Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(5): 89 – 102 (2021)
www.ejabf.journals.ekb.eg

Status of Pathogenic Bacteria with their Antibiotic Susceptibility in Indigenous and Exotic Climbing Perch, *Anabas testudineus* (Bloch, 1972) in North-Eastern Bangladesh

Md. Abdul Baten [1], Md. Motaher Hossain [2], Rakiba Sultana [3], Mohammad Mosarof Hossain [4], Md. Golam Rasul [5], Mohammad Abu Jafor Bapary [2,*]

[1]Department of Fishing and Post Harvest Technology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

[2]Department of Fisheries Technology and Quality Control, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[3]Department of Aquaculture, Sylhet Agricultural University, Sylhet-3100, Bangladesh

[4]Department of Coastal and Marine Fisheries, Sylhet Agricultural University, Bangladesh

[5]Department of Fisheries Technology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh

[*]**Corresponding Author: jafor.ftqc@sau.ac.bd**

ARTICLE INFO

Article History:
Received: March 4, 2021
Accepted: June 19, 2021
Online: Sept. 7, 2021

Keywords:
A. testudineus,
Food safety,
Pathogenic bacteria,
Public health,
Antibiotic resistance

ABSTRACT

Information on pathogenic bacterial contamination and their antibiotic susceptibility to infrequently consumed fishes are largely unknown. This study aimed to assess the status of pathogenic bacteria with their antibiotic susceptibility in two varieties of climbing perch *Anabas testudineus* (capture-based indigenous and culture-based exotic) in North-Eastern Bangladesh. Thirty (30) indigenous and exotic climbing perch were collected separately from ten (10) fish markets of Sylhet Sadar to investigate total viable count (TVC), total coliform count (TCC), total faecal coliform (TFC) count, and a variety of pathogenic bacteria with their antibiogram profile. Significantly ($P<0.05$) higher values of TVC, TCC, and TFC were recorded in indigenous climbing perch than those of the exotic variety. The pathogenic bacteria such as *Escherichia coli*, *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus aureus*, *Salmonella* spp. and *Vibrio* spp. were detected in the studied samples. In comparison to the indigenous climbing perch, the antibiotic sensitivity assay revealed that exotic climbing perch had more multidrug-resistant bacteria. Since the presence of pathogenic bacteria in fresh fish is unsafe for human consumption and considered as rejected for export, the current study emphasizes the importance of assuring the safety and quality of climbing perch at all stages until it reaches consumers.

INTRODUCTION

Copared to other meats and meat products, fish isconsidered an important component of a healthy diet containing high-quality protein, essential nutrients, fatty acids as well as low cholesterol **(Rhea, 2009; Pal, 2010)**. It is generally regarded as a

safe, nutritious and beneficial food item although aquaculture products have sometimes been associated with certain food safety issues **(WHO, 2007)**. It has been well known that both fresh and brackish water fishes can harbour human pathogenic bacteria, particularly the coliform and faecal coliform **(Emikpe *et al.*, 2011)**. The natural habitats of fish such as rivers, *haors*, *baors*, lakes, reservoirs, canals, ponds and others could act as a probable source of microbial contamination due to the increase of water pollution and the indiscrimination of the deposition of human stool, animal excreta and other environmental wastes. Thus, those habitats could be highly prone to the transmission of pathogenic microorganisms **(Doyle, 2006; Pal, 2012)**. The bacterial contamination of fish can also be linked to the environmental condition, careless handling of landed fish, personnel hygiene, materials used for fish processing and preservation as well as preservatives as ice or salt **(Pal *et al.*, 2016)**. The bacterial contamination is also considered as a crucial factor for aquaculture production **(Austin & Austin, 2007)**. Several studies have shown that bacteria belonging to the genera *Aeromonas*, *Corynebacterium*, *Myxobacterium*, *Streptococcus* spp., *Pseudomonas* and *Vibrio* mostly cause infectious diseases in fish **(Ampofo & Clerk, 2010; Emikpe *et al.*, 2011)**. On the other hand, antibiotics are frequently used in fish ponds and/or farms either to treat bacterial diseases or promote the growth of aquatic organisms**(Wamala *et al.*, 2018)**. Due to the use of different antibiotics in aquaculture system, the resistance of different microbial species is daily increasing which, in return, increases the chance of their adaptation in the aquatic environment **(Zampieri *et al.*, 2017)**. The common route of pathogenic bacterial infection to human is post-harvest handling as cleaning and evisceration of fish **(Okerentugba *et al.*, 2012)**.Hence, the consumption of bacterial contaminated fish can cause infection or intoxication to the consumers **(Sanjee & Karim, 2016)** thatmay be responsible for the occureceof several food borne diseases such as, dysentery, typhoid, fever, salmonellosis and cholera **(Christopher *et al.*, 2009; Sichewo *et al.*, 2014)**. It was reported so far that, more than 80 million cases of seafood borne illnesses were detected in the USA per annum; the cost of whichis as high as billions of dollars per year **(Adebayo-Tayo *et al*., 2012)**. It is estimated that one-fourth of the world food supply is hampered due to microbial activity which leads to a huge economic loss **(EEC, 1992)**. Thus, it is an emerging issue to safeguard the quality of aquaculture products. As a consequence, the study of pathogenic bacterial contamination in fishes is very essential for the successful aquaculture production as well as ensuring safe food for the consumers.

