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Identification of pathogenic bacteria from diseased stringing catfish *Heteropneustis fossilis* with their sensitivity to antibiotics

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Abstract
The stringing catfish, *Heteropneustes fossilis* (locally known as Shing) is an important cultured species with excellent taste, market value, live fish, nutritional and medicinal benefits containing high content of Iron and Calcium. The study was performed to identify bacterial pathogens from diseased *H. fossilis* and to assess their sensitivity to antibiotics. Pure culture of bacteria using Slant and Streak plate techniques, and biochemical tests i.e. Gram's Staining, Motility Test, Sugar Fermentation Test, Indole Test, MR-VP Test, etc. were performed to identify the causative agents of the diseased fish. Five antibiotics discs i.e. Ciprofloxacin (5µg), Azithromycin (15µg), Ampicillin/Sulbactam (20µg), Tetracycline (30µg) and Erythromycin (15µg) were used to test the sensitivity of the isolated bacteria. Four pathogenic bacteria such as *Aeromonas hydrophila*, *Flavobacterium columnare*, *Edwardsiella tarda* and *Pseudomonas* sp. were identified in the studied diseased Shing (*H. fossilis*). Among them *F. columnare*, *E. tarda* and *Pseudomonas* sp. were identified from the fresh fish. *Aeromonas hydrophila* was found only in diseased Shing which was responsible for the disease, Motile Aeromonas Septicemia (MAS), also known as Dropsy. The results of the antibiotic sensitivity test showed multi-resistances of the identified bacteria to the tested antibiotics. Ciprofloxacin (5µg) was found sensitive to identified bacteria, and Azithromycin (15µg) and Ampicillin/Sulbactam (20µg) were moderately sensitive, but Tetracycline (30µg) and Erythromycin (15µg) were resistant to the studied bacteria. Ciprofloxacin (5µg) could be used to control MAS or Dropsy disease in fish. The results of this study will be helpful to the fish farmers for the management of bacterial diseases in fish. Further research could be done on using PCR technique for the identification of all bacterial isolates.

Keywords: Stringing catfish, *Aeromonas hydrophila*, antibiotic sensitivity, biochemical tests

1. Introduction
Fish is an important source of animal protein in the diet of the people of most Asian countries including Bangladesh. It provides valuable nutrient and vitamin, and helps to growth of people. Fisheries sector plays a vital role in terms of nutrition, employment, increased GDP growth and foreign exchange earnings. It is one of the major economic sources of our country. Total fish production of Bangladesh in 2016-2017 was about 4.13 million MT where aquaculture contributed 56.44% [1, 2]. Fisheries contributed 3.61% of the GDP, 24.41% of agricultural GDP [1, 2]. The country earned more than 42.88 billion BDT by exporting about 68.31 thousand MT of fish and fisheries products [1, 2]. Per capita annual fish intake is about 19.71 kg, supplementing about 60% of the protein of the daily national diet. The average growth rate of the last 10 years of Bangladesh fisheries is 5.43%. In fisheries sector, about 11% of the total population is directly or indirectly employed [1, 2].
Bangladesh is endowed with diversified fisheries resources, i.e. inland capture, marine capture and aquaculture. The production of aquaculture and capture fisheries of Bangladesh is gradually increasing [3]. We have lot of potentiality to grow fish in haors, baors, lakes, pond and derelict water bodies.
In recent years, the decline in open water fisheries both in inland and marine sector has directed towards aquaculture development because most of the factors affecting the productivity of fish in aquaculture are under heavy pressure as a result of worsening the

environmental conditions; siltation in river beds; water pollution from agricultural, industrial and municipal wastes; fish diseases; construction of embankments for flood protection; irresponsible and destructive fishing practices; and loss of natural breeding grounds through habitat degradation. Therefore, Bangladesh has focused its attention on aquaculture, which has a high potential for development. The country aquaculture contributes about 56% to total fish production in 2014-2015 [3].

The success of potential aquaculture depends on management of good water quality and fish diseases which are greatly influenced by aquatic microorganism along with other factors. Usually, a large number of bacteria are taken by aquatic animals including fish through their food and drinking water, which accumulate in their intestine and cause fish diseases and are constrains for successful implementation of intensive and semi-intensive aquaculture technologies [4].

A large number of microorganisms, causing diseases in farmed aquatic animals, are widely distributed in nature wherever favorable environmental factors are present for their growth. Bacteria are one of the most important microorganisms present in fishes responsible for economic loss due to mortality and contamination of fish and thus farmers are losing interest in fish farming [5]. The different chemicals including antibiotics are commonly used in large quantity in aquaculture for treatment of infectious diseases. Farmers often use excess amount of aqua-drugs due to influence of drug traders. There are several important concerns with regard to the use of chemicals in aquaculture [6]. Some of these chemicals, especially antibiotics are often non-biodegradable and persist in fish muscle and in the aquatic environment as residues. Thus, the use of unapproved drugs or misuse of approved drugs in aquaculture, fish possesses a potential human health hazard.

Among the cultured species, *Heteropneustes fossilis* is an indigenous stinging catfish of South-East-Asia, which is locally known as Shingi or Shing in different parts of Bangladesh, which is a freshwater fish found in small rivers, canal and swamp. It is not only recognized for its excellent taste and market value but is also highly sought after for its nutritional and medicinal benefits. The species has high content of iron (226 mg 100 g-1) and fairly high content of calcium compared to many other freshwater fishes. Due to its high nutritive value the fish is recommended in the diet of the sick and the convalescents. Being a lean fish it is very suitable for people for whom animal fats are undesirable.

Fish is known to harbor of bacteria of public health importance. Aquatic environment are known to influence the bacterial loads of the cultured fish. Bacteria associated with skin lesions and internal organs have been reported in fish reared in different region of Bangladesh and the reported bacteria which they were associated with include: *Edwardsiella tarda, Flavobacterium columnare, Mycobacterium spp., Aeromonas spp., Vibrio spp., Pseudomonas spp.* etc. For the eradication of these pathogenic bacteria various types of chemicals and drugs are used in cultured ponds and in hatcheries. In the advance condition of disease outbreak various commonly known antibiotics also applied and after the long time use of these antibiotics pathogenic bacterium becomes resistant against these antibiotics. As a result, widespread use of antibiotics, especially in hatcheries and cultured pond as prophylactic and therapeutic agents to prevent the bacterial infection or load leads to the development of multiple drug resistances and causes mass mortality of cultured as well as wild fishes due to bacterial infection. However, there is little available literature about bacteria associated with skin as well as the internal organs of diseased catfish and the antibiotic sensitivity profile of the bacterial isolates has not been reported from cultured fishes of Bangladesh yet.

