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Detachment, distinguishing proof of bacterial pathogens from infected Shing (*Heteropneustes fossilis*) cultured in freshwater ponds in Bangladesh

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Abstract

Among the local fishes, Shing (*Heteropneustes fossilis*) is one of the most demandable, popular and highly valuable fish in Bangladesh. A total of 84 clinically infected shing fishes were directly collected by a cultivator from their own ponds between April 2019 and December 2019. In total, eighty four fish-based ponds, 58(69.1%) were in Mymensingh region and the rest 26(30.9%) were in Netrakona districts in Bangladesh. Out of 84 infected fish samples, 74(88.1%) were infected with pathogenic bacteria and 10(11.9%) were with normal flora. A total of 74 pathogenic bacterial strains were isolated and among the isolates *Aeromonas* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Citobacter* spp, and *Vibrio* spp, appeared to be the main pathogen in the diseased fishes. Among the isolated species of bacteria distribution of the largest pathogens *Aeromonas* species was 38 (51.4%), and second the largest *Pseudomonas* spp was 15(20.3%). The rest of isolates were distributed *as Staphylococcus* spp 7(9.4%), *Citobacter* spp 4(5.4%), *Vibrio* spp 3(4.1%) and only 7(9.4%) others namely Bacillus spp, *Edwardsiella* spp, *Enterococcus* spp, *Flavobacterium* spp, *Klebsiella* spp in infected *H. fossilis*. The cultivation of shing (*H. fossilis*) fishes is dramatically increased all over the country. However, bacterial diseases may influence to decrease the production in ponds water. In this work, bacterial pathogens were sensitive against Ciprofloxacin (77%), Cotrimoxazole (97.3%), and Enorfloxacin (97.8%). All the strains showed resistant to 74/74(100%) Amoxicillin, and 63/74(85.1) erythromycin. The intermediate sensitive against Colistin was 35.1% and Doxycycline was 22.9% respectively.

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Introduction

Stinging catfish (*Heteropneustes fossilis) is* an indigenous air-breeding catfishes of South-East-Asia which is locally named as Shing in various parts of Bangladesh. Shing (*H. fossilis*) is extremely well-known and exceptionally important fish species in Bangladesh. In viewpoints, it isn't just perceived for its delightful taste and market esteem but at the same time is profoundly respected for being restorative and healthful. Due to high demand and market price, it is cultured in farms with high stocking density. Despite the fact that Shing (*H. fossilis*) culture has incredible potential in Bangladesh, different illnesses of Shing causes genuine financial misfortunes in view of their high mortality under cultivating conditions. Generally, different species of cultivated and freshwater fishes are infected by *Aeromonas* spp in Bangladesh (Sarker *et al*., 2000). Moreover, Rashid *et al.* (2008) distinguished *A. hydrophila* from epizootic ulcerative syndrome (EUS) influenced shing (*H. fossilis*). Once upon a time, shing was bounteously accessible in the vast water of Bangladesh, yet by and by, it is undermined due to abuse and different environmental changes in its regular natural surroundings. Despite the fact that, new approach of fry and fingerlings of shing fishes has been developed in recent years, but obscure diseases of shing (*H. fossilis)* cause great economic losses because of their high mortality rate. In any case, the production of *H. fossilis* is identified with their aquaculture credits which incorporate capacity to withstand taking care of pressure, ailment opposition, high development rate, fruitfulness and attractiveness (Anyanwu *et al.,* 2014).

Microscopic organism associated to produce infections in fish species have been accounted in various locale of Bangladesh and the revealed microbes were *Aeromonas hydrophila* (Ahamad *et al.,* 2013), *Flavobacterium columnare* in columnaris infection (Declercq *et al.,* 2003), *Edwardsiella* spp in edwardsiellosis (Mohanty and Sahoo, 2007), *Aeromonas salmonicida* in run of the mill furunculosis and *Psudomonas species* (Austin, 2011). The dangerous microbes such as *Pseudomonas* species, *Aeromonas* species, *Staphylococcus* species, *Flavobacterium* species, *Citobacter* species *Edwardsiella* species, and *Vibrio* species that live in every pond causing perilous, bacterial disease, for example, ulcer, blade decay and tail spoil of fishes. In Bangladesh, there is minimal accessible literature about bacterial infected shing fishes and antimicrobial sensitivity patterns of the isolates that have not been accounted for to gather enough information on pond cultured shing fish diseases. Therefore, the current study was embraced to isolate and identify bacteria from the infected pond cultured shing (*H. fossilis)* and observe their antibiotic affectability against various anti-infection agents.

