

Isolation and Identification of *Edwardsiella tarda* from Catla (*Catla catla*), Koi (*Anabas testudineus*) and Tilapia (*Tilapia mosumbicus*)

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Abstract: Edwardsiellosis is a very common fish disease in framed fish caused by *Edwardsiella tarda* which can lead a severe economic loss in aquaculture worldwide. Isolation and identification of the pathogenic bacteria *Edwardsiella tarda* from three fish species: catla *Catla catla*, koi *Anabas testudineus* and tilapia *Tilapia mosumbicus* were undertaken from different area of Mymensingh District. The intestine, liver and kidney of each fish were inoculated into *Salmonella-Shigella* (SS) plates and TSA plates. The colonies of the SS plates were subcultured onto TSA plates and undergone specific identification processes through their morphological and biochemical tests for *Edwardsiella tarda*. They were Gram negative, rod shaped, motile, oxidase negative bacteria that showed positive reactions for catalase and fermented glucose. Quantitative studies of the bacterial flora of the above fishes on TSA plates showed that the minimum total bacterial load of tilapia was 1.50×10^2 cfu/g in the blood and the maximum was 8.20×10^6 cfu/g in kidney. In case of catla, the minimum load was 2.36×10^3 cfu/g in the blood and the maximum was 8.20×10^6 cfu/g in intestine. Koi showed its minimum load of 2.45×10^3 cfu/g in the blood and a maximum of 5.54×10^5 cfu/g in liver. It was understood that the bacteria *Edwardsiella tarda* is an opportunistic pathogen in Bangladesh aquaculture and can, any time be a cause of serious disease condition of culture fishes of Bangladesh at least in catla, koi and tilapia.

Keywords: Pathogenic bacteria; *Edwardsiella tarda*; Catla; Koi; Tilapia.

INTRODUCTION

The bacterium *Edwardsiella tarda* is a Gram-negative motile rod that is pathogenic to a wide range of fishes, reptiles, birds and mammals including humans (Mamnur Rashid, 1997). *Edwardsiella tarda* is peritrichously flagellated, facultatively anaerobic, non-encapsulated, non-spore forming rod which belongs to the family Enterobacteriaceae and was first recognized by Hoshina (1962) as a causative agent of Hireaka Bio (red disease) in Japanese eel, *Anguilla japonica* and named *Paracolobactrum anguillimortiferum*. Later, Ewing *et al.* (1965) proposed the name *Edwardsiella tarda* for the organism. It has two other species named *E. hoshinae* (Grimont *et al.*, 1980) and *E. ictaluri* (Hawke *et al.*, 1981). Mamnur Rashid *et al.* (1997) observed the pathogenesis of experimental edwardsiellosis in Japanese flounder *Paralichthys olivaceus*. Mamnur Rashid *et al.* (1999)

isolate *Edwardsiella tarda* for the first time in Bangladesh from Thai pangas *Pangasius sutchi* and its stock pond.

Edwardsiellosis (emphysematous putrefactive diseases) is one of the most important bacterial diseases in fish. The outbreak of *E. tarda* is the highest in warm water as the bacteria grow between the temperatures of 20°C and 37°C and the optimum temperature for their growth is 25°C (Kodama *et al.* 1987). As Bangladesh has also a warm water environment, there is a great possibility for *E. tarda* outbreak in this country. Hossain (1999) carried out an investigation on the characterization of *E. tarda* and found that it was pathogenic for the rajpunti *Puntius gonionotus*. In spite of this, only a few research works have been performed in Bangladesh though the environmental condition is favorable for *E. tarda* in this country. So, the present study was conducted with the following objectives:

1. To isolate *Edwardsiella tarda* like bacteria from catla *Catla catla*, koi *Anabas testudineus* and tilapia *Tilapia mosombicus*; and
2. To identify and characterize the isolate.

MATERIALS AND METHODS

The experimental fishes were collected from the selected ponds of Tarakanda and Trishal of Mymensingh District (Figure 1).

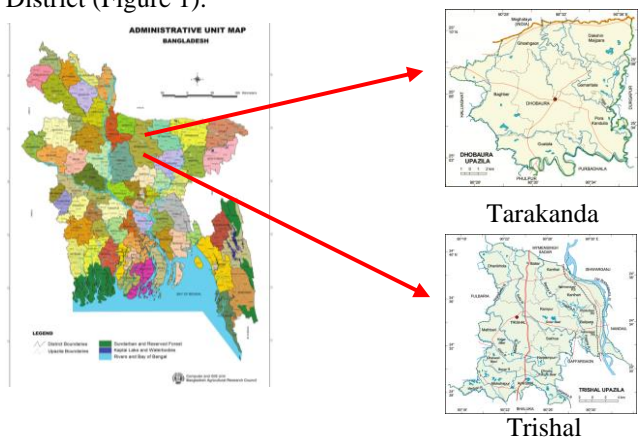


Figure 1. Study location in map

Three fish of each species were subjected to bacteriological investigation. Decimal dilutions of intestine, liver and kidney samples were prepared. Two samples of 0.1 ml from 10^{-2} and 10^{-3} were transferred onto SS plate to get only the presumptive colonies of *Edwardsiella*. Another two samples of 0.1 ml from 10^{-7} and 10^{-8} were transferred onto TSA plates for total bacterial colony counting. TSA slants were prepared for stocking *Edwardsiella* bacteria for further studies. All the plates and TSA slants were incubated at 25°C for 28 h.