Climbing perch (*Anabas testudineus*); commonly known as *Koi* in Bangladesh, usually inhabit the inland waters **(Kohinoor *et al.*, 2016; Barman *et al*., 2018)**. It is one of the delicious fishes with high market demand in Bangladesh **(Hossain, 2009; Hossain *et al.*, 2012)**. Very recently, aqua-farming of *A. testudineus* in earthen ponds and cemented tanks has become very popular and most commercially cultured exotic climbing perch known as Thai Koi and Vietnami Koi. The intensive and unhygenic

culture practices cause some health problems, most of which occurred during post-harvest management of the cultured (exotic) and the captured (native) climbing perch **(Baten *et al.*, 2018)**. Bacteria are ubiquitous in the aquatic environment **(Allen *et al.*, 1983),** but the presence of pathogenic bacteria both in open (*Haor*, *Baor*, lakes, reservoirs, canals, Rivers etc.) and closed (ponds) water bodies is considered poor water quality status of habitats. Moreover, like many other fish species, climbing perch are susceptible to the bacterial infection **(Bonga, 1997; Svobodova *et al.*, 2003)**. The pathogenic bacteria such as *Pseudomonas* spp. and *Aeromonas* spp. which are mostly responsible for ulcer, fin, and tail rot diseases can also be found in climbing perch **(Rahman *et al.*, 2010; Begum *et al.*, 2015)**. These pathogenic bacteria can cause serious health hazards forboth fish handlers and consumers **(Hossain *et al.*, 2017)**. The consumer may be affected due to the intoxication of contaminated climbing perch and may pose serious health hazards, includingdiarrhoea, abdominal cramp, vomiting, nausea, and fever. Though food safety issues are getting prime concern both from the perspectives of fish health management and consumer health, it is essential to have available information on pathogenic bacteriological contamination status with their antibiotic susceptibility in climbing perch.

Nevertheless, limited researches have been conducted on this field, and the information on this food safety and public health concern issue in fishes are still unknown. Yet, literature focusing on assessment of pathogenic bacterial contamination with their antibiotic susceptiblitily in indigenous (wild or captured) and exotic (cultured) variety of climbing perch is extremely scant. Thus, the present reseach aimed to assess the status of pathogenic bacterial contaimination with the antibiotic susceptibility of climbing perch, *A. testudineu*s in the North-Eastern Bangladesh.

MATERIALS AND METHODS

Site profile of the study area

Sylhet located in the North-Eastern region of Bangladesh is one of the most important districts for the availability of both indigenous (wild) and exotic (cultured) climbing perch, *A. testudineus*. Therefore, ten fish markets; namely, Kazir Bazar, Bondor Bazar, Shibgonj, Tilagor, Majortila, Akhalia, Subid Bazar, Amborkhana, Shahi Eidghah and Baluchar Naya Bazar of Sylhet Sadar Upazila were selected randomly for the collection of fish samples. Total thirty samples of each were collected from those fish markets during the experimental period from December 2014 to November 2015.

Collection and preparation of samples

Both indigenous and exotic variety of climbing perch were purchased from the fish markets and then transported to the laboratory of the Department of Microbiology and Immunology, Sylhet Agricultural University (SAU), Sylhet using icebox. Then, the sample was prepared according to the laboratory protocol. Briefly, 25g fish sample was

taken from muscle, gill and intestine and mixed homogeneously with 225 ml distilled water in a Stomacher lab blender (Seaward Stomacher 400 UK). Each sample was mixed aseptically with sterilized distilled water at the ratio of 1:10. Afterwards, the sample was shaken properly to make a homogenous suspension. Later on, 10-fold serial dilutions (1:10) were made to form 10^{-2} to 10^{-9} in accordance to the recommendations of the International Standardization **(ISO, 1995)**. The diluted samples were then taken in nutrient broth and mixed properly. Thereafter, the nutrient broth containing samples was kept into an incubator for 24 hours.