Materials and methods

Collection and transportation of samples

A total of 84 infected shing fishes were directly collected from different regions of Bangladesh. During the collection of fish samples, precautionary measures were maintained to avoid touch and ice box was used to maintain cool chain. The samples were then brought to the laboratory of the Quality Aqua Laboratory, Quality feeds Limited, Mymensingh.

Sample processing and enrichment of bacteria

Three types of specimens of fishes were taken for investigation of a microbiological test such as intestine, skin and gill. These specimens were taken in a sterile chopping board and then minced properly and grind 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 and finally incubated at 37°C for 24 hours.

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 slant stand and incubated overnight at 37°C. Characterization of the pure isolates were performed employing some

common tests that involved colonial characteristics, bacterial cell morphology, alkaline and acidic reaction, H_2S (hydrogen sulfide production) and gas production, motility test, indole, urease test, oxidase test, catalase test, Methyl Red (MR) test, and Voges Praskaure (VP) test were performed. 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).

In-vitro antibacterial sensitivity test

According to the CLSI guidelines (CLSI -2012), the Kirby- Bauer disc diffusion methods were applied to in-vitro antimicrobial susceptibility tests of all the pathogenic bacteria isolates. *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.

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

SN	Name of Antibiotics	Disc Concentration	Interpretation of results (zone in diameter in mm)		
			S	I	R
1	Amoxycillin	10µg	≥22	16 - 21	≤ 23
2	Ciprofloxacin	5µg	≥16	12 - 15	≤17
3	Colistin	10µg	≥17	11-16	≤10
4	Clotetracycline	25µg	≥16	13- 15	≤17
5	Doxycycline	30µg	≥14	09-13	≤15
6	Erythromycin	15µg	≥23	14-22	≤13
7	Cotrimoxazole	25µg	≥16	11-15	≤10
8	Enorfloxacin	5µg	≥21	17-20	≤16

Sl= Serial, No.=Number, µg = Microgram, mm= Millimeter, S=Susceptible, M=Medium susceptible, R=Resistant, ≥ = Greater than or equal to, ≤ = Less than or equal to.

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 37°C for 24 hours. Around the discs, the antibiotic zones of inhibition conformed, estimated in the diameter of a millimeter (mm).The zone span was really scaled from the focal point of the anti-microbial plate as far as possible as a reasonable zone where microscopic organisms could be seen developing. The interpretation of antibiogram was measured in a millimeter (mm) of diameters as sensitive, intermediate and resistant as per the producer's guidelines.

Statistical analyses of experimental data

Data obtained were analyzed by SPSS version 20 and Excel 2016. Descriptive statistics and chi-square tests were used to check the statistical evaluation. To identify statistically significant differences, the p-value of that consideration was <0.5.

Results

Clinical signs and post mortem findings

In infected fishes, some external abnormalities such as equilibrium loss, hemorrhagic ulcerative lesion, rectal protrusion, dropsy, body and tail erosion, reddish discoloration around the eye and mouth, profuse mucous secretion and skin lesions on the body surface were clinically identified (Fig. 1). Postmortem examination also showed that organ enlargement and congestion in the internal organ of the diseased fishes were the cause.

Bacteria isolated from different diseased fishes

A total of 74(88.1%) pathogenic bacterial isolates were secluded from 84 samples of infected shing (*Heteropneustes fossilis*) fishes and only 10(11.9%) isolates were normal flora. The isolated bacterial pathogens found from different infected shing fishes are mentioned in Table 1 and Fig. 1.

Among 74 isolated strains, *Aeromonas* spp, was in the highest number 38(51.4%) and the next found was *Pseudomonas* spp 15(20.3%). The rest of isolates were distributed *as Staphylococcus* spp 7(9.4%), *Citobacter* spp 4(5.4%), *Vibrio* spp 3(4.1%) and only 7(9.4%) others namely *Bacillus* spp, *Edwardsiella* spp, *Enterococcus* spp, *Flavobacterium* spp, *Klebsiella* spp in infected *H. fossilis*.

