	Indole	Urea					
<i>Aeromonas spp</i>	Gram negative	R	Y	+	-	+	+	-	+	±	+	±	+
<i>Pseudomonas spp</i>	Gram negative	R	R	-	-	+	-	±	+	+	-	-	+
<i>Vibrio spp.</i>	Gram negative	R	Y	-	-	+	+	-	+	+	-	±	+
<i>Staphylococcus spp</i>	Gram positive	Y	Y	-	-	+	-	+	-	+	+	+	+
<i>Flavobacterium spp</i>	Gram negative	Y	W	-	-	+	-	-	+	+	+	-	+
<i>Citobacter spp,</i>	Gram negative	R	Y	+	±	+	+	±	-	+	+	-	+
<i>Edwardsiella spp.</i>	Gram negative	R	Y	+	+	+	+	-	-	-	+	-	+

(+)=Positive; (-)=Negative reaction; (±)=Variable; R=Red (Alkaline reaction); Y=Yellow (Acid reaction); W= Weak positive, H₂S=Hydrogen sulphide (Blackening); MR= Methyl Red, VP= Voges Proskauer, KIA=Kligler Iron agar, MIU=Motility indole urea test. Cat=Catalase test, Mot=Motility test,

3.4. Susceptibility to antimicrobial agents’ in-vitro condition - In our experiment, all of the isolated pathogenic bacteria were 84/84(100%) resistant to Amoxicillin and 18/84 (64.3%) resistant to Erythromycin. All the strains showed sensitive to Ciprofloxacin, Cotrimoxazole, Enorfloxacin, Doxycycline, Clotetracycline, and Colistin were 78/84 (92.8%), 76/84 (90.5%), 73/84(86.9%), 67/84 (67.9%), 53/84(63.1%) and 52/84(61.9%) respectively. Colistin, Erythromycin and Clotetracycline expressed intermediate sensitivity were very few such as 33.3%, 26.2% and 15.5% respectively presented in **Fig 2. Table 3-7** showed antibiotic sensitivity patterns all of the individual isolates.

3.5 Physico-chemical characteristic of pond water - The recorded pond water parameters of 95 sub-surface water samples were studied shown in **Table 8**. The average range of temperature, p^H, ammonia, hardness, alkalinity, nitrate and nitrite varied depending on their cultivated fish types.

All of the fish water samples showed ammonia concentration higher than their normal value and that was <0.5mg/L. Only Pangas fish pond water expressed the average hardness and Nitrite (NO₂) were 126.42 and 0.37 mg/L respectively that was higher than ideal value 20-100mg/L and <0.2mg/L respectively **Table 8**.