Therefore, the present study was undertaken to isolate and identify bacteria associated with disease of cultured *H. fossilis* and also to test the sensitivity of the isolated bacteria against different antibiotics to determine the sensitivity and identify the appropriate antibiotics to control the diseases. The information of the study will be useful for the prevention and control of diseases of catfishes and thus, for the increment in aquaculture production. The objectives of the study are to isolate and identify pathogenic bacteria from diseased Shing (*H. fossilis*) fish and to identify the appropriate antibiotics effective to control bacterial disease of Shing (*H. fossilis*).

2. Materials and Methods

The study was performed to isolate and identify bacterial pathogen from stringing catfish, Shing (*Heteropneustes fossilis*) and to test the bacterial sensitivity towards commonly used antibiotics. The whole work was performed during the period of January 2018 to July 2018.

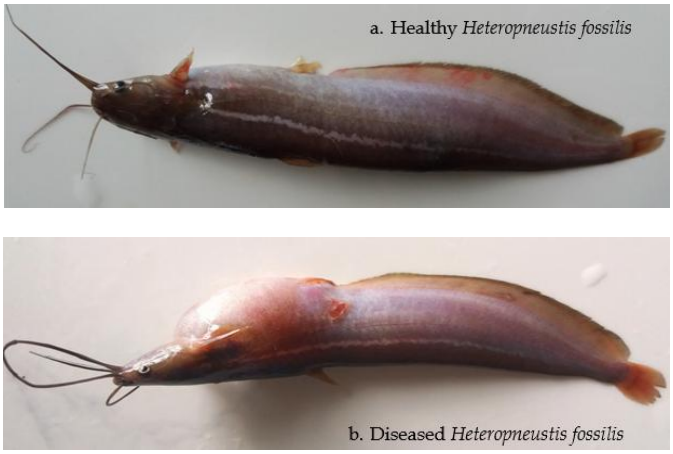

Fig 1: Samples of healthy and diseased *Heteropneustes fossilis*

2.1 Study sample

The study sample was indigenous stinging catfish (*Heteropneustes fossilis*), locally known as Shing or Shingi. A diseased and a fresh Shing were collected from aquaculture farm in Bangladesh (Figure 1).

2.2 Collection and Transportation of Samples

The collected fish (*Heteropneustes fossilis*) samples were transported to the laboratory aquarium of the Department of Fish Health Management, Sylhet Agricultural University (SAU), Sylhet.

2.3 Preservation of Samples

The collected fish samples were preserved in refrigerator at -20 °C to prevent further bacterial contamination.

2.4 Media and reagents used for bacterial culture

2.4.1 Preparation of Solid Media

Nutrient agar medium was prepared by dissolving 28 g of nutrient agar powder in 1 liter of distilled water following standard methods. After sterilization, the medium was poured

into sterile petridishes (5 ml in each petridish) and allowed to solidify and then incubated at 37 °C for overnight to check the sterility and used for cultural characterization or stored at 4 °C in refrigerator for future use.

2.4.2 Preparation of Liquid Media
Nutrient broth medium, Lactose broth medium, Phenol Red Sucrose Broth and Phenol Red Mannitol Broth were prepared following standard methods. Alkaline Peptone water was prepared by adding 30 g of peptone to 1 liter distilled water. MR-VP broth medium was prepared by adding 17 g of MR-VP broth powder in 1 liter of distilled following standard methods.

2.4.3 Chemical reagents
The reagents, used for the study, were phosphate buffer saline (PBS), reagents for Gram`s staining (crystal violet, gram`s iodine, acetone alcohol, safranin), 3% hydrogen peroxide methylene blue and other common laboratory chemicals and reagents. Methyl red (MR) solution, Potassium hydroxide (KOH) solution, Gram's Iodine Solution and Crystal violet or gentian violet (also known as methyl violet 10B or hexamethyl pararosaniline chloride) were prepared following standard methods. After that the crystal violet and ammonium oxalate monohydrate solutions were mixed to make the crystal violet stain. Acetone- Ethanol Solution, Counter stain (Safranin Solution), Normal saline solution and Physiological saline solution (PSS) were prepared following standard methods.

2.5 Preparation of pathogen sample for culture
The live fish was sacrificed for the collection of sub sample. For this purpose, six cotton bars and inoculating loop were taken to collect mucus and slime from whole skin, gill and body cavity of the infected and fresh fish. After that the cotton bars with sample were stricken three times on sterile solid nutrient agar media and inoculated with the loop into nutrient broth media and incubated at 37°C for 24 hours in incubator for observation of different bacterial colonies.

2.6 Isolation and identification of Bacteria
For the isolation and identification of bacteria from the mentioned samples the Morphological (size, shape, arrangement, motility) study was performed by Gram's staining reaction, colony characteristics, Biochemical reaction, catalase test, motility test. The suspected colony from these media was sub cultured in Nutrient agar and Nutrient Broth to promote the growth of a particular type of bacterium. Finally the pure culture was obtained from the selective media. Staining with "Gram's staining" method along with other tests was performed. Strict aseptic measures were maintained during the period of study. Striking on different solid agar was done under laminar air flow. After performing the above mentioned tests, the results were analyzed and the isolated bacteria present in samples were identified.

2.6.1 Isolation and identification of *Aeromonas hydrophila*
For the isolation and identification of *Aeromonas hydrophila*, the samples were first inoculated on Nutrient agar media in petridishes and incubated at 37°C for 24 hours. Then the yellow small spot round shape colonies from Nutrient agar were sub cultured again on Nutrient agar media in petridishes for three times. After that, one unit colony was transferred into Nutrient broth media in slant test tube for pure culture and incubated at 37 °C for 24 hours. Opaque yellowish color colonies were grown on slant media. Again a single colony was transferred in Nutrient broth media in test tube and petridishes for biochemical and antibiotic sensitivity tests.

