Journal of Marine Biology and Aquaculture (J Marine Biol Aquacult)

OPEN ACCESS

ISSN 2381-0750

Research Article

DOI: 10.15436/2381-0750.20.2743

Occurrence and Antimicrobial Susceptibility Profiling of Bacteria Isolated from Cultured Pangas Catfish (*Pangasius pangasius*) and Climbing Perch (*Anabas testudineus*) Fishes

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Abstract

Aquaculture infections in fish are very common in Bangladesh. The aims and objectives of this study are to identify the pathogenic agents infecting aquaculture fish and sensitivity patterns of commonly used antimicrobial agents. A total of 20 infected fish samples were tested, among them pangas catfish (*Pangasius pangasius*) and climbing perch koi (*Anabas testudineus*) fishes were 13 and 7 respectively. Cultural analysis was done by phenotypic examination along with biochemical examination to identify the isolates. Finally, antibiotic sensitivity was tested against conventionally used antibiotics. All the samples were aseptically collected from different Upazilla in Mymensingh region and tests were done in Quality Aqua Laboratory, Quality feeds Limited, Mymensingh between April 2019 to December 2019. The most predominant isolates in pangas fish were *Flavobacterium spp*., (38.5%) and *Edwarsiella spp*., (38.5%) and *Aeromonas spp*., (57.1%) in koi fishes. Like most of the previous reports, *Flavobacterium spp., Edwardsiella spp., Aeromonas spp*., was predominant, which corroborates this study. However the antimicrobial profile of the detected organisms differed compared to studies which were previously done. The isolates were mostly resistant to Amoxycillin. The pathogens showed remarkable amount of sensitivity against Ciprofloxacin, Cotrimoxazole and Doxycycline. The pathogens also showed moderate sensitivity to Erythromycin. For validating more reliability, this research needs further work.

Keywords: Pangas catfish; Climbing perch; Bacterial pathogens; Antibiotic sensitivity profiles

Introduction

Fish is considered as one of the prime sources of food and income globally[1]. It is comparatively cheaper than other meat products; hence, it is more affordable to most people. Bangladesh is the world leading fish producing country. Common aquaculture practices in Bangladesh are mainly the culture of carps, tilapias, catfishes, climbing perches (koi), and shrimps, nile tilapia and pangas catfish are cultured mostly for commercial purpose by entrepreneurial farmers. Pangas catfish fish was first introduced to Bangladesh in 1989 from Thailand, aimed to upsurge overall aquaculture production and meeting the increasing demand for food fish[2].

Presently, this fish is considered as one of the important fishes in aquaculture of Bangladesh because of its fast growth, year-round production, and high productivity. The climbing perch fish (*Anabas testudineus*) is one of the important small *indigenous spp*., (SIS) fresh water fish of Bangladesh, which is locally known as koi fish. In Southeast Asian region, this fish is native and often found in fresh water sources of east India and south China[3]. But in Bangladesh, it is normally found in open water (streams, lakes, floodplain and beels), paddy fields, swamps and its habitats are heavily-vegetated and stagnant waters. Pathogenic micro organisms are a serious threat to fish production in

Received date: July 07, 2020
Accepted date: August 07, 2020
Published date: August , 2020

Citation: Abedin, M.Z., *et al*. Occurrence and Antimicrobial Susceptibility Profiling of Bacteria Isolated from Cultured Pangas Catfish (*Pangasius pangasius*) and Climbing Perch (*Anabas testudineus*) Fishes. (2020) J Marine Biol Aquacult 6(1): 7-12.

all over the world due to high economic importance of diseases they cause. A number of bacterial pathogens have been found to cause diseases in fish worldwide. In fresh water fishes, bacteria were particular importance including *Aeromonas spp., Pseudomonas spp., Streptococcus spp., Flavobacterium spp., Edwardsiella spp., Vibrio spp*., and so on[4].

