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Original article

Microbiological assessment of Nile tilapia *Oreochromis niloticus* collected from different super shops and local market in Dhaka, Bangladesh

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Abstract

The present study was conducted to detect the bacterial contamination of Nile tilapia *Oreochromis niloticus* collected from super shops and local market of Bangladesh. The sample specimens were collected from three super shops in Dhaka city *viz.* Agora, Shwapno and Prince Bazar and one local market (Savar Bazar). Samples were analysed to determine some pathogenic bacterial genera through the isolation and enumeration from different organs. The isolates were identified as *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. by conventional culture method, biochemical tests and PCR assay. Total Viable Bacterial Counts (TVBC) were also determined. Fishes were found to be contaminated within a range between 6×10^4 to 1.6×10^6 cfu g^{-1}. The load of *E. coli* and *Shigella* spp. were higher than that of *Salmonella* spp. and *Vibrio* spp. in all the studied samples. The study concludes that more precautions are required for personnel hygiene during fish processing and handling in order to prevent assemblage of pathogenic bacteria in super shops as well as local fish markets of Bangladesh.

Keywords: Microbiological assessment; Nile tilapia; pathogenic bacteria; super shops.

1 | INTRODUCTION

There are a large number of different types of inland water bodies which develop Bangladesh as one of the leading countries of the world for freshwater aquaculture (Islam and Habib 2013). It has been reported that Bangladesh is the third freshwater fish producing country in the world (DoF 2018**).** Fisheries sector contributes 60% of animal protein to the diet and 8% to total export earnings in Bangladesh (Noor *et al.* 2013).

Microbiological quality study of fish has great importance to public health as it is directly related to spoilage of fish and causes food poisoning. Microbial hazards causing infections and poor health which are closely related to food security concerning with animal proteins derived from marketed food such as fish, fishery products, meat and meat products. This poses a burning question for all consumers with high-risk products related to pathogenic bacterial contaminations concerning for food security challenges. Food borne disease results from the ingestion of bacteria and the toxins produced by microorganisms present in the marketed food, and the severity of the

symptoms may vary with the amount of the contaminated food ingested and susceptibility of the individuals to the toxin (Clarence *et al.* 2009; Nilla *et al.* 2012). Bacterial infestation may be considered the most important cause of fish mortality (El Deen *et al.* 2014). Every year a large amount of fish spoils due to the infestation of many pathogenic bacteria including *Aeromonas hydrophila*, *Citrobacter freundii*, *Escherichia coli*, *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Streptococci* spp. and *Staphylococci* spp. (Noor *et al.* 2013). Aquatic habitat and transportation problem is mainly responsible for the microbial contamination in fish (Frazier and Westhoff 1978). Any mishandling of fish and fish products can lead to the transmission of the pathogen to humans (Apun *et al.* 1999).

Tilapia is one of the most common fishes in Bangladesh as well as entire world. Nile tilapia *Oreochromis niloticus* is the most common farmed species around the world and it is the most commercialized fishes because of its high rate of growth and consumer preferences (Mortuza and Al-Misned 2013). For the first time, it was introduced to Bangladesh from Thailand in 1974 by the United Nations International Children Emergency Fund (UNICEF) and later by the Bangladesh Fisheries Research Institute (BFRI) (Rahman 1985; Gupta 1992). These are freshwater species but most of these fishes are able to tolerate a wide range of salinity. It can grow and reproduce in a wide range of environmental conditions and tolerate stress induced by handling (Tsadik and Bart 2007). It is the most important fish in aquaculture after carp and salmon (El-Sayed 2006) and considered a model to reduce the gap in aquaculture nutrition (Kapinga *et al.* 2014). However, microbial association is much common in Nile tilapia and studies have showed that different organs of this species carry a wide variety of bacteria (Mandal *et al.* 2009; Eissa *et al.* 2010).

The super shops in Bangladesh are mainly concentrated in densely populated urban areas. People are highly interested to move on super shops for their daily shopping. Hassle-free shopping environment, fixed price, one stop service, brand image, quality commodities and convenient location are the key factors provoking the customers to visit the super shops. In Bangladesh super shops have special section for fish selling and equipped with ice, display tray, electric balance, uniformed salesman and attractive packaging. Thus, it is believed that the chance of microbial contamination is relatively lower in super shops. On the other hand the local fish market are well-known for the damp and unhealthy environment, poor storage, display and packaging facilities which may be an effective source of microbial contamination (Begum *et al.* 2010).