2.6.2 Isolation and identification of *Pseudomonas* species
Pseudomonas species were allowed to grow on nutrient agar with creamy whitish small round shape, dense colonies. The samples were inoculated on Nutrient broth media in test tube and incubated at 37 °C for 24 hours for biochemical and antibiotic sensitivity tests.

2.6.3 Isolation and identification of *Edwardsiella tarda*
Edwardsiella tarda was allowed to grow on nutrient agar. On nutrient agar the *Edwardsiella tarda* colonies were translucent, opaque, large irregular whitish color colonies. A single colony was transferred in Nutrient broth media in test tube and petridishes for biochemical and antibiotic sensitivity test.

2.6.4 Isolation and identification of *Flavobacterium columnare*
Flavobacterium columnare were first inoculated on Nutrient agar media in petridishes and incubated at 37 °C for 24 hours. Then the yellow-grey small irregular colonies from Nutrient agar were sub cultured again on Nutrient agar media in petridishes for three times. After that, one unit colony was transferred into Nutrient broth media in slant test tube for pure culture. Yellowish-grey color colonies were grown on slant media. Then a single colony was transferred in Nutrient broth media in test tube and petridishes for biochemical and antibiotic sensitivity test.

2.7 Identification of bacteria
Bacterial identification was performed on the basis of colony morphology; Gram's staining reaction, motility and biochemical tests (including indole test, MR-VP test and sugar fermentation test).

2.7.1 Colony characteristics
Colony characteristics such as: shape, size, surface texture, color and opacity developed after 24 hours of incubation at 37°C were recorded.

2.7.2 Gram's staining test
Gram's staining of the pure culture was done following standard methods and then examined under light microscope (100X) using immersion oil.

2.7.3 Motility test
The motility test was done to distinguish motile bacteria from the non-motile one. A pure culture of the organism was allowed to grow in Nutrient broth. One drop of broth culture was placed on the cover slip and inverted over the concave depression hanging drop slide to make hanging drop preparation. The hanging drop slide was then observed carefully under compound light microscope (100x) using immersion oil.

2.8 Biochemical Tests
2.8.1 Sugar Fermentation test
The sugar fermentation test was performed by inoculating a loop full of NB culture of the organisms into each tube

containing three basic sugars (e.g. sucrose, lactose, and mannitol) separately. Acid production was indicated by the color change of reddish to yellow in the medium and the gas production by the appearance of gas bubbles in inverted Durham's tube.

2.8.2 Indole test
One ml of xylene was inoculated with the 5 ml of bacterial broth culture and incubated at 37 °C for 48 hours. 0.5 ml of Indole reagent was added, shaked well and examined after 1 - 2 minutes. A pink to red color in the reagent layer indicated indole positive. In negative case, there is no development of color.

2.8.3 MR-VP Test
MR-VP broth was used for both MR test and VP test. Only addition of reagent differs, both tests were carried out consecutively.

2.9 Antibiotic sensitivity test
Antimicrobial Susceptibility Testing (AST) was used to determine which specific organism or group of organisms were susceptible to which antibiotics. The standard procedure for assessing antimicrobial activity was the disc diffusion test [7]**.** After incubation period, the diameters of the inhibition zones formed around each disc were measured. The zone radius was actually scaled from the centre of the antibiotic disc to the end of the clear zone where bacteria could be seen growing. The antibiotics, their codes and concentrations were as follows: Ciprofloxacin (5µg), Azithromycin (15µg), Ampicillin/Sulbactam (20µg), Tetracycline (30µg) and Erythromycin (15µg). Inhibition zone diameters were then interpreted into susceptibility categories based on the zone size (Susceptible, Intermediate, and Resistant) [8]. The sensitivity was identified as (a) Sensitive- S: zone inhibition wider than or equal to 18 mm, (b) Intermediate- I: zone inhibition between 13-17 mm and (c) Resistant- R: no zone of inhibition or less than 13 mm.

2.10 Statistical analysis
The tables were prepared using MS Excel. Other statistical analysis and interpretations thereafter were done by using the computer software like Microsoft Excel.

3. Results and Discussion
This study examined the clinical, morphological and biochemical characteristics of different bacteria found in a diseased *H. fossilis*. The isolated and identified bacteria were *A. hydrophila, E. tarda, F. columnare* and *Pseudomonas* spp. All of these bacteria grew on nutrient agar and produced round, smooth, colorless, dew drop like colonies on the petridishes of nutrient agar after incubation of 24 hours. On the other hand, most of the bacteria grew in nutrient broth and produced turbidity and heavy sedimentation in the test tube of nutrient broth after incubation of 24 hours in incubator. For the separation of specific bacterial colony, nutrient agar plate culture for sub-culture and pure culture were used for the growth of *A. hydrophila, E. tarda, F. columnare* and *Pseudomonas* spp. The methods used for the identification of the bacteria were cultural characteristics, staining methods, motility test and biochemical tests. The antibiogram profile was done by culturing bacteria on nutrient agar media and using commercially available antibiotic disc. Most of the bacteria showed highly sensitive, intermediate and some were resistant according to their ring size.

3.1 Clinical pathology
Naturally infected Shing fish lost their normal appearance. Fish showed reddish abdomen and anal region, pale body color, external ulcerative lesions with hemorrhagic and reddened fin bases and skin, mucous secretion, congestion and enlargement with hemorrhage of the internal organs. Moribund fish were found to swim abnormally at water surface with full of fluid in the body cavity and swollen abdomen (Figure 1).

3.2 Isolation of Pathogenic bacteria
Most of the bacteria grow in nutrient broth and produce turbidity and heavy sedimentation in the test tube of nutrient broth after incubation of 24 hours in incubator. On the other hand most of the bacteria grow on nutrient agar and produce round, smooth, colorless, dew drop like colonies on the petridishes of nutrient agar after incubation of 24 hours. For the separation of specific bacterial colony, nutrient agar plate culture for sub-culture and pure culture used for the growth of *A. hydrophila, E. tarda, F. columnare* and *Pseudomonas* species.

