Identification of Pathogenic Bacteria from Diseased Thai Pangas *Pangasius hypophthalmus* with Their Sensitivity to Antibiotics

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MP performed the literature searches, the laboratory work and the statistical analysis. Author MMMA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SMIK, AH, OG, MMR and MAAM managed the analyses of the study. Author MAAM executed the reviewers' comments. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2020/v30i330201

Received 16 February 2020
Accepted 22 April 2020
Published 29 April 2020

ABSTRACT

Thai Pangas (*Pangasius hypophthalmus*) has been emerging as an economically very important species due their faster growth, year round production and high productivity in South-East Asian aquaculture. It has good market value as a food fish due to its good taste and deliciousness with high protein, minerals and fat content. It is also popular as a game fish.

**Aims:** The present study was conducted to identify bacterial pathogens in diseased *P. hypophthalmus* and evaluate their sensitivity to antibiotics.

**Methodology:** To identify the causative agent of diseased fish pure culture of bacteria using slant and streak plate techniques and different biochemical tests such as Gram’s Staining, Motility Test, Sugar Fermentation Test, MR-VP test, Indole Test etc. were performed. To assess the sensitivity of the isolated bacteria to antibiotics five antibiotics disks i.e. Ciprofloxacin (5μg), Azithromycin (15μg), Ampicillin/Sulbactam (20μg), Tetracycline (30μg) and Erythromycin (15μg) were used.

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1. INTRODUCTION

Fisheries sector plays a vital role in food security and economic development of Bangladesh. It is also potential for its significant contributions in the development of agrarian economy of the country. It is also important for employment generation, supplying animal protein, earning foreign currency and poverty alleviation. Global fish production peaked at about 171 million MT in 2016 [1], in which aquaculture contribute 47 percent of the total and 53 percent if non-food uses were excluded. The total sale value of aquaculture and fisheries production in 2016 was estimated as USD 232 billion and USD 362 billion [1], respectively. In per capita terms, food fish consumption grew from 9.0 kg in 1961 to 20.2 kg in 2015, at an average rate of about 1.5 % per year. The fish consumption further reached to about 20.3 and 20.5 in 2016 and 2017 respectively [1]. Asia contributes more than 90 percent of the world aquaculture production, like other farming system. Bangladesh is one of the world’s leading fish producing countries with a total production of 41.34 lac MT in 2016-17 [2]. The fisheries sector plays a very important role in the national economy, contributing 3.69% to the Gross Domestic Product (GDP) of the country and 22.60% to the agricultural GDP [3].

In 2015–2016, Bangladesh was the fifth in world aquaculture production, which accounted for half of the country’s total fish production 55.15% [2]. The fisheries resources of Bangladesh have got position among the richest in the world and the inland fisheries production ranks 4th, where China and India are leading the global position [1]. About 11% people of the total population of Bangladesh are directly or indirectly involved in various activities of fisheries sector to lead their livelihood. Fish supplements about 60% of Bangladeshi people’s daily animal protein intake [2]. More than 17 million people including about 1.4 million women depend on fisheries sector for their livelihoods through fishing, farming, fish handling, and processing. A different survey revealed that more than 80% of laborers engaged in the fish processing industries are women [2]. Over the last few decades pangas culture in Bangladesh become the integral part of aquaculture. Pangasius hypophthalmus (Hamilton, 1822) is a catfish species of the family Pangasiidae under the order Siluriformes. It is locally known as Thai pangas in different parts of Bangladesh. It forms a good fishery of considerable value. Pangas having a good market price food fish due to its good taste and deliciousness with high protein, mineral and fat content in its flesh. It is also popular as a game fish. Recently it has made its entry into ornamental fish markets and has also been documented to be exported from India. In Bangladesh aquaculture contributes about 56% to total fish production in 2014-2015. Pangas production was estimated as 14.59% of the total inland fish production [2].