The bacterial infections which are caused by antibiotic-resistant bacteria increasing all over the world. The main objective of this work was to isolate and identify bacteria from climbing perch (*Anabas testudineus*) and pangas catfish (*Pangasius pangasius*) cultivated in the pond water of different areas in Mymensingh and to determine the level of antibiotic susceptibility rates of the isolated bacteria against seven antibiotics.

Materials and Methods

Study area and Design:

This cross section study involved collection of affected fish samples of climbing perch fish (*Anabas testudineus*) from 3Upazilla (Fulbaria, Muktagacha, Trishal) and pangas catfish (*Pangasius-pangasius*) from 4 Upazilla (Fulbaria, Muktagacha, Trishal, Ishwarganj) of Mymensingh, Bangladesh. Bacteria were isolated from the collected fishes and identified by cultural and biochemical characteristics along with Gram staining technique. Antimicrobial susceptibility test was determined by the Kirby-Bauer disc diffusion method[5].

Figure 1: Map showing the geographical locations of districts sampled in this study.

Sample collection and Transportation:

A total of 07 infected climbing perch fish (*Anabas testudineus*) and 13 infected pangas catfish (*Pangasius pangasius*) samples were taken from different cultivated ponds between April 2019 to December 2019. During the collection, fish samples were maintained avoidance of touch and ice box were used to maintain cool chain. The samples were then brought to the laboratory of the Quality Aqua Laboratory, Quality feeds Limited, Mymensingh.

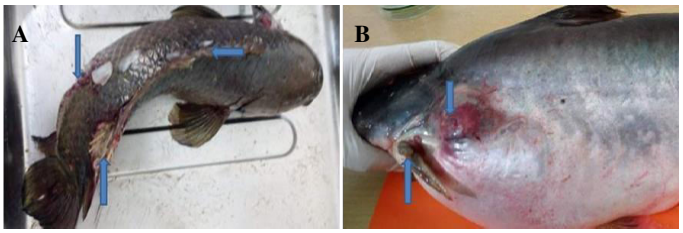


Figure 2: Infected fishes sampled from farmer's ponds; (a) Koi (*Anabas testudineus*) with deep haemorrhagic ulcerative lesion on tail region; (b) Pangas (*Pangasius pangasius*) with spoiled fin.

Processing of sample and enrichment of bacteria:

Aseptic measures were taken during the sampling procedure to prevent contamination. Three types of specimens of fishes were taken for analysis of microbiological test and the specimens are intestine, skin and gill. These specimens were taken on a sterile chopping board and then minced and grinded together. Ten (10) gm of samples were homogenized with 90 milliliters (ml) of freshly prepared 0.1% peptone water and then 0.1 ml of homogenized sample was inoculated according to standard methods on to both selective media and conventional media such as Rimler-Shotts Medium Base agar (for *Aeromonas spp*.), Pseudomonas Baseagar (for *Pseudomonas spp*.), Thiosulfate citrate bile salt sucrose (TCBS) agar (for *Vibrio spp*.), Tryptic Soy Agar for enrichment of bacterial isolates, Blood agar, and MacConkey agar. Then all media were incubated at 37ºC for 24 hours.

Identification of Bacterial Pathogens

Morphological Characterization:

The suspected bacterial colonies obtained from different culture plates were isolated and then streaked on TSA slants, MIU medium, Simon citrate agar slant and then incubated overnight at 37ºC. For morphological characterization, colonial characteristics, and bacterial cell morphology such as size, shape and so on.

Biochemical Characterization:

Various biochemical tests such as alkaline and acidic reaction, H_2S (hydrogen sulfide) production, gas production, MIU (motility , indole, urease), oxidase, catalase, Methyl Red (MR) test, and Voges-Proskauer (VP) test were performed to characterize the pathogens. The biochemical tests were done to identify the pathogens following Bergey's manual of Bacteriological classification[6].

Gram Staining Method:

These methods were done to distinguish between Gram positive and Gram negative bacteria. This technique was done on a clean grease free glass slide from a single bacterial colony by crystal violet solution (1 min), iodine solution (1 min), and safranin (2 min). Then the slide was washed properly by tap water before starting the next step. The slide image analysis was performed using a light microscope (Nikon Co., Tokyo, Japan) at 10X100 with immersion oil.