3.2.1 Isolation of infectious bacteria from *Heteropneustes fossilis*
Bacteria are isolated based on their morphological feature, cultural characteristics etc. There are four (4) types of bacteria isolated from diseased and non- diseased Shing (*Heteropneustes fossilis*). The isolated bacteria are *Aeromonas hydrophila, Edwardsiella tarda, Flavobacterium columnare* and *Pseudomonas* Species.

3.3 Identification of bacterial colonies
Identification of bacteria was done by cultural characteristics, staining methods, motility test and biochemical tests.

3.3.1 Cultural characteristics of *A. hydrophila, E. tarda, F. columnare* and *Pseudomonas* spp.
The cultural characteristics of *Aeromonas hydrophila, Edwardsiella tarda, Flavobacterium columnare* and *Pseudomonas* species were examined by observation of cultured bacterial colonies color, shape and transparency (Table 1; Plates 1-4).

Table 1: Colony characteristics of *A. hydrophila, E. tarda, F. columnare* and *Pseudomonas* spp. observed during the study

Sl. No.	Name of bacteria	Colony characteristics
1	*Pseudomonas spp.*	Creamy whitish, round, opaque colony
2	*A. hydrophila*	Yellow, round, dense colony
3	*E. tarda*	Transparent whitish, irregular dense colony
4	*F. columnare*	Yellowish- grey, irregular opaque colony

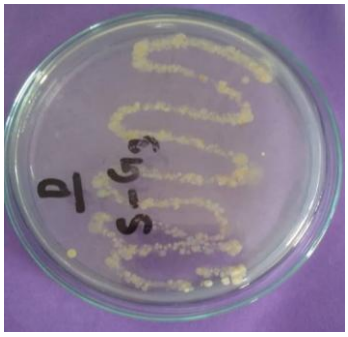

Plate 1: *Pseudomonas spp.* on Nutrient agar

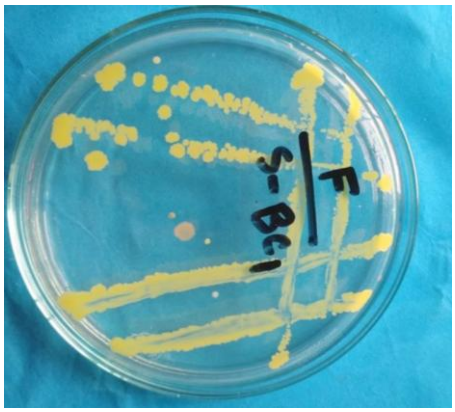

Plate 2: *A. hydrophila* on Nutrient agar

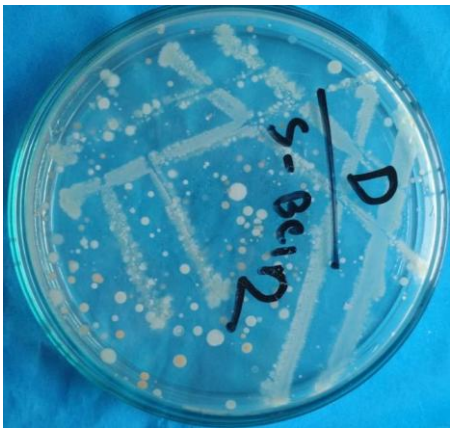

Plate 3: *E. tarda* on Nutrient agar

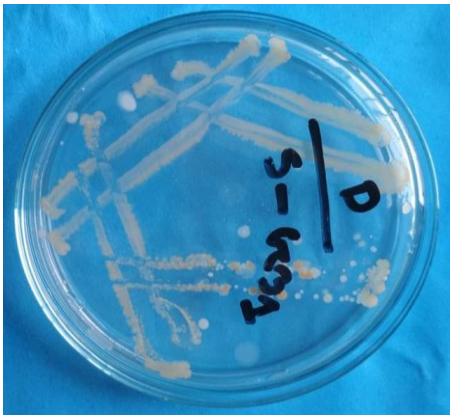

Plate 4: *F. columnare* on Nutrient agar

3.3.2. Gram staining test for *Pseudomonas* species, *A. hydrophila, E. tarda* and *F. columnare* showed in Table 2 and Plates 5-8.

Table 2: Morphology and Gram's staining properties of *Pseudomonas* species, *A. hydrophila*, *E. tarda* and *F. columnare*

Characteristics			Identified bacteria
Shape	**provisions**	**Gram's staining reaction**	
Small rods	Single, paired or in short chain	-ve	*Pseudomonas* spp.
Straight rods with rounded ends	Single, paired or in short chain	-ve	*A. hydrophila*
Short straight rod	Single	-ve	*E. tarda*
Small rods with rhizoidal edges	Single	-ve	*F. columnare*