Enumeration of total viable count (TVC)

1ml of tenfold serially diluted sample was taken into petridish containing plate count agar (PCA) using a pipette and a sterilized glass rod to spread the sample. Then, the sample containing petridishes was incubated at 37˚C for 24-48 hrs **(ISO, 1995)**. Only plates having 30 to 300 colonies were considered for calculation of acceptable number of bacteria in climbing perch. Number of bacteria per gram of the sample (CFU/g) was calculated in accordance to the formula of **Kashem *et al*. (2014)** as CFU/g =

$$\frac{\text{No. of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{Volume of total sample solution}}{\text{Wt. of fish sample (g)}}$$

Enumeration of total coliform count (TCC)

According to the procedure followed by ISO (4831:1991), each of the 10^{-1}, 10^{-2} and 10^{-3} dilutions were further transferred into three separate tubes of Lauryl Sulphate Tryptose Broth (LSTB) containing Durham's tube. Then, the tubes were incubated in an incubator at 37˚C for 48 hrs. After incubation, the positive gas production tubes were recorded. For each set of positive LSTB tubes, one set of brilliant green bile broth (BGBB) tubes (for total coliform enumeration) was prepared. A loopful of broth was taken from each positive LSTB tubes and inoculated into a BGBB tube and incubated at 37˚C for 24 hours. The positive gas production tubes were recorded and the result was computed in accordance with the method approved by **FDA (2011)**.

Enumeration of total faecal coliform (TFC) count

According to **ISO (7251:1993)**, a loopful broth from the tubes of LSTB that were positive for gas production was transferred to an *E. coli* broth (EC) containing Durham's tubes. EC tubes were incubated at 45˚C for 24 hrs in a circulatory water bath. After incubation, positive gas production tubes were recorded. Thereafter, a loopful of broth from each tube was transferred to sterile tryptone water tube and incubated at 37˚C for 48 hrs in an incubator. After incubation, Kovac's reagents were added to determine the presence of indole ring. A positive indole reaction indicates the presence of faecal coliform bacteria. Positive tubes were recorded and the results were computed using MPN chart (**FDA 2011**).

Isolation and detection of pathogenic bacteria

The morphological study (size, shape, arrangement, motility), colony characteristics evaluation, and biochemical test (oxidase, catalase, amylase, gelatinase, lipase, indole, H2S production, and nitrate reduction) were also performed for the isolation and detection of bacteria, using the criteria described in Bergey's Manual of Determinative Bacteriology **(Holt *et al*., 1994; Al Harbi & Uddin, 2012)**. Firstly, the total coliform count (TCC) and total faecal coliform (TFC) were determined by using LSTB and EC. The suspected colony from these media was subcultured in nutrient agar, Eosin methylene blue (EMB), baird parker agar (BPA), m-aeromonas agar, acetamide agar, macc onkey, salmonella-shigella (SS), brilliant green agar (BGA), and thiosulfate citrate bile salt sucrose (TCBS) to promote the growth of a particular type of bacterium. Finally, the pure culture was obtained from the selective media. The aseptic condition was strictly maintained for all the steps during the research. After performing these tests, the results were analyzed and the isolated bacteria were detected.

Antibiogram sensitivity test

The isolated pathogenic bacteria were randomly selected for antimicrobial drug susceptibility test against five commonly used antibiotics, such as ciprofloxacin (5 µg/disc**),** chloramphenicol (30 µg/disc**)**, gentamicin (10 µg/disc), ampicillin (10 µg/disc), and cefalexin (30 µg/disc) by disk diffusion or Kirby-Bauer method **(Bauer *et al*., 1966)**. The results of the antibiotic sensitivity assay were recorded as sensitive, intermediate and resistant following the guidelines of the Clinical Laboratory Standard Institute **(CLSI, 2007)**.

Data analysis

After incubation, colonies were counted upon visualization and data were recorded. For preliminary processing of raw data, the mean and standard deviation of the microbial count was calculated. Statistical differences were analyzed by using IBM SPSS software version (20); Student's t-test was applied to compare the TVC and coliform bacteria in indigenous and exotic climbing perch where P-value of <0.05 was considered as statistically significant.

RESULTS

Total viable count (TVC) in *A. testudineus*

Total viable count (TVC) of collected indigenous and exotic climbing perch was analysed during the study period. It was observed that the TVC value of the fishes differed slightly among markets. When the values were comapared between the fish groups, significantly ($p<0.05$) higher TVC was found in indigenous climbing perch compared to those of exotic variety (Table 1).

Table 1. Calculation of total viable count (TVC) in indigenous and exotic variety of *A. testudineus*[1]

Variety of Climbing perch	Number of Samples	Mean TVC (Log CFU/g±std)[1]
Indigenous	30	7.52 ± 0.08^{a}
Exotic	30	7.32 ± 0.06^{b}

[1]Values are means of data obtained ± Std. Deviation (mean ± SD). Values in the same column with different superscript indicate the statistically significant difference (p<0.05).

Total coliform count (TCC) and total faecal coliform (TFC)

The total coliform count (TCC) and total faecal coliform (TFC) of indigenous and exotic varieties of climbing perch are shown in Fig. (1). It was observed that the mean TCC of indigenous and exotic climbing perch were 163.33±43.33 and 115.67±27.78 MPN/g, whereas the mean TFC of them were 53.37± 16.57 and 35.93±10.17 MPN/g, respectively. The mean total coliform count and total faecal coliform revealed that the indigenous climbing perch contained significantly (p <0.05) higher coliform bacteria than those in exotic variety.