Culture of Thai pangas has become very popular and economically beneficial among the local fish farmers. But diseases are the most common and severe problem in Thai pangas farming of Bangladesh. Diseases cause monetary loss to farmers due to poor growth, death of fish and increased production cost. Due to diseases price of this fish is decreasing, causes financial losing of farmers and livelihood of farmers, traders and many others who depending on the pangas industry are in a great threat. A number of diseases like epizootic ulcerative syndrome, skin loss, gill alteration, tail and fin rot are common in farmed fishes of Bangladesh [4]. In pond aquaculture system, high stocking density and irregularly feed supply is very prone to disease outbreak. Most pond fish farmers do not have a

Results: Three pathogenic bacteria i.e. Aeromonas hydrophila, Edwardsiella ictaluri and Pseudomonas sp. were identified in the studied diseased fish. Only Pseudomonas sp. were identified from Fresh fish. E. ictaluri was found only in diseased pangas which was the causative agent for the disease, Bacillary Necrosis. The results of the antibiotic sensitivity test showed multi-resistances of the identified bacteria to the tested antibiotics. The identified bacteria were 100% sensitive to Ciprofloxacin (5µg), intermediate to Azithromycin (15µg) and Tetracycline (30µg), but resistant to Erythromycin (15µg) and Ampicillin/Sulbactam (20µg).

Conclusion: Ciprofloxacin (5µg) could be used to control bacillary necrosis disease in Thai pangas. The results of this study will be helpful to the fish farmers for the management of bacterial diseases in fish.

Keywords: Bacteria culture; diagnosis; bacterial diseases control; effective antibiotics; fish health management.
good understanding of health and disease issues in their system. Many diseases of fish are secondary to environmental insult, and can be prevented through proper management. The different chemicals including antibiotics are commonly used in large quantity in aquaculture for treatment of infectious diseases by farmers. 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 [5]. 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. However, there is hardly such scientific information available from which rural pond aqua-farmers could be benefited. 

Fish are susceptible to a wide variety of bacterial pathogens. Many of these bacteria are obligatory in nature whereas few are opportunistic. Some of these bacterial pathogens of fishes are fastidious and require special growth media for laboratory culture. Others grow at different temperatures, dependent upon the aquatic environmental temperature of the fish. Some of the more common bacterial pathogens are Edwardsiella ictaluri; Flavobacterium columnare; Mycobacterium sp. Aeromonas spp., Vibrio spp. and Pseudomonas spp. [6,7]. Therefore, the present study was undertaken to isolate and identify bacteria associated with skin muscle and internal organs of cultured P. hypopthalmus and also their sensitivity against different antibiotics to determine and identification of bacterial pathogens cause catfish diseases.

2. MATERIALS AND METHODS

The present study was undertaken to isolate and identify the bacteria from pangas (P. hypopthalmus) and assess the antibiotic sensitivity profile of pathogenic bacteria to compare the bacteriological status in terms of sound aquaculture practice. The whole work was performed in the laboratory of the Faculty of Fisheries and Department of Microbiology and Immunology, Faculty of Veterinary and Animal science, Sylhet Agricultural University, Sylhet, Bangladesh, during the period of June/2018 to November/2018. 

2.1 Fish sample

The fish sample pangas (P. hypopthalmus) was collected from Bangladesh Rural Advancement Committee (BRAC) fish hatchey Sreemangal, Moulvibazar, Bangladesh. Both diseased and healthy (non-diseased) Pangas (P. hypopthalmus) were considered as fish sample for the study (Fig. 1).
2.2 Collection and Transportation of Samples

The collected Thai pangas (P. hypophthalmus) samples were transported to the laboratory aquarium of the Department of Fish Health Management, Sylhet Agricultural University (SAU), Sylhet, Bangladesh.

2.3 Preservation of Samples

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

2.4 Preparation of Solid Media

Nutrient agar medium was prepared by dissolving 28 g of nutrient agar powder in 1 liter of distilled water according to manufacturers’ instructions. After sterilization, the medium was poured into sterile petri dishes (5 ml in each petri dish) 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 further use.

2.5 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 water according to manufacturers’ instructions.

2.6 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 pararosanilin 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 [8].

2.7 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.

2.8 Isolation and Identification of Bacteria

Isolation and identification of bacteria from bacterial colony were characterized by morphological study such as colony size, shape, density and colour and by biochemical test such as Sugar test, Gram staining method, motility test and indole test.