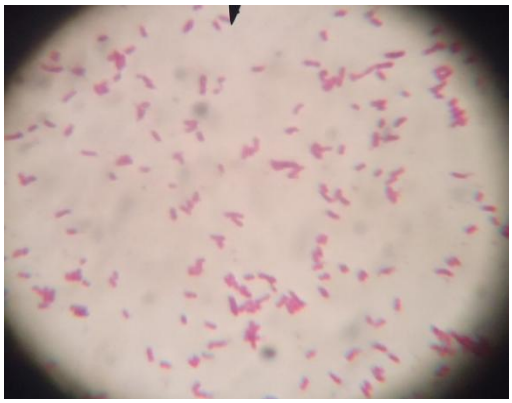

Plate 5: *E. tarda* found gram negative

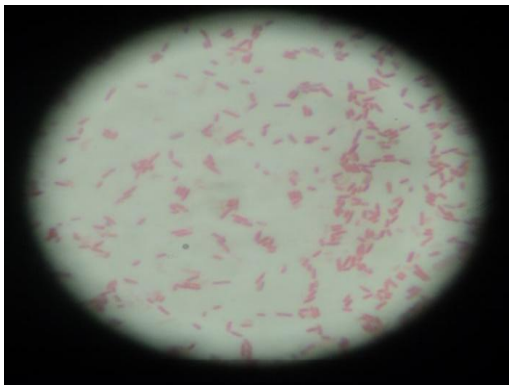

Plate 6: *A. hydrophila* found gram negative

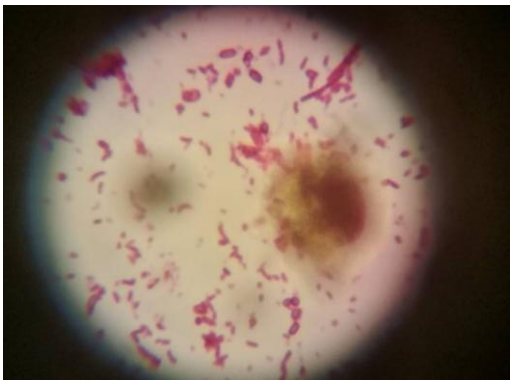

Plate 7: *F. columnare* found gram negative

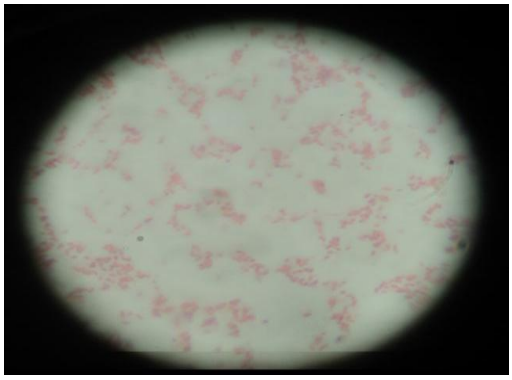

Plate 8: *Pseudomonas* spp. found gram negative

3.3.3 Motility test of *Pseudomonas* species, *A. hydrophila, E. tarda* and *F. columnare*

In hanging drop slide method it was found that *Pseudomonas* species, *A. hydrophila*, *E. tarda* and *F. columnare* were motile that shown in Plates 9-12.

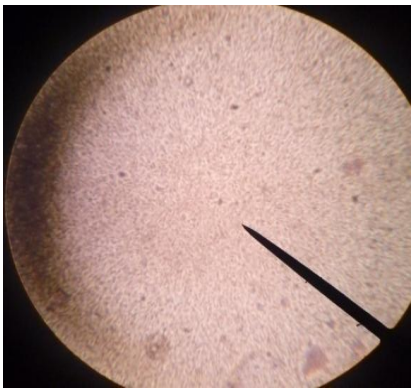

Plate 9: Movement of *Pseudomonas* spp. on microscope

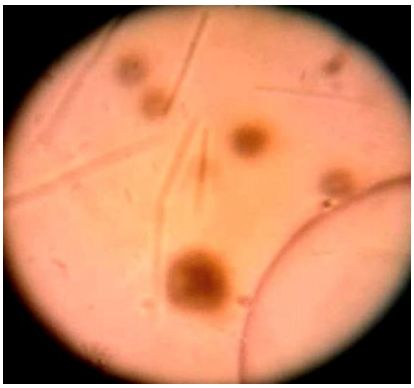

Plate 10: *A. hydrophila* found motile on microscope

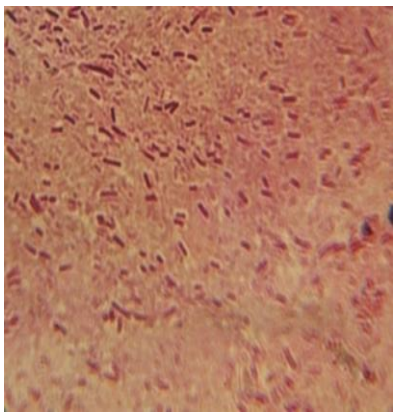

Plate 11: *E. tarda* found motile on microscope

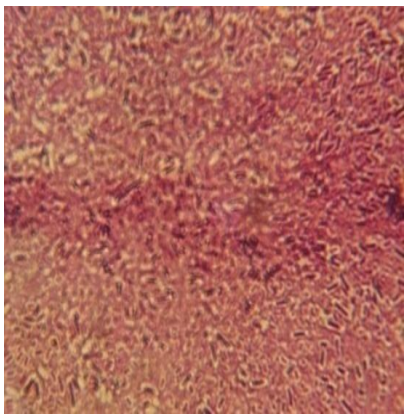

Plate 12: *F. columnare* found motile on microscope

3.3.4 Biochemical tests of *Pseudomonas* species, *A. hydrophila, E. tarda* and *F. columnare*

Results of sugar fermentation tests using three basic sugars such as lactose, sucrose and manitol; acid and gas production were indicated by change of color from red to yellow and presence of gas bubbles. Negative reaction was indicated by no change of color. Biochemical test result is presented on Table 3 and Plates 13-28.

Table 3: Biochemical properties of *A. hydrophila, F. columnare, Pseudomonas* species and *E. tarda*

Carbohydrate fermentation test			MR test	VP test	Indole test	Interpretation of results
Lactose	Sucrose	Mannitol				
Acid	Acid and Gas	Acid	-ve	+ve	+ve	*A. hydrophila*
Acid	Acid	Acid	-ve	-ve	-ve	*F. columnare*
-ve	-ve	Acid	-ve	-ve	-ve	*Pseudomonas* spp
-ve	-ve	-ve	+ve	-ve	+ve	*E. tarda*

3.3.4.1 Biochemical tests of *A. hydrophila*

Plate 13: *A. hydrophila* found positive on sugar fermentation test.

