Microbiology Research Journal International

30(3): 7-21, 2020; Article no.MRJI.56146
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

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
Editor(s):
(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Mostafa M. Abo Elsoud, National Research Centre, Egypt.
(2) Noor ul Akbar, Kohat University of Science and Technology, Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56146

Original Research Article

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

ABSTRACT

Thai Pangas (*Pangasiaus 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 causativeagent 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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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 thedisease, 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 Ampicilin/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.

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 hypopthalmus* (Hamilton, 1822) is a catfsh species of the family Pangasiidae underthe 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

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. hypophthalmus)* were considered as fish sample for the study (Fig. 1).

Fig. 1. *Pangasius hypopthalmus*: a) Healthy fish and b) Diseased fish

2.2 Collection and Transportation of Samples

The collected Thai pangas (*P. hypopthalmus*) 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 byadding 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 pararosaniline chloride) were prepared following standard methods. After thatthe 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 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 thenexamined under light microscope (100X) using immersionoil [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, shaked 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/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) [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 discolouration 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

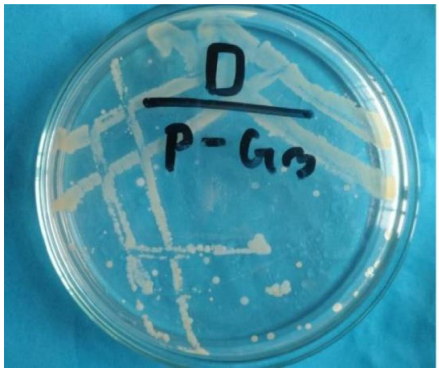

Fig. 3. *Pseudomonas* spp. on Nutrient agar

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.

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).