Antimicrobial susceptibility test:

The Kirby- Bauer disc diffusion methods (CLSI, 2015)[7] were performed for antibiogram patterns of all the pathogenic bacteria which were isolated. In this study, the commonly used antibiotics

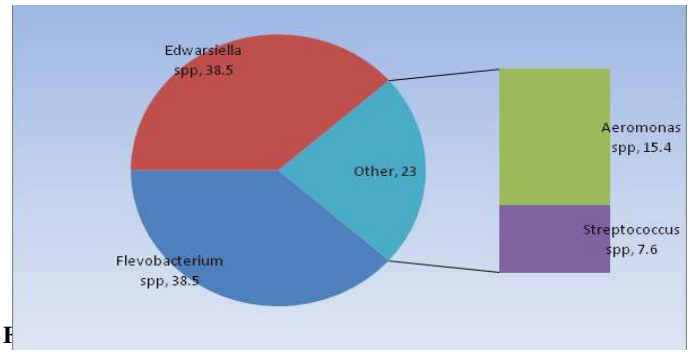
were included: Amoxicillin (10µg), Ciprofloxacin (5µg), Colistin (25µg), Clotetracyclin (30µg), Doxycycline(30µg), Erythromycin (15µg) and Co-trimoxazole (25µg). *Aeromonas hydrophila* (ATCC 7966), *Pseudomonas aeruginosa* (ATCC 27853), *Flavobacterium columnare* (ATCC 23463), *Edwardshiella tarda* (ATCC 15947) were used as standard throughout the study for culture and antimicrobial susceptibility testing.

The suspected isolated colonies of bacteria were taken to sterile PBS (phosphate buffered saline) water and then adjusted to 0.5 McFarland's turbidity standard. The bacterial suspension was spread onto Mueller–Hinton agar (Himedia, India) and then antibiotic discs (Himedia, India) were placed and incubated at 37ºC for 24 hours. Around the discs, the inhibition of antibiotic zone was estimated in diameter of millimeter (mm). Thezone of inhibition was scaled from the focal point of the anti-microbial plate as far as possible of the reasonable zone where microscopic organisms could be seen developing. The interpretation of antibiogram was measured in millimeter (mm) of diameters as resistance, sensitive and intermediate as per the producer's guidelines.

Statistical Analyses of Experimental Data:
Data obtained were analyzed by SPSS version 20 and Excel 2016. Descriptive statistics and chi-square tests were done to check the statistical evaluation. The p-value that considered significant was <0.5.

Results

Occurrence of bacteria in Pangas catfish (*Pangasiuspangasius*)
The bacteria isolated were *Aeromonas spp., Edwarsiella spp., Flavobacterium spp.*, and *Streptococcus* species from 13 infected pangas catfish samples which were identified by using morphological properties, Gram staining, and series of biochemical tests. *Edwardsiella spp*., 5(38.5%) and *Flavobacterium spp*., 5(38.46%) were the most dominant species among the isolates. The rest of 2(15.4%) *Aeromonas spp*., and 1(7.6%) *Streptococcus spp*.,were confirmed from pangas catfishes as indicated in the Figure 3.



Occurrence of Bacteria in Climbing Perch Fish (*Anabas testudineus*)
The bacteria isolated were *Aeromonas spp., Streptococcus spp*., and *Pseudomonas* species from 07 infected climbing perch fish (*Anabas testudineus*) commonly known as koifish samples which were identified using morphological properties, Gram staining, and series of biochemical tests. *Aeromonas spp*., 4(57.14%) was the most dominant isolates and rest of 2(28.6%) *Streptococcus spp*., and 1(14.3%) *Psudomonas* species from fish samples as indicated in Figure 4.