Then the suspected colony was taken and sub cultured both in nutrient agar and nutrient broth media for the purpose of identify the specific type of bacteria. Finally pure culture was done in slant in zigzag method. Different biochemical test such as gram staining, motility test, MR-VP test was done in fully aseptic condition. Antibiotic sensitivity test was done to observe the bacterial sensitivity to antibiotic. Streaking 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.

2.8.1 Colony characteristics

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

2.8.2 Gram’s staining

Gram’s staining of the pure culture was done following standard methods and then examined under light microscope (100X) using immersion oil [8].

2.8.3 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 [8].

2.8.4 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 colour change of reddish to yellow in the medium and the gas production by the appearance of gas bubbles in inverted Durham’s tube.

2.8.5 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, shaken well and examined after 1-2 minutes. A pink to red colour in the reagent layer indicated indole positive. In negative case, there was no development of colour.

2.8.6 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 [9]. 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/Subactam (20μg), Tetracycline (30μg) and Erythromycin (15μg). Inhibition zone diameters were then interpreted into susceptibility categories based on the zone size (Susceptible, Intermediate, and Resistant) [10]. 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 Software IBM SPSS, 21.0 was applied for all statistical calculation. All results were expressed as means ±SE of three replications and subjected to one-way analysis of variance (ANOVA) followed by Duncan’s multiple range tests.

3. RESULTS

3.1 Clinical Pathology

The most prominent clinical signs of diseased fishes included pale gill, reddish discoloration around the mouth as well as eye and anal protrusion (Fig. 2). Missing of the secondary gill lamellae, pale gill, weak body, grayish cotton wool like lesion and red spots in the ventral and dorsoventral region were also noticed. Some fishes had deep ulceration lesion on the body surface. Petechial haemorrhages, reddening of the abdomen and large external ulcerative lesion in P. hypophthalmus infected with As. hydrophila. Moribund fish were found to swim abnormally at watersurface with full of fluid in the body cavity and swollen abdomen.

3.2 Isolation of Pathogenic Bacteria

Bacteria were isolated based on their morphological feature, cultural characteristics etc. There were three (3) types of bacteria isolated from diseased and non- diseased Thai pangas (P. hypophthalmus). The isolated bacteria were A. hydrophila, E. ictaluri, and Pseudomonas sp.

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. ictaluri and Pseudomonas spp.

The cultural characteristics of A. hydrophila, Edwardsiella ictaluri, and Pseudomonas sp. were examined by observation of cultured bacterial colonies colour, shape and transparency that are presented in Table 1 and Figs. 3-5.
Fig. 2. External clinical pathology in sampled Pangas (Pangasius hypophthalmus)

Table 1. Cultural characteristics of A. hydrophila, E. ictaluri and Pseudomonas spp

| Sl. | Name of          | Colony characteristics                      |
|-----|------------------|---------------------------------------------|
| 1   | Pseudomonas sp.  | Creamy whitish, round, opaque colony        |
| 2   | A. hydrophila    | Yellow, round, dense colony                 |
| 3   | E. ictaluri      | Whitish, irregular, dense colony            |

Fig. 3. Pseudomonas spp. on Nutrient agar

Fig. 4. A. hydrophila on Nutrient agar

Fig. 5. E. ictaluri on Nutrient agar
3.3.2 Gram staining test for *Pseudomonas* species, *A. hydrophila* and *E. ictaluri*

The results of the Gram Staining test were shown in Table 2 and Figs. 6-8.

3.3.3 Motility test of *Pseudomonas* species, *A. hydrophila* and *E. ictaluri*

The motility of *Pseudomonas* species, *A. hydrophila* and *E. ictaluri* utilizing hanging drop slide method showed in Figs. 9-11.

| Shape                        | Characteristics | Gram's staining | Identified bacteria |
|------------------------------|-----------------|-----------------|--------------------|
| Small rods                   | Single, paired  | -ve             | *Pseudomonas* sp.   |
| Straight rods with           | or in short     |                 | *A. hydrophila*     |
| rounded ends                 |                 |                 |                    |
| Short straight rod           | Single          | -ve             | *E. ictaluri*       |

Table 2. Morphology and Gram's staining properties of *Pseudomonas* species, *A. hydrophila* and *E. ictaluri*

3.3.4 Biochemical tests of *Pseudomonas* species, *A. hydrophila* and *E. ictaluri*

Biochemical test results are presented in Table 3.