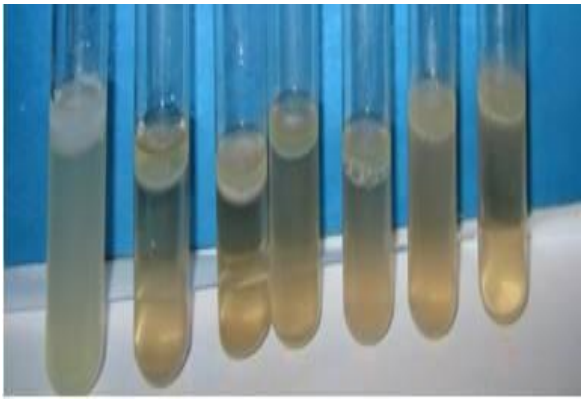

Plate 14: *A. hydrophila* showing negative on MR test

Plate 15: *A. hydrophila* showing positive on VP test

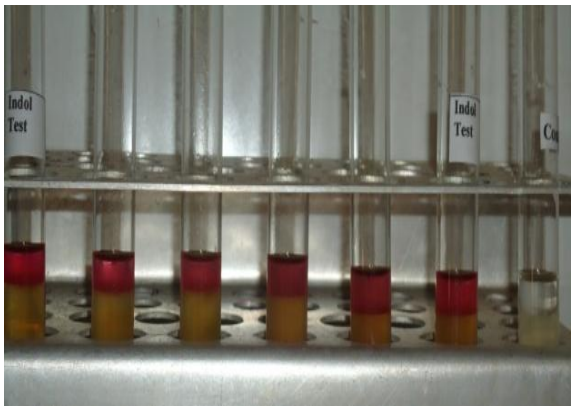

Plate 16: *A. hydrophila* showing positive on Indole test

3.3.4.2 Biochemical tests of *F. columnare*

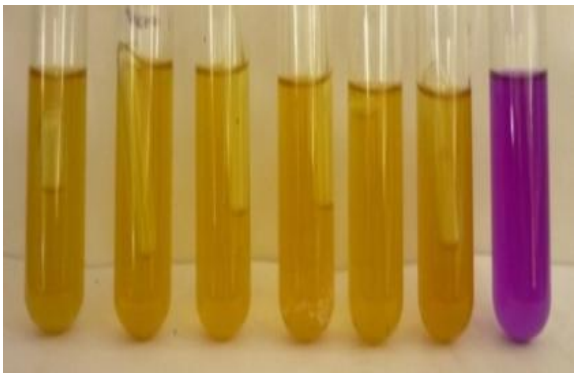

Plate 17: *F. columnare* produces acid on sugar fermentation test.