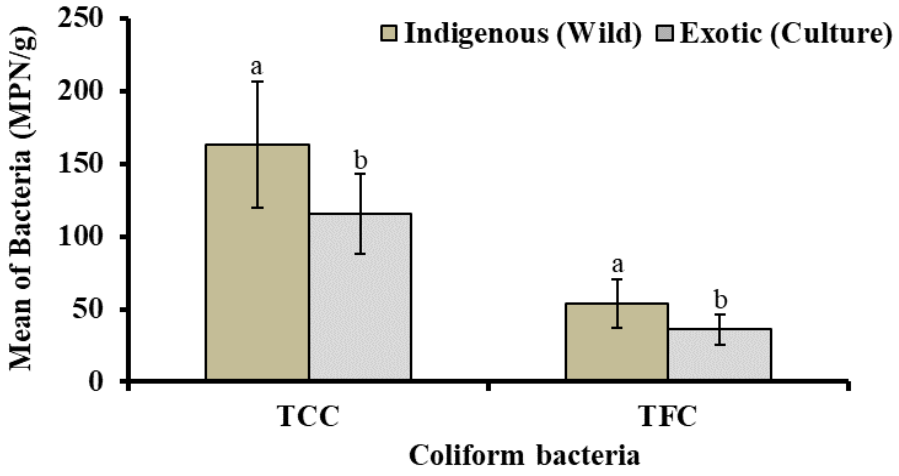

Fig. 1. The TCC and theTFC of indigenous and exotic varieties of climbing perch

Data arepresented as mean±std (n=30), where different superscript letter showing statistically different (p<0.05).

Isolation and detection of pathogenic bacteria from *A. testudineus*

Based on the study of cultural characteristics, biochemical and morphological features, six (6) types of bacteria were isolated from both indigenous and exotic climbing perch (Table 2). The isolated pathogenic bacteria were *Escherichia coli*, *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus aureus*, *Salmonella* spp., and *Vibrio* spp. (Fig. 2).

Table 2. Detection of pathogenic bacteria from both indigenous and exotic varieties of climbing perch, *A. testudineus* by different Bio-chemical tests

Detected Species	Color	Shape	Motility	Gram's reaction	Indole	Catalase	TSI	MR	VP
Escherichia coli	Metallic green	Rod	+	-	+	-	Yellow	+	-
Staphylococcus aureus	Black	Round	-	+	-	+	N/A	+	+
Pseudomonas spp.	Purple	Rod	+	-	-	+	Black	+	-
Aeromonas spp.	Yellow-brown	Rod	+	-	+	+	N/A	+	-
Salmonella spp.	Black	Rod	+	-	-	-	Black	+	-
Vibrio spp.	Yellow/ green	Curve	+	-	+	+	Black	+	-

+ = strains positive, - = strains negative, TSI=Triple Sugar Iron, MR=Methyl Red, VP=Voges-Proskauer

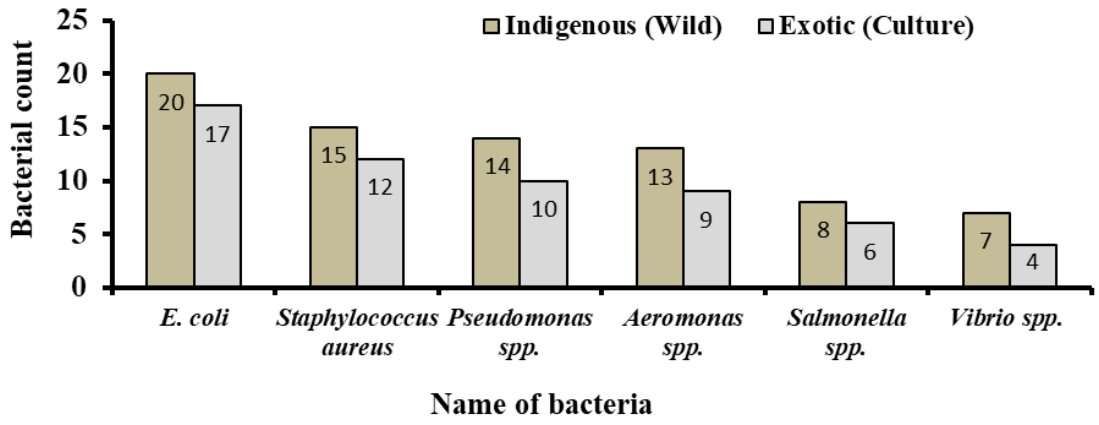

Fig. 2. Isolated pathogenic bacteria from both variety of climbing perch, *A. testudineus*

Antibiotic susceptibility of pathogenic bacteria

Six pathogenic bacteria identified in this research were found sensitive for three antibiotics such as ciprofloxacin, chloramphenicol and gentamicin for both the varieties of climbing perch. The isolated six bacteria were found resistant against ampicillin and cefalexin in exotic climbing perch. On the other hand, *E. coli* and *Salmonella* spp. isolated from indigenous climbing perch showed resistant against ampicillin, whereas other bacteria were found sensitive against the remaining antibiotics (Table 3).