There is limited data on fish microbial research in Bangladesh. A recent study highlighted a comparative microbiological assessment in some selected fish from two different markets of Dhaka city (Begum *et al.* 2010). Another study by Noor *et al.* (2013) assessed microbiological assessment of major sea fishes found in local markets of Dhaka city. However, none focused on the microbiological study in super shops Tilapia. Therefore, the aim of the present study was to isolate pathogenic bacteria in tilapia collected from super shops and local markets in Dhaka, Bangladesh.

2 | METHODOLOGY

2.1 Sample collection

Adult tilapia (N = 33, mean (± SD) length 28 ± 3.35 cm) were collected from each of the three super shops and Savar local fish market (23°51′30″N 90°16′00″E) between March and May 2018. From every super shop, we collected three sample specimens and all the shops were visited two times during the study periods. From Savar local fish market five sample specimens were collected every time and the market was visited three times. All the samples were collected early in the morning and immediately transported to the Department of Microbiology, Jahangirnagar University, Savar, Dhaka, Bangladesh using an insulated ice box to avoid any change in the quality due to microbial degradation.

2.2 Sample processing

Three different parts (muscles, gills and intestines) were randomly taken to determine the presence of four pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. following standard methods (Okoro *et al.* 2006; Begum *et al.* 2010). Briefly, portion of gill, muscle and intestine were dissected using sharp knife rinsed with 70% ethanol and burnt to prevent cross contamination. Each sample was cut finely under complete aseptic conditions. Then, 1 g of each sample was weighed and transferred into a sterile screw cap tube containing 9 ml of autoclaved distilled water. Mixtures of screw cap tube were mixed properly using a vortex machine for 5 minutes at room temperature. 1 ml of the original dilution (10^{-1}) was transferred to 9 ml of autoclaved distilled water to prepare further decimal dilutions up to 10^{-3}. Then these diluted samples were spread on Nutrient agar, MacConkey agar, Salmonella Shigella (SS) agar and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar for colony count. The media used in this study were selected on the basis of experience of the past researchers (Noor *et al.* 2013; Abbas 2014).

2.3 Enumeration of *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp.

An aliquot of 100 µl of each serial dilution was spread on the petriplate containing different medium using a sterile spreader. Then all plates were incubated at 37°C for 24 to 48 hours after Kapute *et al.* (2012). The colonies of bacte-

ria appeared to be pink, black centred, transparent and yellow or green in the medium after incubation. Following incubation, number of colony was counted in cfu g^{-1} units (Cappuccino and Sherman 2005). Total viable bacterial count (TVBC) was observed in Nutrient agar media.

2.4 Isolation of pure bacterial colonies

To isolate pure colonies of bacteria, streaking was done onto nutrient agar plates. After streaking the plates were incubated at 37°C for 24 to 48 hours.

2.5 Gram staining

Gram staining is a quick procedure used to look for the presence of bacteria in tissue samples and to characterise bacteria based on the chemical and physical properties of their cell wall. Briefly, a loop full of tap water was placed on a slide. A small amount of sample of the colony obtained from fresh overnight culture was transferred to the drop to get a thin smear. Then the sample was air dried. Crystal violet solution was added for one minute and washed briefly with tap water. Slide was flooded with Gram's Iodine solution and allows acting for about one minute and washed with tap water. The smear was then decolorized with 95% ethyl alcohol drop by drop until crystal violet fails to be washed out from the smear. Then slides were flooded with safranin solution and allowed counterstaining for 30 seconds and again washed with water. All the slides of bacteria were examined under the oil immersion lens (100X) using a light microscope.

2.6 Biochemical tests

Biochemical tests were used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. All the isolates were presumptively identified using biochemical methods such as catalase test, citrate utilisation test and triple sugar iron (TSI) agar test.

Catalase test: The catalase test is also one of the main three tests used by microbiologists to identify species of bacteria. It is a basic test to differentiate between gram positive *Staphylococci* and *Streptococci*. Catalase is produced by certain bacteria, which acts as a catalyst in breakdown of hydrogen peroxide into water and oxygen. The tests were conducted following the method as previously described (Bitton and Dutka 1983). Briefly, a small amount of bacterial colony was transferred to a surface of clean, dry glass slide. A drop of 3% H_2O_2 was placed on to the slide and mixed well. A positive result was observed with the rapid evolution of oxygen (within 5–10 seconds) as evidenced by bubbling.