Plate 19: *F. columnare* showing negative on VP test

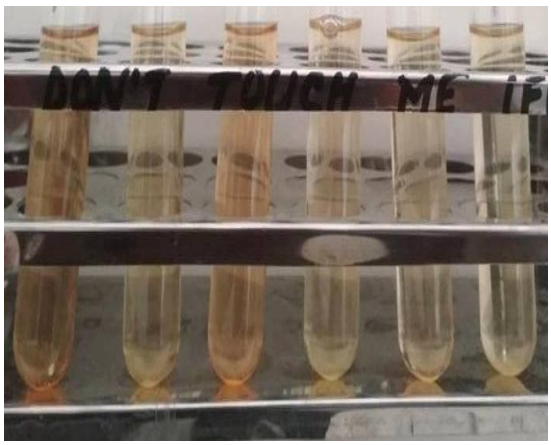

Plate 20: *F. columnare* showing negative on Indole test

3.3.4.3 Biochemical tests of *Pseudomonas* spp

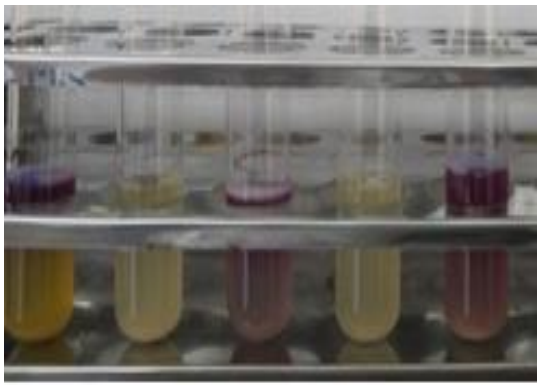

Plate 21: *Pseudomonas* spp found negative on sugar fermentation test

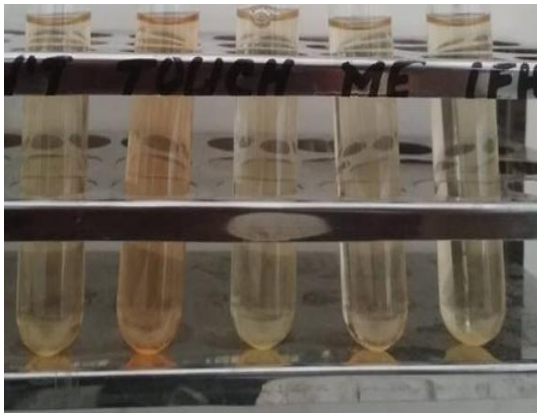

Plate 22: *Pseudomonas* spp found negative on MR test

Plate 23: *Pseudomonas* spp showing negative on Indole test

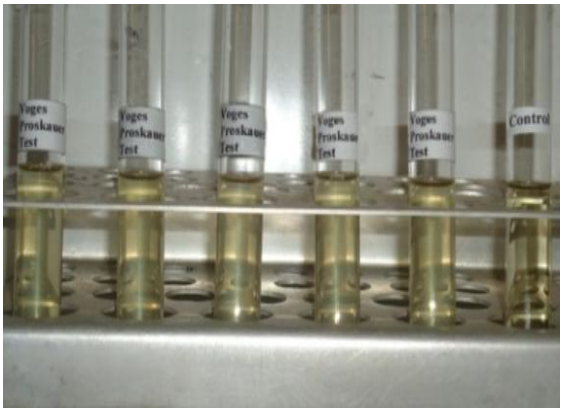

Plate 24: *Pseudomonas* spp found negative on VP test

3.3.4.4 Biochemical tests of *E. tarda*

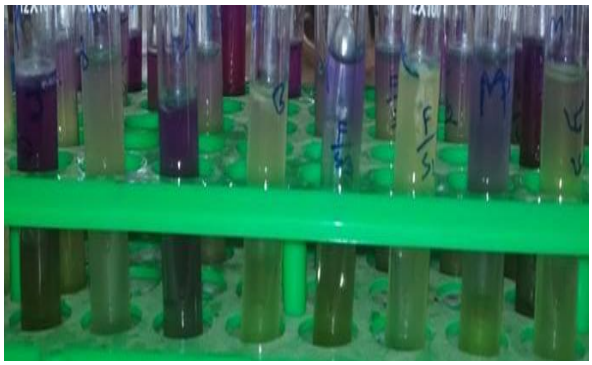

Plate 25: *E. tarda* found negative on sugar fermentation test

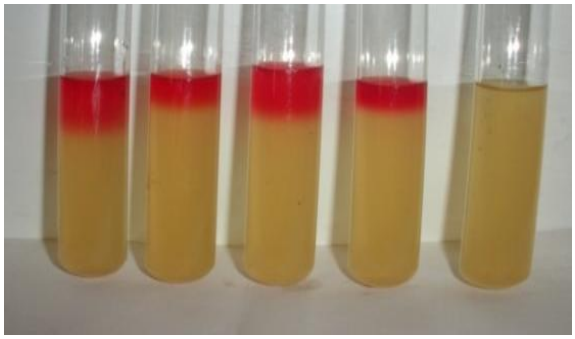

Plate 26: *E. tarda* found positive on MR test

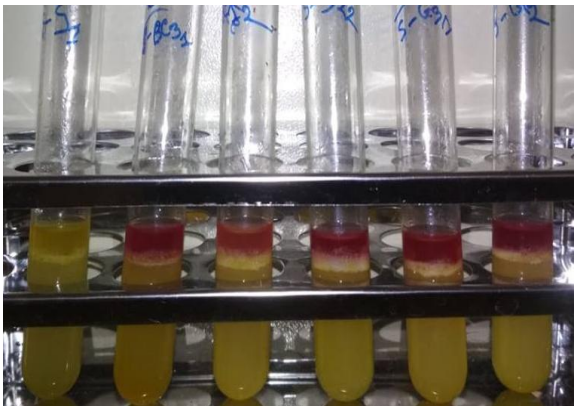

Plate 27: *E. tarda* found positive on Indole test

Plate 28: *E. tarda* found negative on VP test

3.4. Prevalence of pathogenic bacteria in fresh and diseased Shing (*H. fossilis*)

The prevalence of bacteria was higher in diseased fish than the fresh fish. In fresh shing, three bacterial colonies were identified such as *Pseudomonas species*, *E. tarda and F. columnare*, whereas four bacterial colonies like *A. hydrophila*, *Pseudomonas species*, *E. tarda and F. columnare* were present in diseased shing fish. Finally, the study confirmed that *A. hydrophila* was the responsible for the disease in the examined *H. fossilis*. The disease was identified as Dropsy.

3.5. Antibiotic sensitivity Test

The isolated bacterial colonies were tested against five commercially available antibiotics and the results of their sensitivity are presented in Tables 4-5. Most of the bacterial samples were sensitive against to Ciprofloxacin (100%), intermediate to Azithromycin (75%), Ampicillin/Sulbactam (50%) and resistant against to Tetracycline (75%), Erythromycin (100%). Where Ciprofloxacin (100%) found more effective to all the identified bacterial colonies and Azithromycin were intermediate to all isolates bacteria except *Pseudomonas spp* which was sensitive against it. Ampicillin/Sulbactam (50%) was intermediate to *A. hydrophila, F. columnare.* Tetracycline (75%) and Erythromycin (100%) were highly resistant to all the identified bacteria except *E. tarda* which were intermediate to Tetracycline (Figure 2).

Table 4: Antibiotic sensitivity test on isolated bacteria from infected Shing (*H. fossilis*)

Antibiotic (Cons/Disc)	*A. hydrophila*	*F. columnare*	*Psudomonas spp.*	*E. tarda*
Ciprofloxacin (5µg)	+++	+++	+++	+++
Azithromycin (15µg)	++	++	+++	++
Ampicillin/Sulbactam (20µg)	++	++	+	-
Tetracycline (30µg)	-	-	-/+	++
Erythromycin (15µg)	-	+	-	+

-: No inhibition, +: Inhibitory zone less than 13mm, ++: Inhibitory zone between 13- 17mm, +++: Inhibitory zone equal 18mm or above.

Table 5: Antibiogram profile percentages (%) of isolated colonies (n=4)

Antibiotics (conc.)	No percentage (%)		
	Sensitive	Intermediate	Resistant
Ciprofloxacin (5µg)	4 (100)	0	0
Azithromycin (15µg)	1 (25)	3(75)	0
Ampicillin/Sulbactam (20µg)	0	2 (50)	2 (50)
Tetracycline (30µg)	0	1(25)	3 (75)
Erythromycin (15µg)	0	0	4 (100)

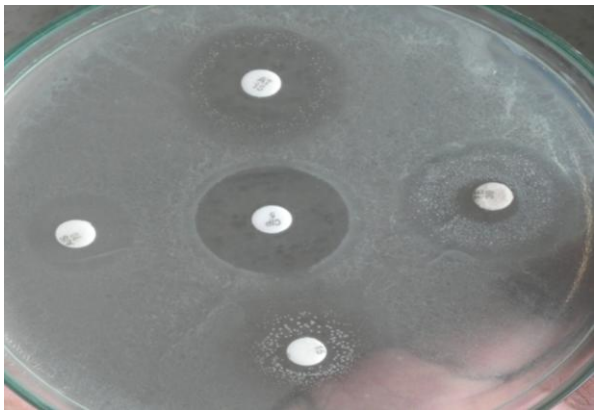

Fig 2: Antibiotic sensitivity and resistant pattern of bacteria isolated from infected *H. fossilis*

Shing (*H. fossilis*) is considered as a high valued and hardy fish species in Bangladesh but production of it in the cultured ponds and farms have been affected by various factors including diseases caused by viral, bacterial and fungal pathogens leads to high mortality of young and adult *H. fossilis* in cultured ponds and farms located in greater Sylhet of Bangladesh. However, the clinical symptoms were loss of equilibrium, skin lesions, mucous secretion, hemorrhages, body and tail erosion, congestion and enlargement with hemorrhage of the internal organs such as body cavity, abdomen which were similar with the findings of other studies [9-12]. Packed liver and internal organs were also observed in the diseased fishes by other researchers[10].