Identification

Identification of *E. tarda* was done based on detailed morphological and biochemical characterization of the isolates. At first, the bacteria were sub-cultured onto TSA plates to obtain fresh 24 hours' culture. They were then streaked onto the selective *E. tarda* isolation medium for preliminary identification of the genus *E. tarda* and discarding the others. Colonies grown on the selective medium were sub-cultured again onto TSA plates and subjected to biochemical tests using commercially available media after autoclaving at 121°C for 15 minutes. The isolated bacteria were morphologically characterized on the basis of gram staining technique, shape of bacteria, motility test. The bacterial isolates were characterized by different biochemical reactions *i.e.*, oxidase, catalase, methyl-red, Voges-Proskauer (VP), indole, H₂S production and citrate utilization for the identification of bacterial species.

RESULTS

Bacterial load in blood, liver, intestine and kidney

Bacterial load in blood, liver, intestine and kidney of catla, koi and tilapia are shown in Table 2. Total bacterial

load was found to be 1.50×10^2 CFU/g in tilapia to 2.45×10^3 CFU/g in koi in blood, 1.33×10^3 CFU/g (tilapia) to 5.58×10^5 CFU/g (catla) in liver, 7.40×10^3 CFU/g (tilapia) to 8.20×10^6 CFU/g (koi) in intestine and 2.90×10^4 CFU/g (koi) to 7.36×10^5 CFU/g (catla) in kidney.

Table 2. Bacterial load in blood, liver, intestine and kidney of catla, koi and tilapia

Name of fish	Blood (CFU/g)	Organ		
		Intestine (CFU/g)	Liver (CFU/g)	Kidney (CFU/g)
Catla	2.36×10^3	8.20×10^6	5.58×10^5	4.80×10^4
Koi	2.45×10^3	5.05×10^5	5.54×10^5	2.90×10^4
Tilapia	1.50×10^2	7.40×10^3	1.33×10^3	7.36×10^5

Morphological and biochemical test results

Streaked samples showed growth on SS agar plate as black colonies which primarily conferred that the bacteria associated with reddish lesions of catla, koi and tilapia fishes were from the genus *Edwardsiella*. The isolates gave rise to yellowish opaque colonies on TSA plates and were smooth, raised and round in shape. Zigzag culture from SS to TSA provided single colonies for stocking. When the bacteria were inoculated to TSI slant it developed characteristic acidic colour at the top of the slant implying that peptone was utilized; alkaline at the middle region expressing that glucose was fermented and black in inoculating area informing that H₂S was produced. The results of morphological and biochemical tests are shown in the table with the result of Sawsan *et. al.* (2012) and Rediet *et. al.* (2022).

Table 3. Morphological and biochemical characteristics of *E. tarda* isolated in this study from catla, koi and tilapia in comparison to Sawsan *et. al.* (2012) and Rediet *et. al.* (2022).

Characters	Present study result	Result of Sawsan <i>et. al.</i> (2012)	Result of Rediet <i>et. al.</i> (2022)
Gram staining	-	-	-
Shape	Rod	Rod	Rod
Motility	+	+	+
Oxidase	-	-	-
Catalase	+	+	+
TSI	+	+	+
H ₂ S production	+	+	+
Urease	-	-	-
Glucose	+	+	+
Sucrose	-	-	-
Indole	+	+	+
Lysine	+	+	+
Methyl red	+	+	+
Citrate	-	-	-
Voges Proskauer (VP)	-	-	-

DISCUSSION

In the present study, *Edwardsiella tarda* bacteria was isolated from diseased catla, koi and tilapia fishes from different areas of Mymensing District. Total bacterial load was found to be 1.50×10^2 CFU/g in tilapia to 2.45×10^3 CFU/g in koi in blood, 1.33×10^3 CFU/g (tilapia) to 5.58×10^5 CFU/g (catla) in liver, 7.40×10^3 CFU/g (tilapia) to 8.20×10^6 CFU/g (koi) in intestine and 2.90×10^4 CFU/g (koi) to 7.36×10^5 CFU/g (catla) in kidney.

The present isolates of *E. tarda* from naturally diseased catla, koi and tilapia showed the same morphological and biochemical characters as were found by of Sawsan et al. (2012) and Rediet et al. (2022).

In the present study *E. tarda* was isolated from catla, koi and tilapia. But its pathogenicity could not be tested in the fish species from which it was isolated. So, it is very essential to test pathogenicity to those fishes. It can be mentioned here that Alam et al. (1999) experienced the experimental pathogenicity of *E. tarda* in cultured pangas *Pangasius sutchi* isolated from the homologous fish.

CONCLUSION

It was concluded that *E. tarda* has been introduced in Bangladesh aquaculture and it may be gradually ubiquitous in this environment. It was also understood that catla, koi and tilapia fishes were susceptible to be infected by the bacteria *E. tarda*.

RECOMMENDATION

This study was limited in the isolation and identification of *E. tarda* in catla, koi and tilapia fishes. So, Further research should be taken to

1. Explore its pathogenicity, ecology, serology and pathogenesis.
2. Investigate the susceptibility to other similar fish species.
3. Investigate the antibiotic sensitivity, prevention and control mechanisms.

Conflict of Interest

There are no conflicts of interest.

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