Table 3. Antimicrobial sensitivity of detected pathogenic bacteria

Names of Pathogenic Bacteria	Varieties of *Anabas testudineus*	Antibiotic sensitivity patterns				
		Ciproflox-acin	Chloramphe-nicol	Gentami-cin	Ampicil-lin	Cefal-exin
E. coli	Indigenous	S	S	S	R	S
	Exotic	S	S	S	R	R
Staphylococcus aureus	Indigenous	S	S	S	S	S
	Exotic	S	S	S	R	R
Pseudomonas spp.	Indigenous	S	S	S	S	S
	Exotic	S	S	S	R	R
Aeromonas spp.	Indigenous	S	S	S	S	S
	Exotic	S	S	S	R	R
Salmonella spp.	Indigenous	S	S	S	R	S
	Exotic	S	S	S	R	R
Vibrio spp.	Indigenous	S	S	S	S	S
	Exotic	S	S	S	R	R

R=Resistance; S=Sensitive

DISCUSSION

Food safety issue is generally considered a prime factor throughout the world **(Rasul *et al*., 2020)**. The presence of pathogenic bacteria in fish food is also regarded as a major threat for the consumer's health **(Akter *et al.*, 2018)**. *Anabas testudineus* (Climbing perch) is familiar as one of the important fish species in Bangladesh due to its delicious taste and high nutritional value. By considering the food safety issue, this study evaluates the status of pathogenic bacteria with their antibiotic susceptibility in climbing perch.

The present study assessed the status of total viable count (TVC), total coliform count (TCC) and total faecal coliform (TFC) bacteria in the studied fish samples collected from the captured and cultured climbing perch. Thismay mean that both the sources are contaminated with pathogenic bacteria that could be a possible source of pathogenic bacteria in the human diet. A considerable number of TVC, TCC and TFC were observed in indigenous (7.52± 0.08 and 7.32± 0.06 log CFU/g), (163.33±43.33 and 115.67±27.78 MPN/g) and exotic climbing perch (53.37± 16.57 and 35.93±10.17 MPN/g) during the study period. These findings also revealed that the indigenous variety of climbing perch contained higher TVC and coliform bacteria than the exotic

variety. According to the International Commission on Microbiological Specifications for Foods **(ICMSF, 1986)**, the acceptable limit of TVC, TCC and TFC are $<10^5<100$ and <3 MPN/g, respectively. This is a standard evident that the present studied sample is not acceptable as it is contaminated with higher coliform bacteria which is not safe for human consumption. In a recent study, greater TVC was observed in indigenous climbing perch (**Baten *et al.*, 2018**). The current findings were also consistent with previous research, which reported the highest total coliform and faecal coliform (>240 MPN/g and 110 MPN/g, respectively) in several fish samples collected from local markets **(Begum *et al.*, 2010)**. The presence of coliform and faecal coliform bacteria in fish samples indicates the probability of having pathogenic bacteria **(Hossain *et al.*, 2010; Hasan *et al.*, 2013)**. Since coliforms are not designated bacterial flora in fish, faecal coliform in fish indicates the extent of pollution in their surroundings **(FAO, 1979; Emikpe *et al.*, 2011)**. Moreover, the presence of coliform bacteria in a higher range suggests the contamination of fish samples before or during the handling, processing and marketing **(Begum *et al.*, 2010)**.

The pathogenic bacteria such as *Bacillus* sp., *Salmonella* spp., *Shigella* spp., *E. coli*, *Pseudomonas* spp. *and Staphylococcus aureus* is responsible for both fish disease and spoilage **(Pal, 2010; Pal, 2016)**. The isolated bacteria from common carp including *S. putrefaciens*, *A. hydrophila*, *Enterobacter* sp., *Streptococcus* sp., *Staphylococcus* sp., *E. coli*, *V. vulnificus*, and *P. fluorescens* are considered as facultative pathogens of food poisoning and spoilage **(Al-Harbi & Uddin, 2012)**. In this study, six different types of bacteria were isolated from both indigenous and exotic varieties of climbing perch, explicitly *Escherichia coli*, *Pseudomonas* spp, *Aeromonas* spp. *Staphylococcus aureus*, *Salmonella* spp. and *Vibrio* spp. These wide variety of pathogenic bacteria were also previously identified in climbing perch fish farm in Bangladesh **(Zaman *et al.*, 2013; Hossain *et al.*, 2017)**. The prevalence of pathogenic bacteria was found higher in indigenous climbing perch than thoseof exotic variety. In Bangladesh, natural water bodies are contaminated by industrial effluents, sewage wastes, diarrheal stools of infected persons that ultimately trigger the abundance of pathogenic bacteria in indigenous fishes **(Rahman *et al.*, 2010; CDCP, 2010)**. The exotic variety of climbing perch mostly grown in controlled aquaculture ponds leads to less possibility of contamination, especially by industrial effluents and sewage wastes. Microbial contamination of fish can also be linked to the external sources, such as poor marketing infrastructure, usage of poor quality water for washing, the use of contaminated materials during handling and transportation, lack of personnel hygiene and sanitation and the use of impure processing tools **(Pal, 2010; Adebayo-Tayo *et al.*, 2012)**. The finfish aquaculture is enormously filled with antibiotics **(Hossain *et al.*, 2017)**. The present study found a higher number of antibiotic resistant bacteria in exotic (cultured) climbing perch. The isolated six pathogenic bacteria were found resistant against ampicillin and cefalexin antibiotics similar to the previous study **ofZaman *et al.* (2013)**. The use of antibiotic in