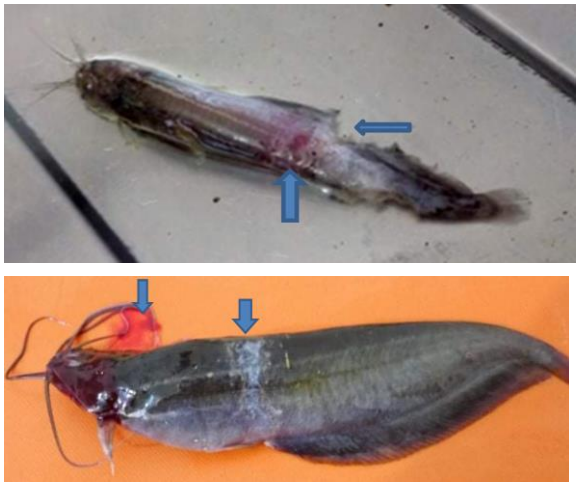

Fig. 1. Bacterial infected shing fish.

Table 1. Bacteria isolated from shing fishes.

Bacteria	*Aeromonas* spp.	*Pseudomonas* spp.	*Staphylococcus* spp.	*Citobacter* spp	*Vibrio* spp	Others bacterial species
N=74 (%)	38 (51.4)	15 (20.3)	7 (9.4)	4 (5.4%),	3 (4.1%)	7 (9.4%)

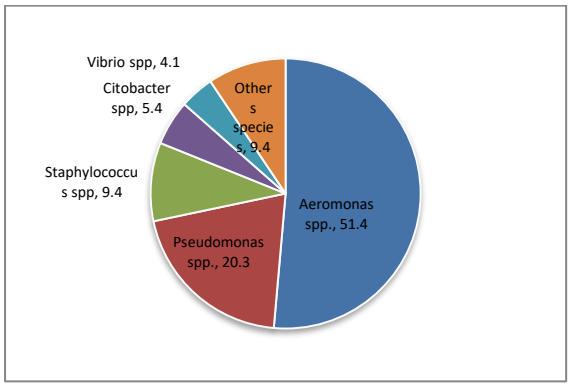

Fig. 2. Prevalence rate (%) of the isolated bacteria from infected *Heteropneustes fossilis.*

The all of the isolated bacterial species were tested against eight commercially available antibiotics and the results of their sensitivity are presented in Table 3.

In-vitro antibacterial susceptibility and resistance testing

In this study, the results of the antibiotic sensitivity testing exhibited that all of the bacterial isolates were sensitive against ciprofloxacin (77%), cotrimoxazole (97.3%), and enorfloxacin (97.8%); resistant against amoxicillin(100%), and erythromycin (85.1%), and intermediate against colistin (35.1%) and doxycycline (22.9%). Table 2 showed antibiotic sensitivity patterns of all of the individual isolates.

Table 2. Antibiogram profiles of isolated bacterial species.

Antibiotics		No(%)		
	Concentration	Resistant	Sensitive	Intermediate
Amoxycillin	10µg	74(100)	0(0)	0(0)
Ciprofloxacin	5µg	17(23.0)	57(77.0)	0(0)
Colistin	25µg	6(8.1)	42(56.8)	26(35.1)
Clotetracycline	25µg	24(32.4)	42(56.8)	8(10.8)
Doxycycline	30µg	11(14.9)	46(62.2)	17(22.9)
Erythromycin	15µg	63(85.1)	4(5.4)	7(9.5)
Cotrimoxazole	25µg	2(2.7)	72(97.3)	0(0)
Enorfloxacin	5µg	4(5.4)	65(87.8)	5(6.8)

Table 3. Antibiotics sensitivity patterns of Gram positive and Gram negative bacillus species from infected shing fishes.

Antibiotics	Aeromonas spp. (n=38)	Pseudomonas spp. (n=15)	Staphyococcus spp. (n=7)	Others pathogens (n=14)
Amoxycillin				
R	38(100)	15(100)	7(100)	14(100)
Ciprofloxacin				
R	1(2.6)	15(100)	1(14.3)	0
S	37(97.4)		6(85.7)	14(100)
Colistin				
R	5(13.2)	0	1(14.3)	0
S	16(42.1)	10(66.7)	2(28.6)	14(100)
I	17(44.7)	5(33.3)	4(57.1)	0
Clotetracycline				
R	13(34.2)	3(20.0)	1(14.3)	7(50.0)
S	21(55.3)	10(66.7)	6(85.7)	5(35.7)
I	4(10.5)	2(13.3)	0	2(14.3)
Doxycycline				
R	4(10.5)	3(20.0)	1(14.3)	3(21.4)
S	24(63.2)	9(60.0)	5(71.8)	8(57.2)
I	10(26.3)	3(20.0)	1(14.3)	3(21.1)
Erythromycin				
R	32(84.2)	15(100)	6(85.7)	10(71.4)
S	3(7.9)		0	1(7.1)
I	3(7.9)		1(14.3)	3(21.5)
Cotrimoxazole				
R		1(6.7)	0	1(7.1)
S	38(100)	14(93.3)	7(100)	13(92.9)
Enorfloxacin				
R	2(5.3)	0	0	2(14.3)
S	33(86.8)	15(100)	6(85.7)	11(78.6)
I	3(7.9)		1(14.3)	1(7.1)

Abbreviations: R=Resistant, S= Sensitive, I=Intermediate sensitive, Data are presented as No.(%).