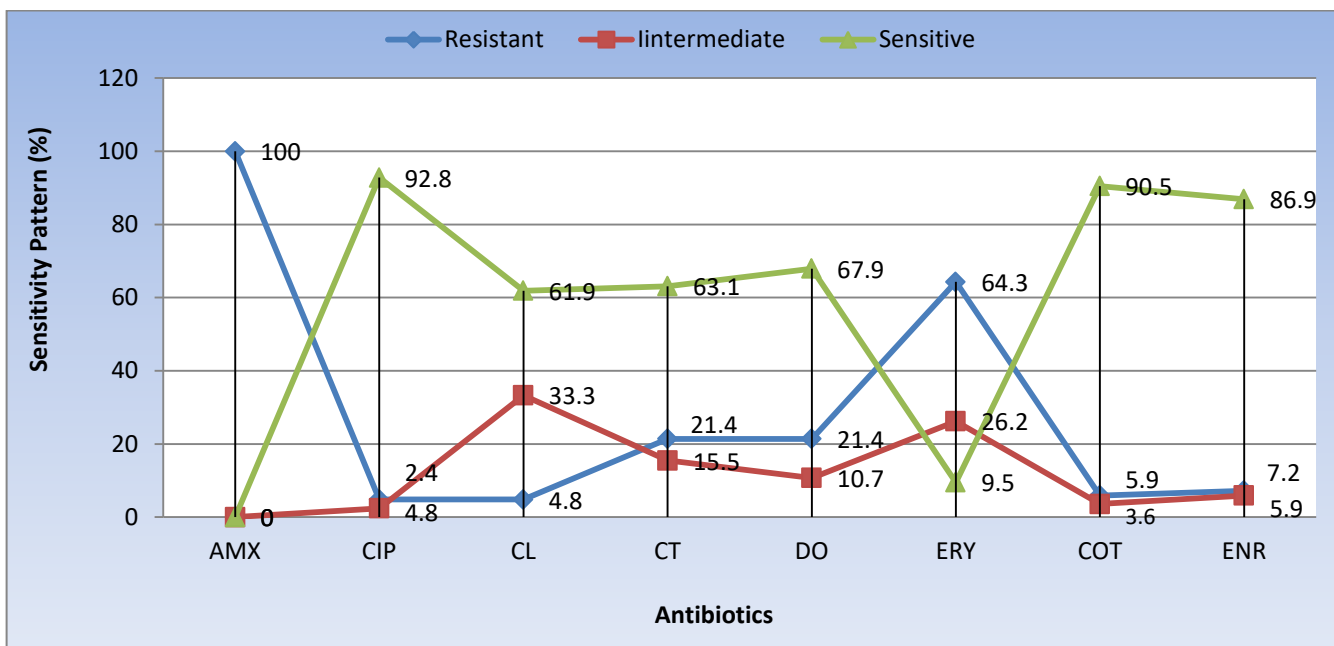


Fig 2: Antibiotic sensitivity pattern of all isolates. AMX=Amoxycillin, CIP=Ciprofloxacin, CL= Colistin, CT=Clotetracycline, DO=Doxycycline, Ery= Erythromycine, COT= Cotrimoxazole, ENR= Enorfloxacin.

Table 3: In-vitro Antibiogram profile of Gram negative and Gram positive bacterial isolates from Shing fishes.

Isolated bacteria		No (%)							
		AMX	CIP	CL	CT	DO	ERY	COT	ENR
<i>Aeromonas hydrophila</i> (n=23)	R	23(100)	0(0)	4(17.4)	7(30.4)	3(13.0)	21 (91.3)	22 (95.7)	1(4.3)
	S	0(0)	22(95.7)	9(39.1)	2(8.7)	6(26.0)	2(8.7)	1(4.3)	2(8.7)
	I	0(0)	1(4.3)	10(43.5)	14 (60.9)	14 (60.9)	0(0)	0(0)	20(87)
<i>Pseudomonas</i> (n=13)	R	13(100)	0(0)	0(0)	2(15.4)	1(7.7)	12 (92.3)	1(7.7)	0(0)
	S	0(0)	13(100)	9(69.2)	8(61.5)	9(69.2)	1(7.7)	12 (92.3)	13(100)
	I	0(0)	0(0)	4(30.8)	3(23.1)	3(23.1)	0(0)	0(0)	0(0)
<i>Staphylococcus</i> (n=5)	R	5(100)	0(0)	1(20)	1(20)	2(40)	5(100)	0(0)	0(0)
	S	0(0)	5(100)	2(40)	3(60)	0(0)	0(0)	5(100)	4(80)
	I	0(0)	0(0)	2(40)	1(20)	3(60)	0(0)	0(0)	1(20)
<i>Flavobacterium</i> (n=2)	R	2(100)	0(0)	0(0)	1(50)	0(0)	1(50)	1(50)	0(0)
	S	0(0)	2(100)	2(100)	1(50)	1(50)	0(0)	1(50)	1(50)
	I	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)	0(0)	1(50)
<i>Citrobacter spp.</i> (n=2)	R	2(100)	1(50)	0(0)	0(0)	0(0)	2(100)	0(0)	1(50)
	S	0(0)	1(50)	2(100)	2(100)	2(100)	0(0)	2(100)	1(50)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Others (n=2, <i>Vibrio spp.</i> & <i>Edwardsella spp</i>)	R	2(100)	0(0)	0(0)	0(0)	0(0)	1(50)	1(50)	0(0)
	S	0(0)	2(100)	1(50)	2(100)	2(100)	0(0)	1(50)	2(100)
	I	0(0)	0(0)	1(50)	0(0)	0(0)	1(50)	0(0)	0(0)

AMX=Amoxycillin, CIP=Ciprofloxacin, CL=Colistin, CT=Clotetracyclin, DO=Doxycillin, ERY=Erythomycin, COT=Trimethoprim, ENR=Enrofloxacin, S= Sensitive, R=Resistant, I=Intermediate

Table 4: In-vitro Antibiogram profile of Gram negative and Gram positive bacterial isolates from Pangas fishes.