the aquaculture industry is increasing day by day resulting higher antibiotics resistant bacteria in aquatic environments of Bangladesh **(Rasul *et al.*, 2015; Rasul *et al.*, 2017; Hossain *et al.*, 2018)**. The antibiotic resistance in fish food would be liable enough to outbreak of fish borne disease **(Noor *et al.*, 2013, Noor *et al.*, 2019)**. The occurrence of pathogenic bacteria with higher antibiotic susceptibility in climbing perch is unsafe for human consumption and it is regarded as rejected for export **(ICMSF, 1986)**.

CONCLUSION

In this study, the higher number of TVC, TCC and TFC were observed in the indigenous variety of climbing perch compared to exotic variety. The indigenous (captured/wild) climbing perch were also found sensitive against five antibiotics except *E. coli* and *Salmonella* that showed resistance against ampicillin, while cultured climbing perch were found resistant against two antibiotic, explicitly ampicillin and cephalexin. The phenomena in indigenous climbing perch might indicate the pollution of the natural inland water habitats from where they were captured. Lower count in exotic variety with higher antibiotic susceptibility may be associated with culture condition in the ponds or tanks and the use of artificial feed and prophylactic items including the use of broad spectrum atibiotics for disease control. On the other hand, our environmental conditions, market infrastructure, poor knowledge about hygiene, poor water supply, and contaminated processing materials might also accelerate the contamination of fishes. Therefore, limiting of water pollution, improving market infrastructure, raising awareness on personnel hygiene and sanitation, restriction in using antibiotics could be some strategy for improving the existing situation. Moreover, the establishment of institutional support system to inspect the fresh fish in market condition should be emphasized to ensure the quality of raw fish from both culture and capture origin.

ACKNOWLEDGEMENT

The authors are grateful to Microbiology laboratory staffs, Department of Microbiology and Immunology, Sylhet Agricultural University for their institutional support. This research was funded by the National Science and Technology fellowship in 2015-2016 under the Ministry of Science and Technology, Bangladesh.

REFERENCES

Adebayo-Tayo, A. C.; Odu, N. N.; Michael, M. U. and Okonko, I. O. (2012). Multi-drug resistant (MDR) organisms isolated from sea-foods in Uyo, south-southern Nigeria. Nature and Science, 10(3): 61-70.

Al-Harbi, A. H. and Uddin, M. N. (2012). Bacterial content in the intestine of frozen common carp *Cyprinus carpio*. African Journal of Biotechnology, 11(30): 7751-7755.

Allen, D. A.; Austin, B. and Colwell, R. R. (1983). Numerical taxonomy of bacterial isolates associated with a freshwater fishery. Journal of General Microbiology, 129(7): 2043-2062.

Akter, T.; Sayeed, M. A.; Rasul, M. G.; Kashem, M. A. and Paul, A. K. (2018). Evaluation of microbiological quality of dried baim (*Mastacembelus armatus*) in Bangladesh. Archives in Agriculture and Environmental Science, 3(4): 344-353.

Ampofo, J. A. and Clerk, G. C. (2010). Diversity of bacteria contaminants in tissues of fish cultured in organic waste-fertilized ponds: health implications. Open Fish Science Journal, 3: 142-146.

Austin, B. and Austin, B. (2007). Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, Praxis Publishing, UK.

Baten, M. A.; Hossain, M. M.; Bapary, M. A. J.; Islam, M. J.; Elahi, A. T. M. M. and Hossain, M. M. (2018). Seasonal variation in bacteriological count between native vs exotic climbing perch, *Anabas testudineus* (Bloch, 1972) at North-Eastern Bangladesh. Bangladesh Journal of Fisheries, 30(1): 123-132.

Barman, A. K. A.; Hossain, M. M.; Rasul, M. G.; Majumdar, B.C. and Rahim, M.M. (2018). Effects of oxytetracycline residues in Thai Koi (*Anabas testudineus* Bloch) collected from Sylhet, Bangladesh. Archives of Agriculture and Environmental Science, 3(2): 174-179.

Bauer, A. T. (1966). Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 149-158.

Begum, M. K.; Chowdhury, M. M.; Haque, W. And Nasrin, T. (2015). Comparative microbiological assessment of export oriented fishes and locally marketed fishes of Bangladesh. IOSR Journal of Pharmacy and Biological Sciences, 10(3): 17-23.

