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Isolation and Identification of *Aeromonas hydrophila* from Carps, Catfishes, Perches, and an Eel from Mymensingh Region of Bangladesh

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Abstract: *Aeromonas hydrophila* is a causative agent of MAS (Motile Aeromonas Septicemia) disease, causing huge mortality of fish all over the country. Isolation and identification of this bacteria, *A. hydrophila* from eight infected fish species: catla (*Catla catla*), rui (*Labeo rohita*), mrigal (*Cirrhina cirrhosus*), tarabaim (*Macrognathus aculeatus*), tilapia (*Tilapia mossambicus*), shing (*Heteropneustes fossilis*), koi (*Anabas testudineus*), and magur (*Clarias batrachus*) were undertaken from Tarakanda and Trishal upazila of Mymensingh District of Bangladesh. Fishes showed pale body color, external hemorrhages, reddish head, and reddish anal region. The intestine, kidney, and liver of each fish were inoculated over the AIM (*Aeromonas* Isolation Medium) and TSA (Tryptone Soya Agar) plates. The colonies of the first plates (AIM) were used for characterizing *Aeromonas* and the colonies of TSA plates were used for quantitative study. TSA plates showed the minimum bacterial load was 1.20×10^2 CFU/g in the kidney of tilapia and a maximum of 8.70×10^6 CFU/g in the intestine of catla. Isolated bacteria were finally identified as *Aeromonas hydrophila* by their physiological, biochemical, and morphological characteristics. They were rod-shaped, gram-negative, and motile. The bacteria showed positive reactions for catalase, oxidase, fermented glucose, and were resistant to vibriostatic agent 0129. A shortcut method of identifying *A. hydrophila* has been developed by this work. Thus, the bacteria as well as MAS disease can be detected easily, and our fish culturists will be able to save their cultured fish by using any recognized anti-*Aeromonas* drug.

Keywords: Pathogenic bacteria; *Aeromonas hydrophila;* Carps; Catfishes; Perches; Eel; Motile Aeromonas Septicemia.

INTRODUCTION

Aquaculture plays an important role in the economy of Bangladesh, contributing significantly to food security and employment. However, the sector faces numerous challenges, including the prevalence of bacterial infections that can severely impact fish health and productivity. Rui, catla, mrigal, koi, tilapia, shing, magur, and tarabaim are traditionally popular fish in Bangladesh. But in the last few years, it has been found that disease is an acute problem in the production of these popular fishes. Although disease history and clinical signs provide valuable clues as to the likely etiological agent, many taxonomically unrelated bacteria induce similar features, and a microbiological examination is generally required to identify a specific pathogen (Frerichs and Millar, 1993). *Aeromonas* are facultatively anaerobic, oxidative-positive, gram-negative, and glucose-fermenting bacteria (Sabur, 2006). Mamnur Rashid *et al*. (2008) identified *A. hydrophila* from the affected shing fish, *Heteropneustes fossilis*. Previously *Aeromonas* was isolated from rui, catla, mrigal, silver carp, and common carp by Sabur (2006), from shing by Mostafa (2007), and Thai pangas by Alam (2009). This study was done to isolate and identify *Aeromonas hydrophila* from carps (rui, catla, mrigal), perches (koi, tilapia), catfishes (shing, magur) and an eel (tarabaim) and to explore the total bacterial load in these fishes. The findings of this research will contribute to the existing knowledge on *A. hydrophila* and mitigate its impact on aquaculture in Bangladesh.

MATERIALS AND METHODS

Study area: The study was conducted in the Tarakanda and Trishal of Mymensingh district, Bangladesh (Figure 1).

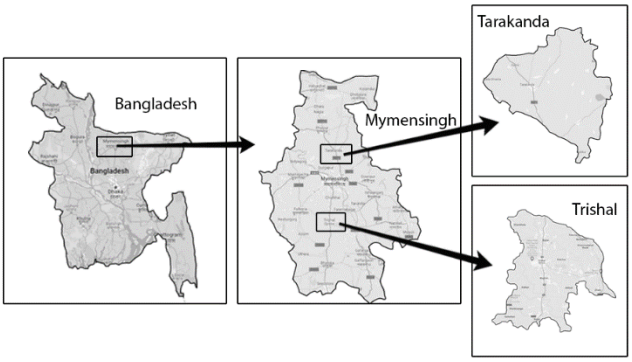

Fig. 1. Study area (Tarakanda and Trishal Upazila of Mymensingh District of Bangladesh)

Infected fishes were collected from the selected ponds of Tarakanda and Trishal of Mymensingh District of Bangladesh (Fig.1). Three fish of each species were subjected to bacteriological investigation. The intestine, liver, and kidney of sampled fishes were prepared for decimal dilutions. Two samples of 0.1 ml from 10^{-2} and 10^{-3} were inoculated onto the AIM agar plate to get the presumptive colonies of *Aeromonas.* The other two samples of 0.1 ml from 10^{-7} and 10^{-8} were inoculated onto TSA plates to count the total bacterial colony. TSA slants were prepared to stock the presumptive *Aeromonas* bacteria for further confirmation up to the species level. All the plates and TSA slants were cultured at 25°C for 28 h.