		No (%)							
Isolated bacteria		AMX	CIP	CL	CT	DO	ERY	COT	ENR
<i>Edwarsiella</i> (n=5)	R	5(100)	0(0)	0(0)	1(20)	0(0)	3(60)	0(0)	0(0)
	S	0(0)	4(80)	3(60)	3(60)	4(40)	0(0)	5(100)	5(100)
	I	0(0)	1(20)	2(40)	1(20)	1(20)	2(40)	0(0)	0(0)
<i>Flavobacterium</i> (n=5)	R	5(100)	0(0)	0(0)	0(0)	0(0)	2(40)	0(0)	0(0)
	S	0(0)	5(100)	4(80)	5(100)	4(40)	3(60)	5(100)	4(40)
	I	0(0)	0(0)	1(20)	0(0)	1(20)	0(0)	0(0)	1(20)
<i>Aeromonas</i> (n=2)	R	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	2(100)	1(50)	2(100)	2(100)	0(0)	2(100)	2(100)
	I	0(0)	0(0)	1(50)	0(0)	2(100)	2(100)	0(0)	0(0)
<i>Staphylococcus</i> <i>spp</i> (n=1)	R	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)
	S	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)
	I	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)

Table 5: In-vitro Antibiogram profile of Gram negative and Gram positive bacterial isolates from Koi fishes.

		No (%)							
Isolated bacteria		AMX	CIP	CL	CT	DO	ERY	COT	ENR
<i>Aeromonas</i> (n=4)	R	4(100)	1(25)	1(25)	0(0)	2(50)	0(0)	0(0)	2(50)
	S	0(0)	3(75)	2(50)	2(50)	2(50)	2(50)	4(100)	2(50)
	I	0(0)	0(0)	1(25)	2(50)	0(0)	2(50)	0(0)	0(0)
<i>Staphylococcus</i> <i>spp.</i> (n=2)	R	2(100)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	2(100)	0(0)	1(50)	2(100)	0(0)	1(50)	2(100)
	I	0(0)	0(0)	1(50)	1(50)	0(0)	2(100)	1(50)	0(0)
<i>Pseudomonas</i> (n=1)	R	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)
	I	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)

Table 6: In-vitro Antibiogram profile of Gram negative and Gram positive bacterial isolates from Pabda fishes.

		No (%)							
Isolated bacteria		AMX	CIP	CL	CT	DO	ERY	COT	ENR
<i>Vibrio spp.</i> (n=5)	R	5(100)	0(0)	0(0)	1(20)	0(0)	1(20)	0(0)	0(0)
	S	0(0)	5(100)	4(80)	4(80)	4(80)	2(40)	5(100)	5(100)
	I	0(0)	0(0)	1(20)	0(0)	1(20)	2(40)	0(0)	0(0)
<i>Aeromonas</i> <i>spp.</i> (n=1)	R	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)
	S	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)

Table 7: In-vitro Antibiogram profile of Gram negative and Gram positive bacterial isolates from other fishes.

Isolated bacteria		No (%)							
		AMX	CIP	CL	CT	DO	ERY	COT	ENR
<i>Aeromonas spp.</i> (n=6)	R	6(100)	1(16.7)	0(0)	2(33.3)	1(16.7)	6(100)	1(16.7)	0(0)
	S	0(0)	5(83.3)	4(66.7)	3(50)	2(33.3)	0(0)	5(83.3)	6(100)
	I	0(0)	0(0)	2(33.3)	1(16.7)	3(50)	0(0)	0(0)	0(0)
Other isolates (n=5)	R	5(100)	0(0)	0(0)	0(0)	1(20)	3(60)	1(20)	1(20)
	S	0(0)	5(100)	5(100)	4(80)	4(80)	0(0)	4(80)	4(80)
	I	0(0)	0(0)	0(0)	1(20)	0(0)	2(40)	0(0)	0(0)

Table 8: Physico-chemical parameters of water samples collected at a time during April to December 2019.