3.3.4.1 Biochemical tests of *A. hydrophila*

*Aeromonas hydrophila* was found positive on sugar fermentation test, negative on MR test, positive on VP test and positive on Indole test (Figs. 12-15).

Fig. 6. *E. ictaluri* found gram negative  
Fig. 7. *A. hydrophila* found gram negative  
Fig. 8. *Pseudomonas* spp. found gram negative
Table 3. Biochemical properties of *A. hydrophila*, *Pseudomonas* sp. and *E. ictaluri*

| Carbohydrate fermentation test | MR test | VP test | Indole test | Interpretation of results       |
|--------------------------------|---------|---------|-------------|---------------------------------|
| Lactose                        |         |         |             |                                 |
| Acid                           | Acid & Gas | Acid | +ve | +ve | *A. hydrophila*                 |
| -ve                            | -ve | -ve | -ve | -ve | *Pseudomonas* spp.              |
| Mannitol                       |         |         |             |                                 |
| Acid                           | Acid | -ve | -ve | -ve | *E. ictaluri*                   |
| -ve                            | -ve | +ve | -ve | +ve |                                |
3.3.4.2 Biochemical tests of Pseudomonas sp.

*Pseudomonas* sp. was found negative on sugar fermentation test, negative on MR test, negative on VP test and negative on Indole test (Figs. 16-19).

3.3.4.3 Biochemical tests of *E. ictaluri*

*E. ictaluri* was found negative on sugar fermentation test, positive on MR test, negative on VP test and positive on Indole test (Figs. 20-23).

3.4 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 (100%) against Ciprofloxacin (Radius of the clear zone- 16.5 mm) intermediate (66%) to Azithromycin (Radius of the clear zone- 12.5 mm), Erythromycin (Radius of the clear zone- 11 mm), and resistant against Tetracycline (66%; Almost no clear zone was observed) and Ampicillin/Sulbactam (100%; No clear zone was observed). Thus, Ciprofloxacin (100%) was 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. Erythromycin was intermediate to *A. hydrophila*, Tetracycline (66%) and Ampicillin/ Sulbactam (100%) were highly resistant to all the identified bacteria except *E. ictaluri* which were intermediate to Tetracycline (Fig. 5).
Fig. 18. *Pseudomonas* sp. found negative on VP test

Fig. 19. *Pseudomonas* sp. showing negative on Indole test

Fig. 20. *E. ictaluri* found negative on sugar fermentation test

Fig. 21. *E. ictaluri* found positive on MR test

Fig. 22. *E. ictaluri* found positive on Indole test

Fig. 23. *E. ictaluri* found negative on VP test
Table 4. Antibiotic sensitivity test on isolated bacteria from infected Thai pangas

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

*: 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=3) based on Table 4

| Antibiotics (conc.) | Sensitive | Intermediate | Resistant |
|---------------------|-----------|--------------|-----------|
| Ciprofloxacin (5µg) | 3(100)    | 0            | 0         |
| Azithromycin (15µg) | 1(34)     | 2(66)        | 0         |
| Erythromycin (15µg) | 0         | 2(66)        | 1 (34)    |
| Tetracycline (30µg) | 0         | 1(34)        | 2 (66)    |
| Ampicillin/Sulbactam (20µg) | 0        | 0           | 0         |

Fig. 24. Antibiotic sensitivity and resistant pattern of bacteria isolated from infected Thai pangas
4. DISCUSSION

Aquaculture in Bangladesh is growing rapidly with respect to both quantity and variety of species. Thai pangas (P. hypophthalmus) is one of the fish species which plays a vital role in Bangladesh. Pangas (P. hypophthalmus) are cultured in ponds mostly as commercial basis by farmers but they faces different types of problem like diseases caused by bacterial, viral and fungal pathogens which leads to high mortality of P. hypophthalmus in cultured ponds and farms located in Sylhet, Bangladesh. However, the clinical symptoms, similar to the other studies [10,11], 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 and abdomen.