Identification of *Aeromonas* bacteria: Colonies grown on the selective medium (AIM) for *Aeromonas* were incubated on TSA plates for 24-hour culture. The isolated bacteria were morphologically characterized based on the gram staining technique, motility test, and shape of the bacteria. Physiological studies were done by observing the pattern of growth of each isolate at temperatures of 4°C, 37°C, and 40°C. Biochemical characters were confirmed by performing different tests like catalase, such as oxidase, oxidative-fermentative, esculin hydrolysis, 0129 test, lactose, glucose, sucrose, methyl-red, mannitol, Voges-Proskauer (VP), decarboxylase, H_2S production, and citrate utilization.

RESULTS

Bacterial load in different organs of sampled fish: Counted bacterial load in blood, liver, intestine, and kidney of rui, catla, mrigal, koi, tilapia, tarabaim, magur, and shing are shown in Table 1. Counted bacteria were found to be 2.45×10^3 (koi) to 7.36×10^5 (tilapia) CFU/g in blood, 1.33×10^3 (tilapia) to 5.58×10^5 (catla) CFU/g in liver, 7.40×10^3 (tilapia) to 8.70×10^6 (koi) CFU/g in intestine, and 1.20×10^2 (tilapia) to 3.20×10^4(rui) CFU/g in kidney.

Table 1. Total bacterial load in blood, liver, intestine, and kidney of rui, catla, mrigal, koi, tilapia, tarabaim, magur, and shing.

Name of fish	Blood (CFU/ml)	Organ		
		Intestine (CFU/g)	Liver (CFU/g)	Kidney (CFU/g)
Rui	3.20×10^3	8.16×10^5	5.60×10^4	5.20×10^4
Catla	2.36×10^4	8.70×10^6	5.58×10^5	4.80×10^4
Mrigal	7.10×10^5	5.28×10^5	3.20×10^3	6.80×10^3
Koi	2.45×10^3	5.05×10^5	5.54×10^5	2.90×10^4
Tilapia	7.36×10^5	7.40×10^3	1.33×10^3	1.20×10^2
Tarabaim	1.65×10^4	2.43×10^4	2.24×10^3	1.85×10^3
Magur	5.29×10^4	7.68×10^6	4.37×10^4	2.83×10^3
Shing	2.30×10^4	4.31×10^4	1.60×10^4	7.44×10^3

Morphological, physiological, and biochemical test results: A comparative result of the present study of morphological, physiological, and biochemical tests with the findings of Popoff (1984) and Sabur (2006) is shown in Table 2.

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Table 2. Comparison of the characteristics of *A. hydrophila* isolates with those shown by Popoff (1984) and Sabur (2006).

Characters		Popoff (1984)	Sabur (2006)	Present Isolates
Gram stain		-[1]	-	-
Shape		Rod	Rod	Rod
Motility		+[2]	+	+
0129		ND[3]	ND	-
Oxidase		+	+	+
Catalase		+	+	+
OF test		F[4]	F	F
Acid and gas production from Glucose		+	+	+
Acid production from	**Lactose**	+	+	+
	Sucrose	+	+	+
	Manitol	+	+	+
Esculin hydrolysis		ND	ND	+
Methyl-red test		-	-	-
Voges-Proskaur test		+	+	+
H_2S production		+	+	
Ornithine decarboxilation		-	-	-
Citrate utilization		+	+	+
Growth at	**4°C**	-	-	-
	5°C	+	+	+
	37°C	+	+	+
	40°C	-	-	-

[1]: Negative; [2]: Positive; [3]: Not done; [4]: Fermentative

The presumptive *Aeromonas* colonies were Gram-negative and Oxidase-positive. They were fermented glucose and negative for the 0129 test. Thus, they were identified as *Aeromonas* bacteria (Fig. 2). Further, they produced gas from glucose and hydrolyzed esculin. In this way, they were finally identified as *Aeromonas hydrophila* (Fig. 3). From the above test results, two schematic diagrams were established for the confirmatory identification of *Aeromonas* and *A. hydrophila* (Fig. 2 and 3).