Discussion

In spite of the fact that, shing (H. fossilis) is hardy fish due to their ability to adapt to a wide range of water parameters but cultivation in fresh water ponds has been influenced by different bacterial ,viral, and parasitic pathogens. The bacterial infections of shing fishes and their antibiotics sensitivity and resistant patterns were identified collecting infected

shing fishes from various regions of greater Mymensingh and Netrakona districts of Bangladesh.

A few numbers of bacterial species were/are pathogenic to fishes, which incorporated *Aeromonas species Pseudomonas species, Vibrio* species, *Staphylococcus* species, *Flavobacterium* species, *Edwardsiella* species, *Citobacter* species, and *Enterobacter* species (Abedin *et al.,* 2020; Anshary *et al.,* 2014).

However, in this study, the main isolated pathogenic bacteria were *Aeromonas species, Pseudomonas* spp, *Staphylococcus* spp, *Citobacter* spp, *Vibrio* spp and others were *Bacillus* spp, *Edwardsella* spp, *Enterococcus* spp, *Flavobacterium* spp, *Klebsiella* spp. These comparative outcomes and isolates were observed by Ahmed and Shoreit (2001). The motile bacteria *Aeromonas* species were identified aspredominant responsible factorfor infections in catfishes and its high mortality rates in Southeast Asia and different countries of the world (Anyanwu*et al.*, 2014). Similar results were observed in our work that was 51.4%. Sarkar and Rashid 2012 recognized *Aeromonas hydrophila from* different injuries of epizootic ulcerative disorder of various fishes. *Pseudomonas* spp were isolated from infected fishes from various sorts of water bodies (Hossain *et al.*, 2011) and another researcher Yanong (2011), noted those Gram negative bacteria in freshwater fish were responsible for skin sore of shing fishes. *Aeromonas* spp. and *Pseudomonas* species were isolated from diseased shing fish by Ahmed and Shoreit (2001). In this investigation, *Aeromonas* species, *Pseudomonas* species and *Staphylococcus species* isolates were analyzed against eight antibiotics.

All bacterial strains of *Aeromonas* spp and *Pseudomonas* species were seen to be 100% sensitive to cotrimoxazole and enorfloxacin, whereas ciprofloxacin and colistin were found 100% sensitive within other bacterial species such as *Citobacter* spp, *Vibrio* spp, *Bacillus* spp, *Edwardsiella* spp, *Enterococcus* spp, *Flavobacterium* spp, and *Klebsiella* spp. In our study, it was found that *Aeromonas* spp was highly sensitive to Ciprofloxacin (97.4%) and enorfloxacin (86.8%); *Pseudomonas* spp to cotrimoxazole (93.3%); *Staphyococcus* spp to ciprofloxacin, clotetracycline and enorfloxacin (85.7%). Only 92.9% Cotrimoxazole was sensitive to other bacterial species. The antibiogram of all bacteria isolates of *Pseudomonas* spp were found to be resistant 100% to amoxicillin, ciprofloxacin and erythromycin. The isolates of *Aeromonas species* were sensitive to cotrimoxazole and ciprofloxacin were reported by Abedin *et al.,* 2020.

Conclusions

This study has shown that bacterial diseases of shing (*Heteropneustes fossilis*) could be a major factor to suffer local fish farmers of Bangladesh considerable economic loss. A number of bacterial species including *Aeromonas* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Citobacter*spp, and *Vibrio* spp appeared to be the main pathogens and major causes of bacterial diseases to shing species. Indiscriminate uses of these antibiotics in shing fish cultured ponds cause multidrug resistance to different bacterial species. Isolation and identification of the causative agent and determination of the antimicrobial profile of bacterial agents associated with infections is necessary for effective antimicrobial treatment. A careful consideration should be given before deciding the antibiotic for treatment so as to prevent the emergence of antibiotic resistance.

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Conflict of interest: None to declare.

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