Infected fishes samples Ponds	Temp (0C) (Ideal Value: 30-32 ⁰ C)	PH (Ideal Value: 7.8-8.5)	Ammonia (Ideal Value: <0.5mg/L)	Hardness (Ideal Value: 20-100mg/L)	Alkalinity (Ideal Value: 50 - 160mg/L)	Nitrate [NO3 (mg NO3/D)] (Ideal Value: <10mg/L)	Nitrite[NO2 (Ideal Value: <0.2mg/L)
Shing fishes ponds water	27.3	7.6	0.87	73.68	94.4	7.24	0.18
Pangas fishes ponds water	26	7.2	0.9	126.42	139.85	10.7	0.37
Pabda fishes ponds water	27.3	7.8	0.88	88.00	127.11	6.88	0.22
Thai Koi fishes ponds water	27.0	7.7	1.87	76.28	140.14	7.64	0.18
Others fishes ponds water	26.0	7.5	1.17	99.14	126.42	9.14	0.21

4. DISCUSSIONS

Now not only in Bangladesh, but all over the world our local fishes have great demands in the global market due to their high nutritional quality. The cultivation of these fishes in pond water has been badly influenced by different factors including various bacterial, viral and fungal contagious pathogens (Sharif et al., 2019). The mortality rate in adult Shing, Pangas, Thai Koi, Pabda and others local fishes like Shol, Magur, Tilapia and Tengara were noticed in freshwater cultured ponds situated in Bangladesh. A few numbers of bacterial species are pathogenic to fishes, which incorporated *Aeromonas spp.*, *Pseudomonas spp.*, *Vibrio spp.*, *Staphylococcus spp.*, *Flavobacterium spp.*, *Edwardsiella spp.*, *Citobacter spp.*, and *Enterobacter spp.* (Shayoet et al., 2012; Anshary et al., 2014).

However, a total of 84 (88.4%) pathogenic bacteria and 11(11.6%) non- pathogenic bacteria were isolated, among pathogenic bacteria Gram positive cocci and Gram negative bacilli were 9(10.7%) and 75 (89.7%)

respectively. A wide range of bacterial species has been recovered from local fishes in our work including *Aeromonas spp.*, *Pseudomonas spp.*, *Vibrio spp.*, *Staphylococcus spp.*, *Flavobacterium spp.*, *Edwardsiella spp.*, with frequency of 36 (42.9%), 15(17.9%), 7(8.3%) 9(10.7%) 7(8.3%) and 7(8.3%) respectively and others were 3(3.6%) *Citobacter spp.* and *Enterobacter spp.*, similar findings have also been reported from other districts of Bangladesh (Al-Harbi and Uddin, 2005; Amal et al., 2010; and Marcel et al., 2013).

Aeromonas spp., *Pseudomonas* strains and other isolates were identified from aquaculture pond fishes reported by Hossian and Chowdhury (2009). In Bangladesh, detached and distinguished some *Pseudomonas* strains from unhealthy cultivated fishes of Bangladesh. The different types of bacterial species of the pond water fishes (Shing) were isolated by numerous scientists (Shewan, 2000; and Okaeme, 2006). Ahmed and Shoreit (2001) also reported that

mixed bacterial contaminations occurred by *Aeromonas spp* and *Pseudomonas spp*. Shing (*H. fossilis*) is most demandable and highly valuable fish in Bangladesh. Although, the mortality rate of Shing is very low but its cultivation in pond water is influenced by different factors such as bacterial pathogens (Shahen et al., 2019). In any case, the clinical manifestations were loss of balance, hemorrhages, skin sores, body and tail disintegration, mucous discharge, and blockage and development with drain of the inside organs which were comparable with the discoveries of Alicia et al. 2005; Khalil et al. 2010; and Mastan, 2013. However, the predominant pathogenic bacteria isolated from the pond cultured Shing were Gram-negative bacteria (50.0%), as correspondingly observed by Marcel et al., 2013. Moreover, in Shing fish, the isolated pathogenic bacteria were *Aeromonas spp* 23(48.9%), *Pseudomonas spp* 13(27.7%), Gram positive cocci *Staphylococcus spp* 5(10.6%) and other pathogens including *Vibrio spp*, *Flavobacteriem spp*, *Citobacter spp* and *Edwardsella spp* were 6(12.8%). *Aeromonas spp* were the highly isolated bacteria in this study. Among the pathogenic bacterial diseases, *Aeromonas spp* were the major pathogens that induced plagues of ulcerative ailment in fish in Southeast Asia and different areas of the world (Anyanwu et al., 2014).