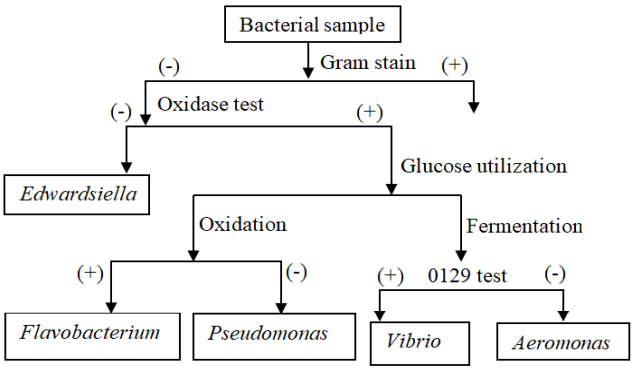

Fig. 2. Schematic diagram for primary identification of *Aeromonas.*

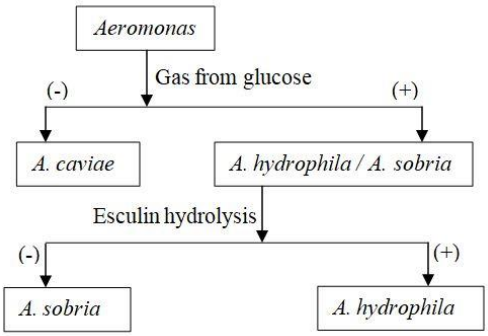

Fig. 3. Schematic diagram for final identification of three species of *Aeromonas.*

DISCUSSION

To identify pathogenic bacteria as well as diseases of fish, bacteriological study is very important. So, this study was done to isolate and identify the causative bacteria from apparently diseased rui, catla, mrigal, koi, tarabaim, tilapia, shing, and magur to find out the total bacterial load in the kidney, liver, and intestine of the tested fishes. Mamnur Rasid *et al*. (2008) also isolated *Aeromonas hydrophila* from EUS-affected shing from Mymensingh, Bangladesh. Sabur (2006) isolated and identified *Aeromonas* from five indigenous and exotic carps: rui (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhina cirrhosus*), silver carp

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(*Hypophthalamus molitrix*) and common carp (*Cyprinus carpio*). They were identified up to the species level as *A. hydrophila*, *A. sobria*, *A. caviae*, *A. veonii* and *A. jandaei*.

In the present study, bacterial count was found to be 2.45×10^3 (koi) to 7.36×10^5 (tilapia) CFU/g in blood, 1.33×10^3 (tilapia) to 5.58×10^5 (catla) CFU/g in liver, 7.40×10^3 (tilapia) to 8.70×10^6 (koi) CFU/g in intestine, and 1.20×10^2 (tilapia) to 3.20×10^4(rui) CFU/g in kidney. Mamnur Rasid *et al.* (2008) found 1.67×10^4 to 6.4×10^8 CFU/g, 1.71×10^3 to 1.18×10^9 CFU/g, and 1.47×10^4 to 3.70×10^8 CFU/g of bacteria in the liver, kidney and intestine of infected Thai pangas respectively. Mostofa (2007) isolated *A. hydrophila* from *Heteropneustes fossilis*. The highest bacterial load was found to be 2.42×10^7 CFU/g from the liver, and the lowest was 2.1×10^2 CFU/g from the kidney. Alam (2009) identified *A. hydrophila* from Thai pangas. The total bacterial load was 2.6×10^6 to 3.6×10^7 CFU/g in the liver, 4.8×10^6 to 7.2×10^7 CFU/g in the intestine, and 2.4×10^3 to 3.70×10^6 CFU/g in the kidney. Rahman and Chowdhury (1996) identified *A. hydrophila* from the kidneys of carp fishes. The total bacterial load in the kidneys of different fishes was 2.6×10^5 to 1.7×10^6 CFU/g. Iqbal *et al.* (1996) found the total load of *A. hydrophila* 5.4×10^3 to 4.7×10^7 CFU/g in the slime of *Cirrhinus mrigala*.

It was the first time in Bangladesh to identify *Aeromonas hydrophila* bacteria by testing their sensitivity to the vibriostatic agent 0129 and esculin hydrolysis. Hence, a new schematic diagram for the confirmation of *Aeromonas hydrophila* was established as Gram-negative, produces acid and gas from glucose, is resistant to vibriostatic agent 0129, and hydrolyzes esculin.

CONCLUSION

A. hydrophila is a causative agent of MAS disease that has been causing mass mortality of fish all over the country. A shortcut method for the identification of *A. hydrophila* has been developed by this work. As a consequence, the bacteria can be identified rapidly by this method, and the disease can be detected easily. Therefore, our fish culturists will get benefit from the result of this work and can save their cultured fish by using any recognized anti-*Aeromonas* drug.

Conflict of Interests: The authors declared there is no conflict of interest.

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