The study on bacterial diseases of Pangasianodon hypophthalmus cultured in earthen pond of Vietnam clearly showed that the majority of infection was caused by Edwardsiella ictaruli counting for 87.9% of all isolates [12]. E. ictaluri was first reported by who had isolated and described this bacteria from pond-cultured channel catfish (Ictalurus punctatus) in U.S.A and named this disease “Enteric Septicemia of Catfish” (ESC) [8]. ESC was believed to be infectious only to ictalurids. The susceptibility of channel catfish to E. ictaluri was high [13]. The results from this study clearly indicated that P. hypophthalmus was highly susceptible to this enteric bacteria. This disease was firstly reported in Pangasius sp. in Vietnam in 2001, including several species of bacteria from affected fish i.e. Bacillus causing the disease “bacillary necrosis” [14]. The same disease was identified in Vietnam in P. hypophthalmus and the pathogen as Edwardsiella ictaluri was confirmed with slightly different biochemical characteristics from two type strains from UK [11]. Both reports indicated the same clinical signs of affected fish as found in this study including multifocal irregular white lesions in liver, spleen and kidney.

Aeromonas hydrophila was also another pathogenic bacteria that could cause disease in P. hypophthalmus, but the incidence of this Aeromonad septicemia was very low (3.03%) when compared with E. ictaruli cases. The external and internal clinical signs of these two diseases were quite similar including hemorrhage in the infected organs, and swollen eyes [15] and the difference between these two pathogens, only E. ictaruli had the specific internal clinical sign including severe necrosis in the trunk kidney and many small white spots of lesion with the 1-3 mm diameter developing in the severe infected organ including liver, kidney and spleen [16]. A. hydrophila sp. is a ubiquitous pathogenic bacterium in fresh water that causes significant losses to the aquaculture industry. The present study examined the occurrence of Aeromonas sp. infection in juvenile P. hypophthalmus. Ten strains of A. hydrophila were isolated, identified and characterized from clinically diseased fish. An intensive search in literature reported that several pathogenic bacterial genera Aeromonas, Micrococcus, Edwardsiella, Pseudomonas, Coryneformes, Flavobacterium, Enterobacteriaceae, Acinetobacter, Achoromobacter and Alcaligenes were reported in farmed Thai pangas in Bangladesh. However, we showed the first comprehensive report for the isolation and characterization of A. hydrophila from exotic farmed pangasius in Bangladesh. Previously, the same bacterial species were isolated/ identified from naturally infected farmed silver carp in Bangladesh [17]. In another study, A. hydrophila were isolated and identified from EUS affected stringing catfish (shing) Heteropeneustes fossilis [18]. Several authors around the world, reported isolation and identification of Aeromonas hydrophila from carp, perch and catfishes [19] gold fish [20] prawns [21].

Some Pseudomonas strains were isolated and identified from diseased farmed fish of Bangladesh [22]. Several Prumondad isolates were also identified from diseased fish from different types of water bodies [23]. P. anguilliseptica was also isolated from O. niloticus fishes affected with Pseudomonas septicemia in Bangladesh [24]. P. fluorescens was also isolated from various diseased fishes [25,26]. Pseudomonas showed more or less similar morphological and biochemical results from diseased shing fish [23,27]. For gelatin there were 4 isolates with positive reaction instead of negative [10]. However, there were 41 isolates that gave the positive reaction on Simmons’ citrate while the other reported negative reaction [10]. Four pathogenic bacteria such as A. hydrophila, F. columnare, Edwardsiella tarda and Pseudomonas spp. from diseased Shing (H. fossilis) were isolated and identified [28]. Streptococcus agalactiae, F. columnare and E. tarda in farmed diseased tilapia, and Aeromonas sp. and S. iniae in healthy tilapia were isolated and identified [29].
In the antibiotic sensitivity test, most isolates (over 60%) of *E. ictaluri* were sensitive to 6 antibiotics where ciprofloxacin showed the highest antimicrobial activity (54 isolates, 91.4%) followed by ampicillin and amoxyccilin, florfenicol, doxycycline, and oxytetracycline. On the other hand, all 58 isolates showed resistant to sulphamethoxazole, erythromycin and potentiated sulphonamide (sulphamethoxazole + trimethoprim). Some isolates showed resistant to oxytetracycline (36.2%), florfenicol (25.9%) and ampicillin and amoxyccilin (15.5%). This resistance might suggest the status of non-control use of drugs to treat the bacterial disease that lead to the high resistance of bacteria especially with the drug that had been used for a long period of time.