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

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

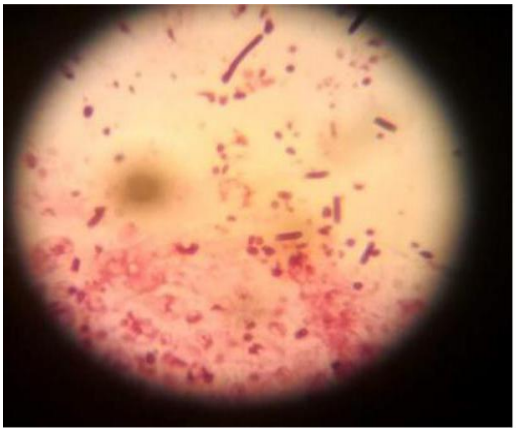

Fig. 6. *E. ictaluri* found gram nagative

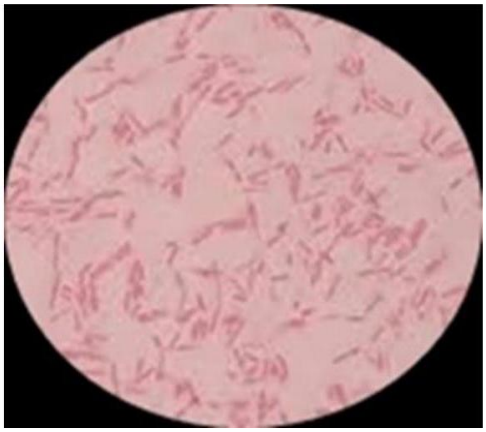

Fig. 7. *A. hydrophila* found gram negative

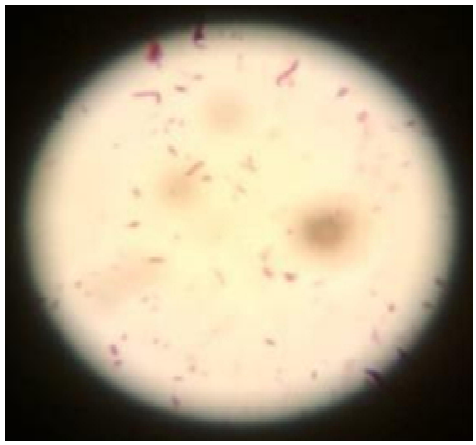

Fig. 8. *Pseudomonas* spp. found gram negative

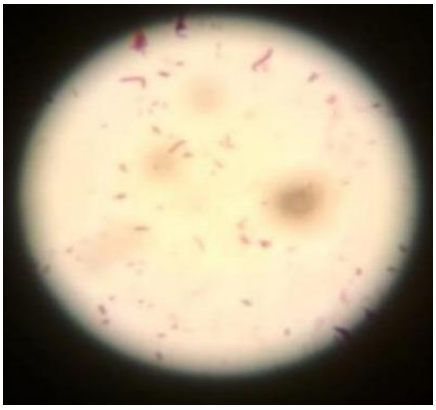

Fig. 9. Movement of *Pseudomonas* spp. in microscope

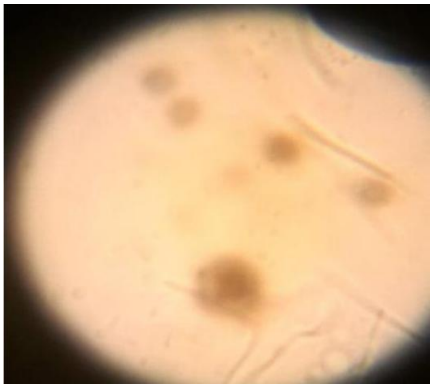

Fig. 10. *A. hydrophila* found motile on microscope

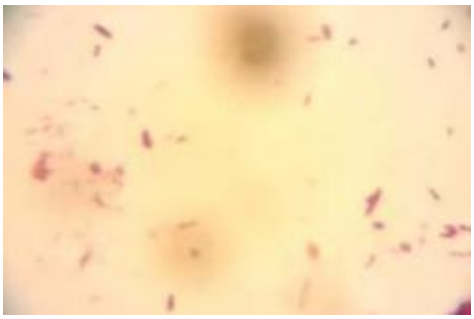

Fig. 11. *E. ictaluri* found motile on microscope

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	**Sucrose**	**Mannitol**				
Acid	Acid & Gas	Acid	-ve	+ve	+ve	*A. hydrophila*
-ve	-ve	Acid	-ve	-ve	-ve	*Pseudomonas* spp.
-ve	-ve	-ve	+ve	-ve	+ve	*E. ictaluri*

Fig. 12. *A. hydrophila* found positive on sugar fermentation test

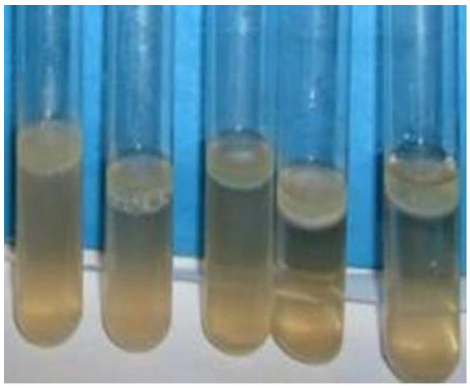

Fig. 13. *A. hydrophila* showing negative on MR test

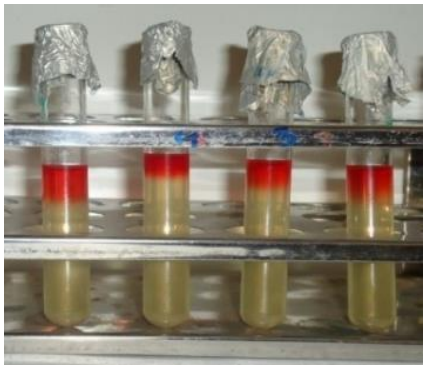

Fig. 14. *A. hydrophila* showing positive on VP test

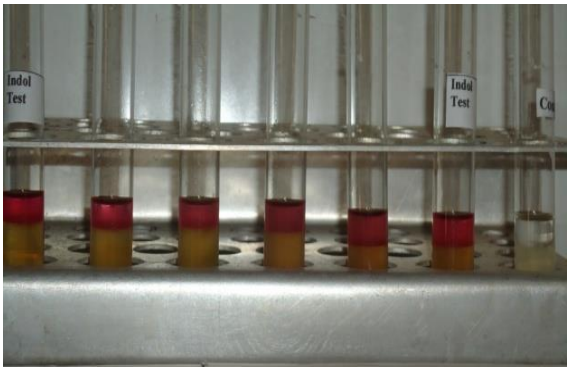

Fig. 15. *A. hydrophila* showing positive on Indole test

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 Ampicilin/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 Ampicilin/ sulbactum (100%) were highly resistant to all the identified bacteria except *E. ictaluri* which were intermediate to Tetracycline (Fig. 5).