The isolated bacteria were *Aeromonas hydrophila, Pseudomonas, F. columnare* and *E. tarda*. More or less similar results and isolates were recorded by other researchers [13, 10, 14]. Among the bacterial diseases, the motile *Aeromonas* caused mass mortality and serious epidemics of ulcerative disease in catfish in Southeast Asia and other regions of the world [15, 16]. The important bacterial pathogens frequently isolated from the diseased fishes throughout the world as well as in Bangladesh were *Aeromonas* and *Pseudomonas* [17]. Additionally, *Aeromonas* is a very common pathogen in carps and other freshwater fishes [18]. *Aeromonas hydrophila* was commonly observed in various species of diseased farmed and wild freshwater fishes in different locations of Bangladesh [19]. It was known as a causative agent of ulcer type disease like Motile Aeromonas Septicemia (MAS) occurred in farmed fishes [20]. Reddish head and anal region, pale body colour, external ulcerative lesions on body surface with hemorrhage, skin erosion and redden fin bases were found from motile Aeromonas septicemia (MAS) like diseased silver carp (*H. molitrix*) in Mymensingh [21]. *A. hydrophila* were frequently isolated from various lesions of epizootic ulcerative syndrome (EUS) of different fishes [22]. Five species of *Aeromonas* spp. were isolated and identified from the diseased fishes of *Labeo rohita, Cyprinus carpio, Cirrhinus cirrhosus, Catla catla* and *Hypophthalmichthys molitrix* [23]. In this study, the morphological and biochemical characteristics of the isolated *A. hydrophilla* were quite similar with those that reported by other studies [24, 25, 23].

Some *Pseudomonas* strains were isolated and identified from diseased farmed fish of Bangladesh [20]. Pseudomonad isolates were also identified several from diseased fish from different types of water bodies [17]. They observed that *P. anguilliseptica* was also isolated from *O. niloticus* fishes affected with *Pseudomonas* Septicemia in Bangladesh. *P. fluorescens* was also isolated from various diseased fishes [26, 27]. *Pseudomonas* showed more or less similar morphological and biochemical results from diseased shing fish [15, 28].

Flavobacterium columnare isolated from symptomatic cat fish (*Clarius batrachus*) in Himalayan and Sub- Himalayan regions [29]. The isolation, characterization of pathogenic bacteria *Pseudomonas* and *Flavobacterium* were associated with bacterial fish disease [30]. *Flavobacterium columnare* were isolated from diseased *Cirrhinus mrigala* and *Carassius auratus* [31]. Biochemical properties of *Flavobacterium columnare* isolated from Carp and Gold fish were similar [31].

Edwardsiella tarda were isolated from freshwater catfish and their environment [32]. More or less similar morphological and biochemical results were found for *Edwardsiella tarda* from catfish and their environment [32, 33].

The most skin lesion-causing organisms in freshwater fish are gram-negative bacteria [34]. Many researchers have isolated different species of bacteria from the skin of the fresh water fish (catfish) [35, 36]. All bacterial infections were found as mixed infections. Mixed bacterial infections with *Aeromonas* spp. & *Pseudomonas* spp. was also reported [10].

In this study, *Aeromonas hydrophila, Pseudomonas, F. columnare* and *E. tarda* isolates were conducted by disc diffusion method against five antibiotics where, all of the isolates were found to be sensitive to Ciprofloxacin (100%), intermediate to Azithromycin (75%), Ampicillin/Sulbactam (50%) and resistant against to Tetracycline (75%), Erythromycin (100%). Where Ciprofloxacin (100%) found more effective to all the identified bacterial colonies and Azithromycin was intermediate to all isolates bacteria except *Pseudomonas spp* which was sensitive to it. Ampicillin/Sulbactam (50%) was intermediate to *A. hydrophila, F. columnare.* Tetracycline (75%) and Erythromycin (100%) were highly resistant to all the identified bacteria except *E. tarda* which were intermediate to tetracycline. Ciprofloxacin was highly effective against *Aeromonas* sp. and *Pseudomonas* sp. which were similar to those that previously studied [25]. Where, Tetracycline and Erythromycin were fully resistant since indiscriminately used in fish culture ponds as well as farmers don't maintain recommend applying dose and resulting in transfer of resistance genes to the isolated bacterial strains. Tetracycline group enhances the production of plasmid-mediated resistance in aquatic bacteria resulting in increased frequency of new Tetracycline resistant isolates [37].

4. Conclusion

The present study was conducted to isolate and identify pathogenic bacteria in Shing (*Heteropneustes fossilis*) with their sensitivity to commonly used antibiotics. Both fresh and diseased Shing (*Heteropneustes fossilis*) samples were utilized. Bacteria samples were cultured on sterile solid nutrient agar media for observation of different bacterial colonies. The isolated pathogenic bacteria were *Aeromonas hydrophila, Pseudomonas, F. columnare* and *E. tarda*. In fresh shing, three identified bacterial colonies such as *Pseudomonas spp., E. tarda and F. columnare* were found where *A. hydrophila* along with above bacterial species were found in diseased shing fish. *Aeromonas hydrophila* was responsible for Motile Aeromonas Septicemia (MAS), also known as Dropsy in Stinging catfish (*H. fossilis*) leads to swollen kidney and spleen with semi-fluid yellowish contents in body cavity, skin lesion, large, red hemorrhages on external and internal organ, fin base erosion, necrotic tissue and abdominal ascites. Secondary infections could be occurred by *Pseudomonas, F. columnare* and *E. tarda*. The isolated bacteria were tested against five commercially available antibiotics. Most of the bacterial samples were sensitive against to Ciprofloxacin (100%), intermediate to Azithromycin (75%), Ampicillin/Sulbactam (50%) and resistant against to Tetracycline (75%) and Erythromycin (100%). Ciprofloxacin (5µg) could be administrated to control the Motile Aeromonas Septicemia (MAS) disease in catfish (*H. fossilis*). The result of this study will be beneficial for the fish farmers who are regularly culturing catfish for diagnosing and controlling diseases by the administration of specific antibiotics. Further research could be done using PCR techniques for identification of all bacterial isolates.

5. Acknowledgement

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drugs and chemicals to control fish diseases through proper diagnosis using plate culture, histopathology and PCR techniques" project of Ministry of Science and Technology, Bangladesh.

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