Citrate utilization test: Citrate agar was used to test an organism's ability to utilise citrate as a source of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts as the sole source of nitrogen. When the bacteria metabolise citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromothymol blue indicator in the medium from green to blue above pH 7.6. Simmon's citrate agar media was used in citrate utilisation test (Ghosh *et al.* 2002). Briefly, Simmons citrate agar were inoculated lightly on the slant by touching the tip of a needle to a colony that was 18 to 24 hours old and incubated at 37°C for 18 to 24 hours. A colour change from green to blue along the slant was observed.

TSI test: This test was used to determine if bacteria could ferment glucose and lactose or sucrose and if they could produce hydrogen sulfide and other gases. These characteristics help distinguishing various Enterobacteriacae. On TSI, a yellow slant indicated the organism capable to ferment lactose or sucrose and a yellow butt capable to ferment glucose. Black precipitate in the butt indicated H_2S production. Production of gases other than H_2S was indicated either by cracks or bubbles in the media or the media being pushed away from the bottom of the tube (Cappuccino and Sherman 2005). Briefly, TSI media were allowed to warm at room temperature prior to inoculation. A pure culture of the organism was obtained to be tested. With an inoculating needle, the well-isolated colonies were picked from solid media. Then the centre of the medium was stabbed into the deep of the tube within 3–5 mm from the bottom. Withdrawing the inoculating needle and the surface of the slant was streaked and incubated aerobically at 37°C for 18 to 48 hours. The tubes were observed for acid production of the slant/butt, gas, and hydrogen sulfide reactions (Dorella *et al.* 2006).

2.7 Molecular Characterization

2.7.1 Boil DNA extraction

Freshly cultured bacterial colony was used in every 1.5 ml eppendrof tube containing 500 µl of sterile distilled water and mixed properly by vortex machine. Then the mixture was heated in a heating block (Grant QBT1) at 100°C for 10 minutes. After heating it was cooled at 0°C for 10 minutes. Tubes were then placed in a centrifuge machine and centrifuged for 8 minutes at 13000 rpm. Supernatant from those tubes were then collected by micropipette into new fresh tubes which were used as template DNA and stored at –20°C until further analysis (Dashti *et al.* 2009; Uddin *et al.* 2019).

2.7.2 PCR assay

Universal primers for bacterial 16S rRNA gene 8F (5'-AGT TTG ATC CTG GCT CAG-3') and 1492R (5'-ACC TTG TTA CGA CTT-3') were used for PCR. PCR mix was prepared with the addition of G2 green master mix, nuclease free water, forward primer, reverse primer. 21 µl mixture and 4 µl template (DNA from boiling method) were transferred into the PCR tube. PCR mix was prepared by main-

taining ice-cold condition. After mixing the mixture was centrifuged briefly to spin down the contents. Negative control reaction was also performed (Joshi and Deshpande 2010). The PCR tubes were then placed in a thermal cycler. All the PCR tubes containing mixtures were heated at 94°C for 4 minutes in the thermal cycler to ensure the denaturation of all DNA templates, followed by 94°C for 30 sec, 50°C for 30 sec 72°C for 45 sec and then 36 cycles of these segments were repeated with a final extension of 8 minutes at 72°C. Finally 5µl PCR products were subjected to 1% agarose gel electrophoresis.

2.7.3 Agarose gel electrophoresis

The amplified products were visualized by horizontal electrophoresis in 1% (for 16S rRNA gene) of agarose gel with a 100 bp DNA ladder (Promega, USA) was prepared in 1X TAE buffer. The gel was viewed by UV-Trans-illuminator.

3 | RESULTS

Four genera of bacteria including *E. coli*, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. were isolated from all the studied fish samples. These pathogens from various organs of fish samples were also enumerated. The load of *E. coli* and *Shigella* spp. were higher than that of *Salmonella* spp. and *Vibrio* spp. in fish samples.

3.1 Colony characteristics

On MacConkey agar plate, colonies of *E. coli* were found medium, circular and pink in colour. On SS agar plate colonies of salmonella were found fine, semi-transparent with black centred and colonies of *Shigella* were found medium, rounded and colourless. On TCBS plate, colonies of *Vibrio* were found medium, rounded, yellow and green in colour. Colonies that were found on nutrient agar plate was in great variation including white or yellow in colour and large, small, medium, pinpoint, irregular and rounded shape.

3.2 Total viable bacterial count (TVBC)

The TVBC of different organs of sample in different super shops and local market were ranged from 6×10^4 to 1.6×10^6 cfu g^{-1} (Table 1) and bacterial count was higher in local market than that of super shops (Figure 1).