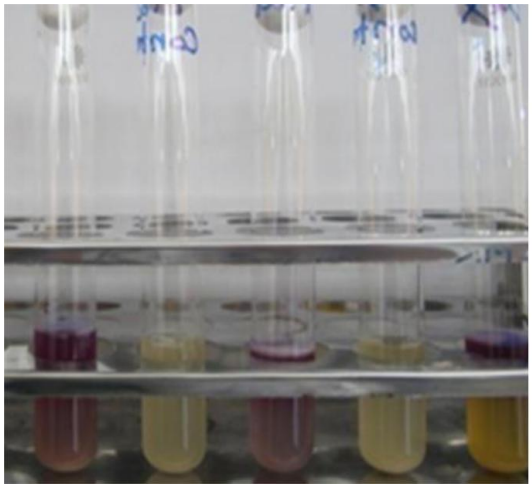

Fig. 16. *Pseudomonas* sp. found negative on sugar fermentation test

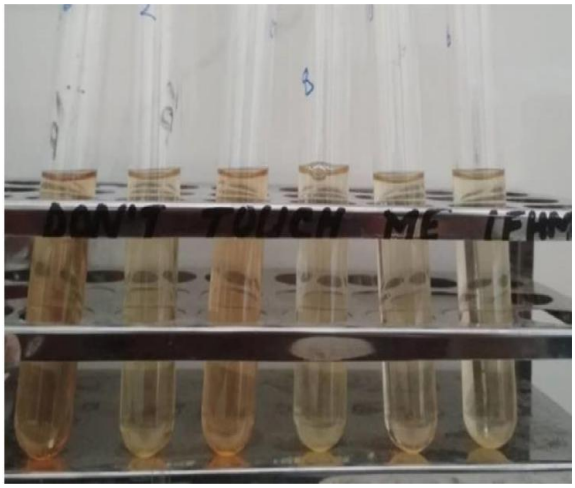

Fig. 17. *Pseudomonas* sp. found negative on MR test

Fig. 18. *Pseudomonas* sp. found negative on VP test

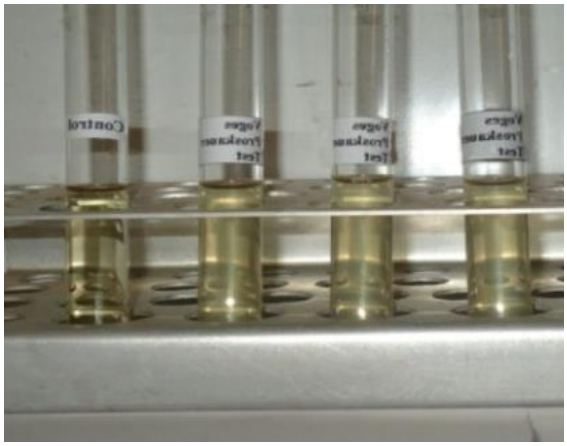

Fig. 19. *Pseudomonas* sp. showing negative on Indole test

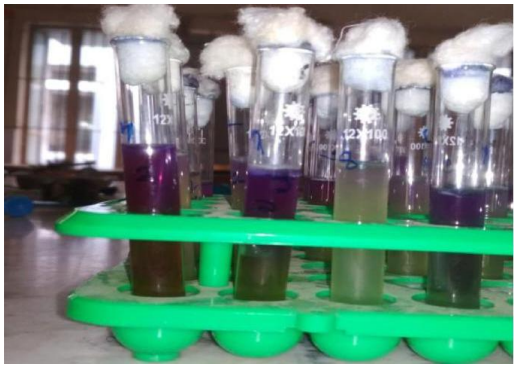

Fig. 20. *E. ictaluri* found negative on sugar fermentation test

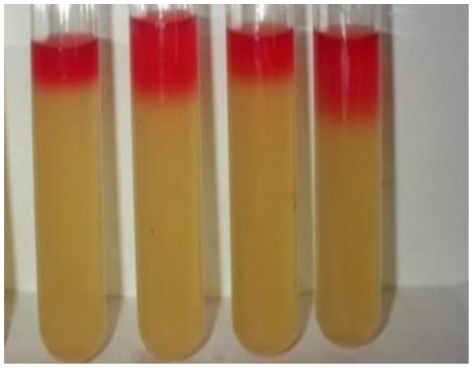

Fig. 21. *E. ictaluri* found positive on MR test

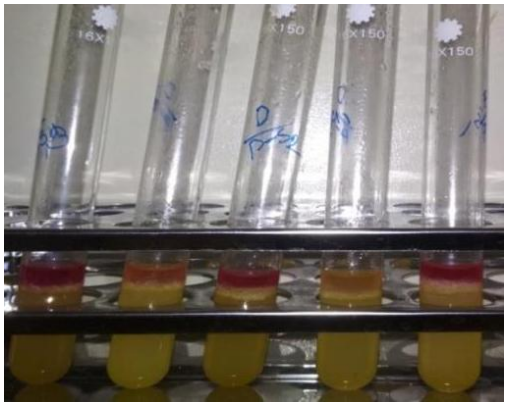

Fig. 22. *E. ictaluri* found positive on Indole test

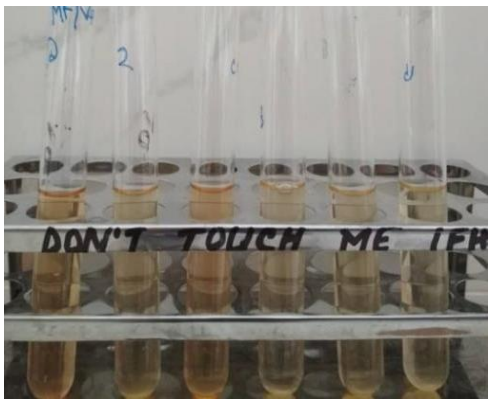

Fig. 23. *E. ictaluri* found negative on VP test

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.)	No. of bacteria (% of sensitivity)		
	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 frompond-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 severalspecies 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, weshowed 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 infectedfarmed silver carp in Bangladesh [17]. In another study, *A. hydrophila* were isolated and identified from EUS affected farmed 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 Pseudomonad 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*sp. 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. ictaruli* were sensitive to 6 antibiotics where ciprofloxacin showed the highest antimicrobial activity (54 isolates, 91.4%) followed by ampicillin and amoxycillin, florfenicol, doxycycline, and oxytetracycline. On the other hand, all 58 isolates showed resistant tosulphamethoxazole, erythromycin and potentiated sulphonamide (sulphamethoxazole + trimethoprim). Some isolates showed resistant to oxytetracycline (36.2%), florfenicol (25.9%) and ampicillin and amoxycillin (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 amoxycillin. Oxytetracyline 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. ictaruli* [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%) Ampicilin/Sulbactam (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. Erythromycin was intermediate to *A. hydrophila,* Tetracycline (70%) and Ampicilin/sulbactum (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/Sulbactam (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. hypophthamus* 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.

REFERENCES

1. FAO. The State of World Fisheries and Aquaculture2018-Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO; 2018.