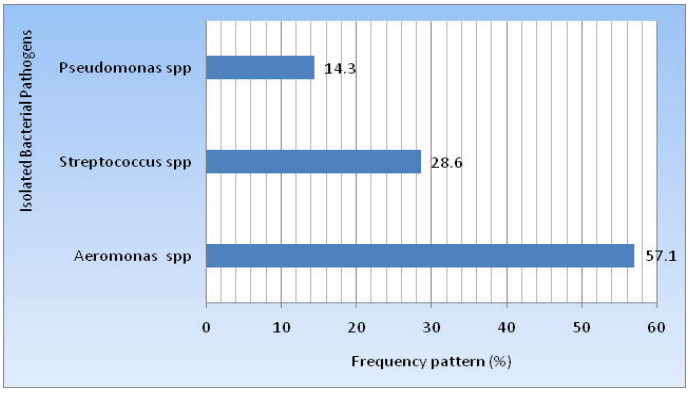


Figure 4: Prevalence of the isolated bacteria in climbing perch fish.

Biochemical tests for Bacterial Identification
The identification of pure bacterial isolates were done by biochemical characteristics which were alkaline reaction, acidic reaction, H_2S (hydrogen sulfide) production, Gas production, Motility test, indole production, urea hydrolysis, catalase test, oxidase test, Methyl -Red (MR) test, Voges- Proskauer (VP) test are presented in Table 1.

Table 1: Result of biochemical tests along with gram staining of the isolated bacterial spp., from infected fishes.

		KIA				MIU medium							
Bacterial isolates	Gram reaction	Slant	Butt	Gas	H2S	Mot	Indole	Urea	Oxidase	S. citrate	MR	VP	Cat
Aeromonas spp	Gram negative	R	Y	+	-	+	+	-	+	±	+	±	+
Pseudomonas spp	Gram negative	R	R	-	-	+	-	±	+	+	-	-	+
Streptococcus spp	Gram positive	Y	Y	-	-	+	-	+	-	+	+	+	+
Flavobacterium spp	Gram negative	Y	W	-	-	+	-	-	+	+	+	-	+
Edwardsiella spp	Gram negative	R	Y	+	+	+	+	-	-	-	+	-	+

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

Overall Antimicrobial Susceptibility of Pangas Catfish:

In this experiment, all of the isolated pathogenic bacteria showed 13/13(100%) resistant to Amoxicillin and Erythromycin. All the strains showed sensitive to Enrofloxacin, Ciprofloxacin, Doxycycline, Cotrimoxazole and Colistin were 12/13(92.3%), 12/13 (92.3%), 11/13(84.6%), 12/13(92.3%) and 8/13(61.5%) respectively. Colis- tin and Chlortetracycline showed intermediate sensitivity such as 5/13(38.5%) and 2/13(15.4%) respectively (Table 2 and Figure 5).

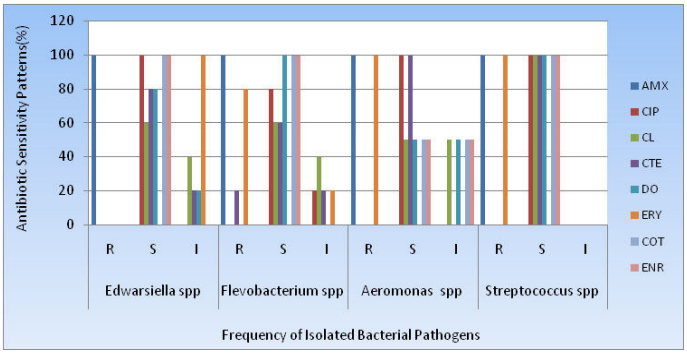


Figure 5: Antimicrobial profiling of catfish pathogens; R: Resistant; S: Sensitive; I: Intermediate sensitive;AMX: Amoxycillin; CIP: Ciprofloxacin; CL: Colistin; CT: Chlortetracycline; DO: Doxycyclline; ERT: Erythromycin; COT:Cotrimoxazole; ENR: Enrofloxacin.