3.3 Morphological characterization

All isolates were tested for gram reaction and observed under light microscope with 100X magnifications. Immersion oil was also used for clear observation. The samples were gram negative as pink colour was seen because gram negative bacteria retain safranin stain during the decolourisation process (Table 2).

3.4 Biochemical characterisation

All isolates were characterised by biochemical tests. These tests were citrate utilisation test, catalase test and triple sugar iron agar (TSI) test (Table 3).

TABLE 1 Microbiological assessment of fish samples collected from three super shops and a local fish market.

Sampling sites	Source	Microbial count (cfu g^{-1})				Occurrence
		TVBC	*E. coli*	*Shigella* spp.	*Vibrio* spp.	*Salmonella* spp.
Local market	Muscle	1.6×10^6	1.2×10^5	4×10^4	2×10^4	=
	Intestine	1.5×10^5	2.7×10^5	3×10^4	5×10^4	=
	Gill	6×10^4	8.4×10^5	6×10^4	9×10^4	±
Agora	Muscle	4.3×10^5	9×10^4	5×10^4	1×10^4	=
	Intestine	7.8×10^5	2.2×10^5	3×10^4	2×10^4	=
	Gill	3.6×10^5	5.6×10^5	6×10^4	4×10^4	=
Swapno	Muscle	4.7×10^5	7×10^4	3×10^4	3×10^4	=
	Intestine	6.9×10^5	1.9×10^5	4×10^4	2×10^4	=
	Gill	4.7×10^5	6.5×10^5	7×10^4	5×10^4	=
Prince Bazar	Muscle	5.1×10^5	1×10^4	2×10^4	3×10^4	=
	Intestine	7.6×10^5	2×10^4	3×10^4	1×10^4	=
	Gill	2×10^5	1×10^4	7×10^4	1×10^4	=

Total Viable Bacterial Count (TVBC), colony forming unit (cfu), '±' or '=' reveals the presence or absence of *Salmonella* spp.

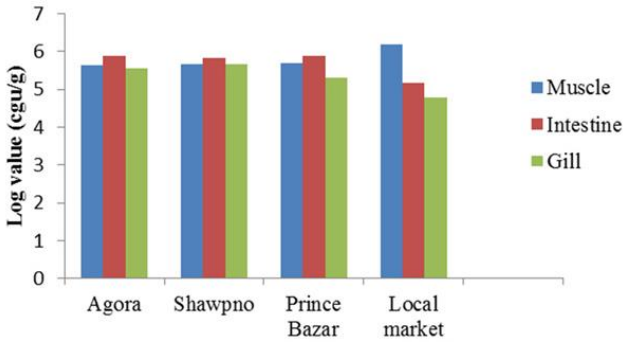

FIGURE 1 Showing comparative analysis of TVBC in three different organs of fish sample collected from super shops and local market.

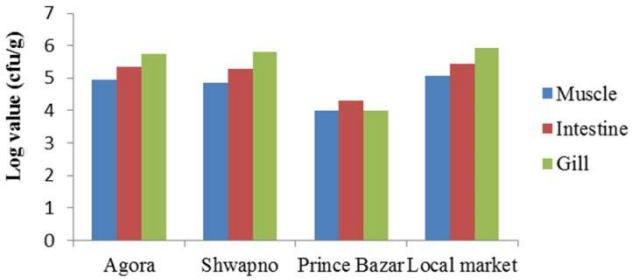

FIGURE 2 Showing comparative analysis of total *E. coli* count in three different organs of fish sample collected from super shops and local market.

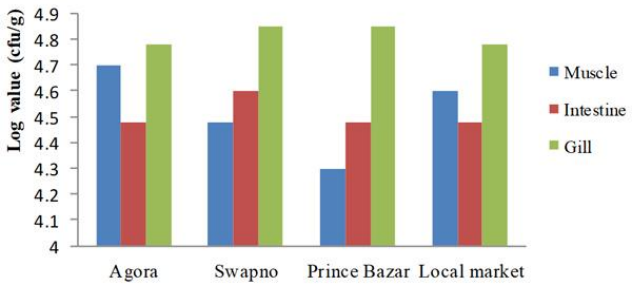

FIGURE 3 Showing comparative analysis of total *Shigella* spp. count in three different organs of fish sample collected from super shops and local market.