2. DoFa (Department of Fisheries). National Fish Week 2017 Compendiun (In Bangali). Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh. 2017;148.
3. DoFb (Department of Fisheries). Yearbook of Fisheries Statistics of Bangladesh 2016-17. Fisheries Resources Survey System (FRSS), Department of Fisheries, Bangladesh. 2017;34:129.
4. Faruk MAR, Sarker MMR, Alam MJ, Kabir MB. Economic loss from fish diseases on rural freshwater aquaculture of Bangladesh. Pak. J. Biol. Sci. 2004;12: 2086-2091.
5. Subasinghe R, Barg U, Tacon A. Chemicals in Asian aquaculture: Need, usage, issues and challenges. Use of Chemicals in Aquaculture in Asia. Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia; 2000.
6. Farmer BD. Improved methods for the isolation and characterization of *Flavobacterium columnare*. A MS thesis Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College. 2004;62.
7. Austin B, Austin, DB. Bacterial fish pathogens in farmed and wild fish. 2nd Ed. Ellis Horwood Ltd, Chichester, England; 1993.
8. Khuntia, BK, Basic microbiology: A illustrated laboratory manual. Daya Publishing House, New Delhi; 2011.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966;45(4):493-6.
10. Hawke JP. A bacterium associated with disease of pond – cultured channel catfish. J Fish Res Board Can. 1979;36:1508-1512.
11. Crumlish M, Dung TT, Turnbull JF, Ngoc NTN, Ferguson HW. Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. J. Fish Dis. 2002; 25(12):733-736.
12. Ho TT, Areechon N, Srisapoome P, Mahasawasde S. Identification and antibiotic sensitivity test of the bacteria isolated from tra catfish (*Pangasianodon hypophthalmus* (Sauvage, 1878)) in pond cultured in Vietnam. Nat. Sci. 2008;4:54-60.
13. Plumb J, Sanchez D. Susceptibility of 5 species of fish to *Edwardsiella ictaluri*. J. Fish Dis.1983;6:261-266.
14. Ferguson HW, Turnbull JF, Shinn A, Thompson K, Dung TT, Crumlish M. Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. J. Fish Dis. 2001; 24(9):509-513.
15. Angka SL, Lam TJ, Sin Y. Some virulence characteristics of Aeromonas hydrophila in walking catfish (*Clarias gariepinus*). Aquaculture. 1995;130:103-112.
16. Dung T, Crumlish M, Ngoc N, Thinh N, Thy D. Investigate the disease caused by the genus *Edwardsiella* from Tra catfish *(Pangasianodon hypophthalmus*). J. Sci. Can Tho Univer. 2004;1:23-31.
17. Rashid MM, Hossain MS, Ali MF. Isolation and identification of *Aeromonas hydrophila* from silver carp and its culture environment from Mymensinghregion. J. Bangladesh Agric. Univ. 2013;11(2):373-376.
18. Rashid MM, Hasan MA, Mostafa K, Islam MA. Isolation of *Aeromonas hydrophila* from EUS affected shing *Heteropeneustes*