Bacterial isolates of *Pseudomonas spp* from Shing (*H. fossilis*) were 100% susceptible to Enrofloxacin and Ciprofloxacin, *Staphylococcus spp* to Ciprofloxacin and Cotrimoxazole, *Flavobacterium spp* to Ciprofloxacin and Colistin, *Citrobacter spp* to Colistin, Clotetracyclin, Doxycycline, Cotrimoxazole and *Vibrio spp* and *Edwardsella spp* to Ciprofloxacin, Clotetracyclin, Doxycycline, and Enrofloxacin. Highly sensitive to Ciprofloxacin (95.7%) and Cotrimoxazole (92.3%) were observed in *Aeromonas spp* and *Pseudomonas spp* respectively and poor sensitive to Colistin (69.2%), Clotetracyclin (61.5%), Doxycycline (69.2%) in *Pseudomonas spp.*, and Clotetracyclin (60%) and Enrofloxacin (80%) in *Staphylococcus spp* were found. In our study, the highly intermediate sensitive were to Clotetracyclin (60.9%), Doxycycline (60.9%), and Enrofloxacin (87.0%) in *Aeromonas spp*; Doxycycline (60.0%) in *Staphylococcus spp* was observed. In our investigation was observed all bacterial isolates were highly resistance to amoxicillin

(100%). Moreover, *Staphylococcus spp* and *Citobacter spp* were 100% resistant to Erythromycin in our study. The resistant pattern of Erythromycin (91.3%) and Cotrimoxazole (95.7%) were found in *Aeromonas spp*; Erythromycin (92.3%) in *Pseudomonas spp*.

Now a day, in Bangladesh, most available cultivated fishes are panagas (*P. pangasius*). The isolated bacteria were *Flavobacterium spp* 5(38.5%), *Edwardsella spp* 5(38.5%), *Aeromonas spp* 2(15.4%). The 100% sensitive bacterial isolates were *Edwardsiella spp* to Cotrimoxazole and Enrofloxacin; *Flavobacterium spp* to Clotetracyclin, Ciprofloxacin, and Cotrimoxazole; *Aeromonas spp* to Clotetracyclin, Ciprofloxacin, Doxycycline, Cotrimoxazole and Enrofloxacin, and only one strain of *Staphylococcus spp* to Doxycycline, Cotrimoxazole and Enrofloxacin, where *Edwardsiella spp.* showed poor sensitive to Ciprofloxacin (80%), Colistin (60%), and Clotetracyclin (60%), and only one strain of *Flavobacterium spp* showed 60% sensitivity to Enrofloxacin. In this investigation, only one strain of *Edwardsiella spp* was observed to 60% resistant to Erythromycin. The resistant pattern of *Staphylococcus spp* was 100% against Clotetracyclin and Erythromycin (Islam et al., 2020).

Thai Koi (*A. testudineus*) is the most available and cheapable fishes in Bangladesh. The pond water cultivated Thai Koi fishes were infected with some bacterial pathogens namely by *Aeromonas spp* were 4(57.1%) and secondly by *Staphylococcus spp* were 2(28.6%) in this study. Comparable isolation rates were accounted by Shittu et al. (2009). In our investigation, *Aeromonas spp* expressed 100% sensitivity to Cotrimoxazole and 75% to Ciprofloxacin, whereas *Staphylococcus spp* were 100% sensitive to Ciprofloxacin, Doxycycline and Enrofloxacin, Cotrimoxazole; third one *Pseudomonas spp* were 100% sensitive to Ciprofloxacin, Cotrimoxazole and Enrofloxacin. The parameters of intermediate sensitivity were 100% to Erythromycin in *Staphylococcus spp*, and Colistin, Doxycycline and Erythromycin were observed in *Pseudomonas spp*. The significant level of resistance from regularly utilized anti-microbials is similar with Hussain et al. (2014) and Mostafa et al. (2008).