Begum, M.; Ahmed, A. T. A.; Das, M. and Parveen, S. (2010). A Comparative Microbiological assessment of five types of selected fishes collected from two different market. Advance in Biological Research, 4: 259-265.

Bonga, S. E. W. (1997). The stress response in fish. Physiological review, 77(3): 591-625.

CDCP. (2010). *Escherichia coli*. Center for Disease Control and Prevention, FAQ. Website:http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.html. Accessed on 10-05-2010.

Clinical and Labratory Standards Institute (CLSI, formerly NCCLS). (2007). Performance standards for antimicrobial susceptibility testing. 17th Informational Supplement document M100-S17: 27: 1. Wayne, Pennsyslvania, pp. 32-50.

Christopher, E. A.; Vincent, O.; Grace, I.; Rebecca, E. and Joseph, E. (2009). Distribution of heavy metals in bones, gills, livers and muscles of (Tilapia)

Oreochromis niloticus from Henshaw Town Beach market in Calabar Nigeria. Pakistan Journal of Nutrition, 8(8): 1209-1211.

Doyle, M. P. and Erickson, M. C. (2006). The faecal coliform assay, the results of which have led to numerous misinterpretations over the years, may have outlived its usefulness. Journal of Microbiology, 4: 162-163.

EEC. (1992). Opinion of the scientific committee on veterinary measures relating to public health on *Vibrio vulnificus* and *Vibrio parahaemolyticus* (in raw and undercooked seafood) (adopted on 19-20 September 2001).

Emikpe, B. O.; Adebisi, T. and Adedeji, O. B. (2011). Bacteria load on the skin and stomach of *Clarias gariepinus* and *Oreochromis niloticus* from Ibadan, South West Nigeria: Public health implications. Journal of Microbiology and Biotechnology Research, 1(1): 52-59.

FAO (Food and Agricultural Organization). (1979). Manuals of food quality control: 4. Microbiological Analysis. FAO Food and Nutrition paper 14 (4).

FDA (2011). Fish and Fishery Products Hazards and Controls Guidance, 4th Edition, Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition Office of Seafood, Washing-ton, D.C. 240: 402-2300.

Hasan, M. R.; Hassan, M. M.; Sen, K.; Kohinoor, A. and Rahman, M. M. (2013). Microbiological risk assessment of frozen fishes in relation to their effects of different processing treatments. International Journal of Bioscience, 3(7):169-176.

Holt, J. G.; Krieg, N. R.; Sneath, P. H. A. and Williams, S. T. (1994). Bergey's manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore.

Hossain, A.; Mandal, S. C.; Rahman, M. S.; Rahman, M. M. and Hasan, M. (2010). Microbiological quality of processed frozen black tiger shrimps in fish processing plant. World Journal of Fish and Marine Science, 2(2): 124-128.

Hossain, M. A.; Sultana, Z.; Kibria, A. S. M. and Azimuddin, A. K. (2012). Optimum dietary protein requirement of a Thai strain *of climbing* perch, *Anabas testudineus* (Bloch, 1792) fry. Turkish Journal of Fisheries and Aquatic Sciences, 12(2): 217-224.

Hossain, M. M. (**2009).** Effects of partial replacement of fishmeal by fermented soybean meal and squid by-product blend in the diet of climbing perch, *Anabas testudineus* (Bloch). MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Hossain, A.; Nakamichi, S.; Habibullah-Al-Mamun, M.; Tani, K.; Masunaga, S. and Matsuda, H. (2017). Occurrence, distribution, ecological and resistance risks of antibiotics in surface water of finfish and shellfish aquaculture in Bangladesh. Chemosphere, 188: 329-336.

Hossain, M. S.; Hashem, S.; Halim, M. A.; Chowdhury, P.; Sultana, S. and Khan, M. N. A. (2017). Bacterial community structure and infection in cultured Koi

(*Anabas testudineus*) fish species. International Journal of Fisheries and Aquatic Studies, 5(3): 520-524.

Hossain, F. E.; Chakraborty, S.; Bhowmick, N. C.; Rahman, M. A. and Ahmed, F. (2018). Comparative analysis of antibiotic resistance pattern of bacteria isolated from fish of cultured and natural ponds: A study based on Noakhali region of Bangladesh. Bioresearch Communications, 4(2): 586-591.

ICMSF (International Commission on Microbiological Specifications for Foods). (1986). Microorganisms in food (Vol. 2). Sampling for microbiological analysis: principles and specific applications. University of Toronto Press, Canada.

ISO (International Standard Organization). (1995). Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC-36/Sc-6. The Netherlands.

ISO. 4831:1991 (E). Microbiology-General guidance for the enumeration of coliforms-Most probable number technique.

ISO. 7251:1993 (E). Microbiology-General guidance for the enumeration of presumptive Escherichia coli-Most probable number technique.