fossilis from afish farm of Mymensingh. Progress. Agric. 2008;19(1):117-124.

19. Ahmed MB. Isolation and identification of *Aeromonas hydrophila* from carps, perch and catfishes. MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh; 2009.
20. Mamun MAA, Nasren S, Abhiman PB, Rathore SS, Sowndarya NS, Ramesh KS, Shankar KM. Investigation of production, formation and characterization of biofilm cells of *Aeromonas hydrophila* for oral vaccination of fish. J. Exp. Zool. India. 2019;22(2):1115-1123.
21. Thayumanavan TG, Vivekanandhanal K, Savithamani R, Subashkumar, P. Lakshmanaperumalsamy. Incidence of haemolysin-positive and drug-resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of coastal South India. Federation of European Microbiological Societies Immunol. Medical Microbiol. 2003;36:41–45.
22. Chowdhury MBR. Involvement of Aeromonads and Pseudomonads in diseases of farmed fish in Bangladesh. Fish Pathol. 1998;33:247-254.
23. Khatun H, Hossain MD, Jahan SN, Khanom DA. Bacterial infestation in different fish at Rajshahi. J. Sci. Found. 2011;9(1&2):77-84.
24. Khan AW, Hossain SJ, Uddin SN. Isolation, identification and determination of antibiotic susceptibility of *Vibrio parahaemolyticus* from shrimp at Khulna region of Bangladesh. Res. J. Microbiol. 2007;2:216-227.
25. Fayed AA, Khaten AA, Omyima A, Abdound Y. *Pseudomonas septicemia* encountered in some Ornamental fish. Alex. J. Vet. Sci. 1997;13:93-100.
26. Swain PA, Behura, Dash S, Nayak SK. Serum antibody response of Indian major carp, *Labeo rohita* to three species of pathogenic bacteria *Aeromonashydrophila*, *Edwarsiella tarda* and *Psesudomonas fluorescens*.Vet Immunol Immunop. 2007; 117:137-141.
27. Monir MS, Bagum N,Kabir SML, Borty SC, Ud-Doulah MA. Isolation, molecular identification and characterization of *Aeromonas hydrophila* from infected air-breathing catfish Magur (*Clarias batrachus*) cultured in Mymensingh, Bangladesh. Asian Australas. J. Food Saf. Secur. 2017;1(1):17-24.
28. Goni O, Alam MMM, Khalil SMI, Bari SM, Hamom A, Parven M, Mamun MAA. Identification of pathogenic bacteria from diseased stringing catfish *Heteropneustis fossilis* with their sensitivity to antibiotics. Int. J. Fish. Aquat. Stud. 2020;8(1):291-301.
29. Hamom A, Alam MMM., Iqbal MM, Khalil SMI, Parven M, Sumon TA, Mamun MAA. Identification of pathogenic bacteria from diseased nile tilapia Oreochromis niloticus with their sensitivity to antibiotics. Int. J. Curr. Microbiol. Appl. Sci. 2020;9(03):716-1738.
30. Plumb JA, Vinitnantharat S. Biochemical, biophysical, and serological homogeneity of *Edwardsiella ictaluri*. J. Aquat. Anim. Health. 1989;1:171-175.

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