Vibrio spp (83.3%) were the most predominant Gram negative bacteria and *Aeromonas spp* (16.3%) were the second common isolated bacteria found in Pabda fish (*Ompok spp*) in our work. In this study, bacterial isolates *Vibrio spp* were 100% sensitive to Cotrimoxazole and Enorfloxacin; *Aeromonas spp* were 100% sensitive to Cotrimoxazole and Ciprofloxacin; *Flavobacterium spp* were 100% sensitive to Colistin, Ciprofloxacin, and Cotrimoxazole. *Vibrio spp* were highly sensitive (80%) to Colistin, Clotetracyclin and Doxycycline, whereas highly intermediate sensitive were to only Erythromycin (100%). Our findings are not correspondent with different examinations led by Simu et al. (2019).

In our examination of other fish spp, 6 (54.5%) of *Aeromonas spp* were found in other spp of deshi fishes such as Shol (*C. striata*), Magur (*Clarias spp*), Tilapia (*O. niloticus*), and Tengara (*M. cavasius*) and the rest 5(45.5%) isolates were *Pseudomonas spp*, *Vibrio spp*, *Staphylococcus spp*, *Edwardsella spp*, and *Enterobacter spp*. In other fish spp such as Shol, Magur, Tilapia, and Tengara, bacterial isolates mainly *Aeromonas spp* were 100% sensitive to Enorfloxacin and low sensitive to Ciprofloxacin (83.3%), Colistin (66.7%), and Cotrimoxazole(83.3%). Other isolates (*Citobacter spp*, *Enterobacter spp*) were 100% sensitive to Ciprofloxacin and Colistin, and low sensitive to 80% Clotetracyclin, Doxycycline, Cotrimoxazole and Enorfloxacin. All of the strains showed 100% resistant to Amoxycillin. Similar results were recorded by Hussain et al. (2014) and Mostafa et al. (2008).

The water quality influenced by the encompassing of ecological conditions may affect the pond cultured fishes. This in the end diminishes the insusceptible status, activating bacterial contamination prompting infection episodes (Amal et al., 2015). The fish wellbeing status and pond cultured environments were reflected due to several bacterial flora. Similar study was conducted by Pakingking et al. (2015). The lake water's physiochemical parameters such as ammonia, hardness, alkalinity, nitrate, nitrite, temperature, and pH were significant in our study area (Ahmad et al., 2018). In this study, every inspecting site had distinctive characteristics that were related with the nearness of microorganisms. This phenomenon was

also observed in multiple works done by other investigators. This investigation uncovered that alkali impacted the presence of different pathogenic bacterial spp at all of the sampling areas. It is believed that the ammonia in ponds water comes from fish excrement and exorbitant feed given to the fish.

5. CONCLUSION

This study has clarified that poor water quality and bacterial diseases could be a major cause of considerable economic loss to fish farmers in greater Mymensingh, Bangladesh. A number of bacterial spp including *Aeromonas spp*, *Pseudomonas spp*, *Vibrio spp*, *Staphylococcus spp*, *Flavobacterium spp*, *Edwardsiella spp*, *Citobacter spp*, and *Enterobacter spp* were the major causes of bacterial diseases to different fish spp. Clinical examination of diseased fishes indicated that due to bacterial infection some severe damages were found in fish bodies such as equilibrium loss, hemorrhagic ulcerative lesion, rectal protrusion, dropsy, body and tail erosion, reddish discoloration around the eye and mouth, skin lesions on body surface and profuse mucous secretion. The presence of antibiotic resistant isolates imposes a serious concern about the drug of choice for treatment in fishes. A careful consideration should be given before deciding the antibiotic for treatment so as to prevent the emergence of antibiotic resistance and properly maintain the physiochemical parameters in ponds water to reduce the mortality of aquaculture fishes in ponds water. Moreover, disease prevention of various local fishes should be carried out by employing better culture practices and health management to ensure the highest yields and the optimum quality of the products.

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7. CONFLICTS OF INTEREST

The authors announce that there is no conflict of interest with respect to the publication of this article.

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