Kashem, M. A.; Uddin, M. N.; Hossain, M. M.; Hasan, M. T.; Haque, S. A.; Khan, M. N. A. and Hossain, F. M. A. (2014). Effect of Oxytetracycline on bacterial load of Labeo rohita (Rohu) fish in culture pond. Global Advance Research Journal of Microbiology, 3(2): 018-024.

Kohinoor, A. H. M.; Rahman, M. M.; Islam, M. S. and Mahmud, Y. (2016). Growth and production performance of climbing perch Thai Koi and Vietnamese Koi Strain (*Anabas testudineus*) in Bangladesh. International Journal of Fisheries and Aquatic Studies, 4(1): 354-357.

Noor, R.; Acharjee, M.; Ahmed, T.; Das, K. K.; Paul, L.; Munshi, S. K.; Urmi, N. J.; Rahman, F. and Alam, M. Z. (2013). Microbiological study of major sea fish available in local markets of Dhaka City, Bangladesh. Journal of Microbiology, Biotechnology and Food Sciences, *2*(4): 2420-2430.

Noor, R,; Maniha, S. M.; Murshed, T. and Rahman, M. M. (2019). Effectiveness of Antibiotics: Anti-Bacterial Activity or Microbial Drug Resistance? Bangladesh Journal of Microbiology, 36 (2): 111-114.

Okerentugba, P. O.; Innocent-Adiele, H. C. and Okonko, I. O. (2012). Benefits, public health hazards and risks associated with fish consumption, New York Science Journal, 5(9): 33-61.

Pal, M.; Ketema, A.; Anberber, M.; Mulu, S. and Dutta, Y. (2016). Microbial quality of Fish and Fish Products. Beverage and Food World, 43 (2): 46-49.

Pal, M. (2010). Fish hygiene. MSc Lecture Notes. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

Pal, M. (2012). Food spoilage. Ph.D. Lecture Notes. Addis Ababa University, College of Veterinary Medicine, Debre Zeit, Ethiopia.

Rasul, M.G.; Yuan, C. and Shah, A. (2020). Chemical and Microbiological Hazards of Dried Fishes in Bangladesh: A Food Safety Concern. Food and Nutrition Sciences, 11(6): 523-539.

Rasul, M. G.; Majumdar, B. C. and Akter, T. (2017). Aqua-chemicals and antibiotics used in freshwater aquaculture of Sylhet, Bangladesh. Journal of Agricultural Science and Engineering, 3(2): 20-26.

Rasul, M. G.; Akter, S.; Barman, P. P; Islam, J.; Shah, A. K. M. A.; Sayeed, A. and Rahman, M. (2015). Effects of Antibiotic Treated Fish Diet on Fish Muscle, Bacterial Flora and Water Quality Parameters under Pond Experimental Condition. World Journal of Fish and Marine Sciences, 7 (2): 135-140.

Rahman, M. S.; Hasan, M.; Mahmud, Z. H. and Islam, M. S. (2010). Bacterial load in twelve freshwater fishes of four feeding habits in Bangladesh. Dhaka University Journal of Biological Science, 19 (2): 145-150.

Rhea, F. (2009). Microbiology handbook: Fish and seafood. Leatherhead Food International Ltd. Surrey, United Kingdom.

Sanjee, S. A. and Karim, M. (2016). Microbiological quality assessment of frozen fish and fish processing materials from Bangladesh. International Journal of Food Science, 1-6.

Sichewo, P. R.; Gono, R. K.; Muzondiwa, J. and Mungwadzi, W. (2014). Isolation and identification of pathogenic bacteria in edible fish: A case study of rural aquaculture projects feeding livestock manure to fish in Zimbabwe. International Journal of Current Microbiology and Applied Sciences, *3*(11): 897-904.

Svobodova, Z.; Lloyd, R. and Vykusova, J. (2003). Water quality and fish health. Food and Agriculture Organization of the United Nations, Rome, Italy,

Wamala, S. P.; Mugimba, K. K.; Mutoloki, S.; Evensen, Ø.; Mdegela, R.; Byarugaba, D. K. and Sørum, H. (2018). Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda. Fisheries and Aquatic Sciences, 21(1): 1-10.

WHO. (2007). Food safety issues associated with products from Aquaculture. Report of a Joint FAO/NACA/WHO study Group, WHO Technical reprint Series: 883, Geneva.

Zaman, B. S.; Khatun, M. M.; Islam, M. A.; Sharmin, S.; Kulsum, U. and Hoq, M. E. (2013). Bacterial flora of Koi (*Anabas testudineus*) harvested from ponds and their antibiogram. Microbes and Health, 2(1): 8-11.

Zampieri, B. D. B.; Oliveira, R. S. D.; Pinto, A. B.; Andrade, V. D. C.; Barbieri, E.; Chinellato, R. M. and Oliveira, A. J. F. C. (2017). Comparison of bacterial densities and resistance in different beach compartments: should water be our main concern?. O Mundo da Saúde, 40: 461-482.