For *A. hydrophila*, both isolates showed sensitivity to oxytetracycline, potentiated sulphonamide, sulphamethoxazole, ciprofloxacin, erythromycin, enrofloxacin, florfenicol and doxycycline but they were resistant to ampicillin and amoxyccilin. Oxytetracycline was the choice to treat *E. ictaluri* infection because all of 40 tested isolates showed sensitive result, while erythromycin also showed good inhibition of *E. ictaluri* [30]. Clinical signs associated with the Aeromonad infection observed in the present study were bilateral exophthalmia with superficial reddening around the eyes and mouth. Hemorrhagic ulceration was also noticed at the base of the fins. Internally, the kidney and liver were swollen and rounded enlarged spleen was observed.

In this study, *A. hydrophila*, *Pseudomonas* and *E. ictalori* isolates were conducted by disk diffusion method against five antibiotics where, all of the isolates were found to be sensitive to Ciprofloxacin (100%), intermediate to Azithromycin (70%), Erythromycin (65%) and resistant against to Tetracycline (70%) Ampicillin/Subbactam (100%) where Ciprofloxacin (100%) found more effective to all the identified bacterial colonies and Azithromycin were intermediate to all isolates bacteria except *Pseudomonas sp* which was sensitive against it. Erythromycin was intermediate to *A. hydrophila*, Tetracycline (70%) and Ampicillin/subbactum (100%) were highly resistant to all the identified bacteria except *E. ictaluri* which were intermediate to Tetracycline. In a study Ciprofloxacin (5μg) was found sensitive to *A. hydrophila*, *F. columnare*, *Edwardsiella tarda* and *Pseudomonas* sp. from diseased Shing (*H. fossilis*), and Azithromycin (15μg) and Ampicillin/Subbactam (20μg) moderately sensitive, but Tetracycline (30μg) and Erythromycin (15μg) resistant to the studied bacteria [28]. In another study, tetracycline and ciprofloxacin were found more sensitive to *S. agalactiae, F. columnare, E. tarda, Aeromonas* sp. and *S. iniae* isolated from farmed Nile tilapia, azithromycin and streptomycin were moderately sensitive, and ampicillin was resistant [29].

5. CONCLUSION

Culture of Pangas (*P. hypophthalmus*) in fresh water has become very popular and economically beneficial for fish farmers. However, production of this species has been reduced by bacterial diseases outbreak. The findings of this study showed that pathogenic bacteria such as *E. ictaluri*, *A. hydrophila* and *Pseudomonas* spp are the major cause of bacterial diseases of this species. In addition, *Aeromonas* spp. and other isolates from the diseased *P. hypophthalmus* have developed multidrug resistance to antibiotics probably due to indiscriminate or abuse of these antibiotics in aquaculture. Ciprofloxacin (5μg) could be administrated to control of bacillary necrosis and other diseases in *P. hypophthalmus*. However, farmer should avoid the application of antibiotic for its negative consequences. Disease prevention should be carried out by means of good health management practice to ensure the best quality of the products. In order to develop sustainable pangasius culture in Bangladesh further research could be done using molecular techniques for proper identification and characterization of pathogens which will help in development of farmer oriented disease control and health management packages.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.
ETHICAL APPROVAL
As per international standard written ethical permission has been collected and preserved by the author(s).

ACKNOWLEDGEMENT
The authors express their sincere thanks to “PCR and histopathology based diagnosis and optimization of dosage of aqua-drugs to control diseases in Tilapia and Thai pangas” project of Sylhet Agricultural University Research System (SAURES), Sylhet and “Optimization of dosage of aqua 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 for the financial support during the study period.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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