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

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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

Compared to other meats and meat products, fish is considered 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) that may be responsible for the occurrence of 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 which is 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 unhygienic
cultural 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 for both 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, including diarrhea, 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 susceptibility in indigenous (wild or captured) and exotic (cultured) variety of climbing perch is extremely scant. Thus, the present research aimed to assess the status of pathogenic bacterial contamination with the antibiotic susceptibility of climbing perch, A. testudineus 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, Akhali, 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 these 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)**

1 ml 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:

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

**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 ciprofloxacine (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 compared 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

<table>
<thead>
<tr>
<th>Variety of Climbing perch</th>
<th>Number of Samples</th>
<th>Mean TVC (Log CFU/g±std)\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigenous</td>
<td>30</td>
<td>7.52 ± 0.08\textsuperscript{a}</td>
</tr>
<tr>
<td>Exotic</td>
<td>30</td>
<td>7.32 ± 0.06\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{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.

![Bar chart showing TCC and TFC](image)

**Fig. 1.** The TCC and the TFC of indigenous and exotic varieties of climbing perch

Data are presented 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

<table>
<thead>
<tr>
<th>Detected Species</th>
<th>Color</th>
<th>Shape</th>
<th>Motility</th>
<th>Gram’s reaction</th>
<th>Indole</th>
<th>Catalase</th>
<th>TSI</th>
<th>MR</th>
<th>VP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Metallic green</td>
<td>Rod</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Yellow</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Black</td>
<td>Round</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>Purple</td>
<td>Rod</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Black</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Aeromonas spp.</em></td>
<td>Yellow-brown</td>
<td>Rod</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>N/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Black</td>
<td>Rod</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Black</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Vibrio spp.</em></td>
<td>Yellow/green</td>
<td>Curve</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Black</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = 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

<table>
<thead>
<tr>
<th>Names of Pathogenic Bacteria</th>
<th>Varieties of <em>Anabas testudineus</em></th>
<th>Antibiotic sensitivity patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Indigenous</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Exotic</td>
<td>S</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Indigenous</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Exotic</td>
<td>S</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>Indigenous</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Exotic</td>
<td>S</td>
</tr>
<tr>
<td><em>Aeromonas</em> spp.</td>
<td>Indigenous</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Exotic</td>
<td>S</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Indigenous</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Exotic</td>
<td>S</td>
</tr>
<tr>
<td><em>Vibrio</em> spp.</td>
<td>Indigenous</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Exotic</td>
<td>S</td>
</tr>
</tbody>
</table>

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. This may 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
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 those of 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 of Zaman 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 cephalixin. 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.

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