Overall Antimicrobial Susceptibility Patterns of Climbing Perch Fish:

In this experiment, all of the isolated pathogenic bacteria showed 7/7(100%) resistant to Amoxicillin and 3/7 (42.9%) resistant to Chlortetracycline. All the strains showed sensitive to Ciprofloxacin, Doxycycline, Cotrimoxazole and Colistin were 6/7(85.7%), 4/7(57.1%), 6/7(85.7%) and 2/7(28.6%) respectively. Erythromycin and Colistin showed intermediate sensitivity such as 5/7(71.4%) and 2/7(28.6%) respectively (Table 3 and Figure 6).

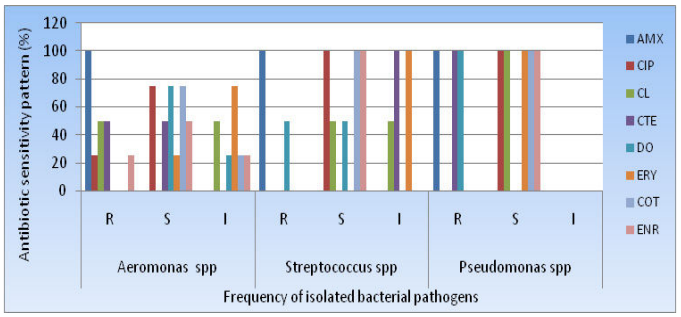


Figure 6: Antimicrobial profiling of climbing perch fish pathogens;R: Resistant; S: Sensitive; I: Intermediate sensitive;AMX: Amoxycillin; CIP: Ciprofloxacin; CL: Colistin; CT: Chlortetracycline; DO: Doxycycline; ERT: Erythomycin; COT:Cotrimoxazole; ENR: Enrofloxacin.

Table 2: Antibiotic sensitivity patterns all of the individual isolates.

Bacterial isolates	Sensitivity pattern	AMX	CIP	CL	CTE	DO	ERY	COT	ENR
Edwardsiella spp (n=05)	R	100	0	0	0	0	100	0	0
	S	0	100	60	80	80	0	100	100
	I	0	0	40	20	20	0	0	0
Flavobacterium spp (n=05)	R	100	0	0	20	0	80	0	0
	S	0	80	60	60	100	0	100	100
	I	0	20	40	20	0	20	0	0
Aeromonas spp (n=02)	R	100	0	0	0	0	100	0	0
	S	0	100	50	100	50	0	50	50
	I	0	0	50		50	0	50	50
Streptococcus spp (n=01)	R	100	0	0	0	0	100	0	0
	S	0	100	100	100	100	0	100	100
	I	0	0	0	0	0	0	0	0

N: number; R: Resistant; S: Sensitive; I: Intermediate sensitive; AMX: Amoxycillin; CIP: Ciprofloxacin; CL: Colistin; CT: Chlortetracycline; DO: Doxycyclline; ERT: Erythromycin; COT:Cotrimoxazole; and ENR: Enrofloxacin.

Table 3: Antibiotic sensitivity patterns all of the individual isolates.

Bacterial isolates	Sensitivity pattern	AMX	CIP	CL	CTE	DO	ERY	COT	ENR
Aeromonas spp (n=04)	R	100	25	50	50	0	0	0	25
	S	0	75	0	50	75	25	75	50
	I	0	0	50	0	25	75	25	25
Streptococcus spp (n=02)	R	100	0	0	0	50	0	0	0
	S	0	100	50	0	50	0	100	100
	I	0	0	50	100	0	100	0	0
Pseudomonas spp (n=01)	R	100	0	0	100	100	0	0	0
	S	0	100	100	0	0	100	100	100
	I	0	0	0	0	0	0	0	0

N: number; R: Resistant; S: Sensitive; I: Intermediate sensitive;AMX: Amoxycillin; CIP: Ciprofloxacin; CL: Colistin; CT: Chlortetracycline; DO: Doxycyclline; ERT: Erythromycin; COT:Cotrimoxazole; and ENR: Enrofloxacin.

Discussions

Fish farming is such an important sector which has been contributing tremendously to our economy. It is one of the most promising industries with the brightest future for our country. Though this sector has such potentiality, fish farming is confronted with acute problem of disease like bacterial, fungal, viral disease and also skin ulcer which can be caused by different factor. Those factors can cause great harm to the production cycle. Bacterial agents are responsible mostly for skin ulcer. In both species of pangas and climbing perch fishes,the most predominant pathogens were *Aeromonas spp., Edwardsiella spp*., and *Flavobacterium spp*., similar findings have been also reported in Mohantyet al[4] and Hemstreet[8].

Pangas (*Pangasius pangasius*) is the most frequently available fishes in Bangladesh. In this study, pond water cultivated pangas fishes were infected with several types ofbacterial pathogens. The most isolated bacteria were *Flavobacterium spp*., 5(38.5%) and *Edwarsiella spp*., 5(38.5%) followed by *Aeromonas spp*., 2(15.4%) and *Streptococcus spp*., 1(7.7). In our study, all bacterial isolates of *Edwarsiella spp*., showed 100% sensitive to Cotrimoxazole and Ciprofloxacin; *Flavobacterium spp*., were 100% sensitive to Doxycycline, Co-trimoxazole and Enrofloxacin; *Aeromonas spp*., were 100% sensitive to Chlortetracycline, Ciprofloxacin and only one strain of *Streptococcus spp*., was 100% sensitive to Doxycycline, Cotrimoxazole, Ciproloxacin. Chlortetracycline, Amoxycillin. On the other hand, all organisms showed high degree of resistance to Erythromycin.

Climbing Perch fish (*Anabas testudineus*) is the most available and cheap fish in Bangladesh. In our research, Climbing Perch fish cultivated in pond water were infected by bacterial pathogens of *Aeromonas spp*., 4(57.1%), *Streptococcus spp*., 2(28.6%) and *Pseudomonas spp*., 1(14.3%). This type of comparable isolation rate was also reported by Shittuet al[9]. In our investigation, *Aeromonas spp*., was the largest isolated bacterial pathogens and the highest sensitive to 75% Cotrimoxazole, Doxycycline and Ciprofloxacin each.

The second largest isolates were *Streptococcus spp*., which was 100% sensitive to Ciprofloxacin, Co-trimoxazole and Enrofloxacin; third one of *Pseudomonas spp*., was 100% sensitive to Ciprofloxacin, Colistin, Erythromycin, Cotrimoxazole and Enrofloxacin. *Streptococcus spp*., was 100% resistant to Amoxycillin and *Pseudomonas spp*., showed 100% resistant to Amoxycillin, Chlortetracycline and Doxycycline. The overall study showed that Amoxycillin was the most resistant antibiotic against all bacterial isolates. The significant level of resistance from regularly utilized antibiotics is similar with Hussainet al[10] and Mostafaet al[11]. Further research is needed to better understand the real situation of aquaculture fish infection and treatment efficacy in greater Mymensingh.

Conclusions

This study intended to ascertain the existing situation of aquaculture infection and drug resistance among Pangas and climbing perch fish. From this study, it has been concluded that *Flavobacterium spp., Edwarsiella spp*., and *Aeromonas spp*., were the predominant aquaculture infection followed by *Streptococcus spp*., and *Pseudomonas spp*., Ciprofloxacin, Cotrimoxazole and Doxycycline were found as a reliable therapeutic intervention for the investigated pathogens because of their broad spectrum activity in the current study. Antibiotic selection should be guided by culture and sensitivity test and empirical drug must be decided on the recent antibiogram of a particular geographical area.

Acknowledgements
The authors would like to thank Department of Microbiology, School of Biomedical Science, Khwaja Yunus Ali University, Sirajganj and Aqua Laboratory Quality Feeds Limited Mymensingh, Bangladesh for assistance this research.

Irreconcilable situations Statement
The authors announce that there is no irreconcilable situation with respect to the publication of this article.

Monetary Disclosure Statements
This investigation was without supported

Animal Rights Statement
No animal experiments were conducted.

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