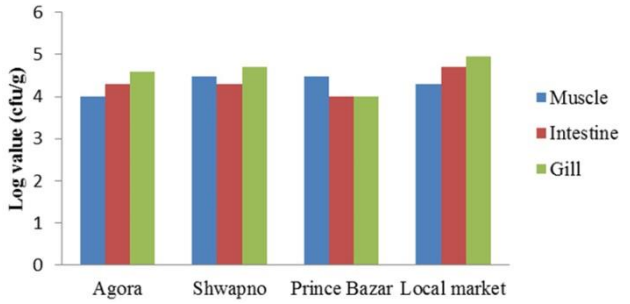

FIGURE 4 Showing comparative analysis of total *Vibrio* spp. count in three different organs of fish sample collected from super shops and local market.

TABLE 2 Colony morphology and microscopic observation of isolates.

Sample ID	Size	Shape	Colour	Texture	Cell form	Gram staining
A	Medium	Round	pink	Smooth	Rod	-
B	Medium	Round	pink	Smooth	Rod	-
C	Small	Round	Pink	Smooth	Rod	-
D	Large	Irregular	Whitish	Mucoid	Rod	-
E	Medium	Round	Colourless	Smooth	Rod	-
F	Large	Round	Black	Smooth	Rod	-
G	Medium	Round	Whitish	Smooth	Rod	-
H	pinpoint	Round	Colourless	Smooth	Rod	-
I	Medium	Round	Blackish	Smooth	Rod	-
J	Pin point	Round	Reddish	Smooth	Rod	-
K	Medium	Round	Green	Smooth	Rod	-
L	Medium	Round	Yellow	Smooth	Rod	-
M	Medium	Round	Yellow	Smooth	Rod	-
N	Medium	Round	Green	Smooth	Rod	-

'–' sign reveals the negative results of Gram staining.

TABLE 3 Biochemical characterization of bacteria isolated from fish samples.

ID	Catalase	Citrate	Triple sugar iron agar test(TSI)			
			Slant	Butt	H_2S	Gas
A	+	-	Yellow	Yellow	-	-
B	+	-	Yellow	Yellow	-	-
C	+	-	Yellow	Yellow	-	-
D	+	-	Yellow	Yellow	-	-
E	+	-	Red	Red	-	-
F	+	+	Red	Yellow	-	-
G	+	-	Red	Yellow	-	-
H	+	-	Red	Yellow	-	-
I	+	+	Red	Yellow	-	-
J	+	-	Red	Red	-	-
K	+	+	Yellow	Yellow	-	-
L	+	+	Yellow	Red	-	-
M	+	+	Yellow	Red	-	-
N	+	+	Yellow	Yellow	-	-

'+' and '–' signs reveal the positive and negative results respectively.

Isolates from different plates were characterised. Based on their characteristics four different genera were identified. After microscopic (morphological) and biochemical characterisation we may presume that isolate A, B, C, and D were *E. coli*, isolate E, G, H and J were *Shigella* spp., isolate F and I were *Salmonella* spp. and isolate K, L, M and N were *Vibrio* spp.

3.4 Molecular Identification of the isolates

Templates DNA from eight isolates were subjected to amplification of their 16S rRNA gene using universal primers 8F and 1492R. The amplified products were visualized in Agarose gel electrophoresis under UV light exhibited amplification of approximately 1484 bp product. 100 bp DNA ladder was used (Figure 5).

4 | DISCUSSION

Fishery products have been recognised as a major carrier of food-borne pathogens (Yücel and Balci 2010). Pathogenic bacteria which are associated with fish and fishery products can be categorised into three general groups (a) indigenous bacteria that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*), (b) enteric bacteria (nonindigenous bacteria) that are present due to faecal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Staphylococcus aureus*), and (c) bacterial contami-

nation during processing, storage or preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp.) (Rehbein and Oehlenschlager 2009).

FIGURE 5: Universal 16S rRNA PCR for isolates. 100 bp DNA ladder marker and negative control was used. Among the 8 isolates, strongly positive results were seen in four isolates.

Most of the cases bacterial count was always higher in local market than that of super shops (Table 1). The total bacterial load of different parts of fish from different super shops and local market ranged from 6×10^4 to 1.6×10^6 cfu g^{-1} or 4.78 to 6.2 log cfu g^{-1}. The results depict that the microbial load in tilapia is higher in local market than the super shops (Table 1; Figures 1–4). Fish in super shops was found with the highest TVBC of 7.8×10^5 cfu g^{-1} and it was 1.6×10^6 cfu g^{-1} for fish from local market. In super shops and local market microbial load was mostly associated with intestine and muscle respectively. Kapute *et al.* (2012) assessed quality of Lake Malawi Tilapia from local and super markets in Malawi and their study revealed that fish from local markets had significantly higher numbers of TVBC (9.5×10^8 cfu g^{-1}), which were above the acceptable limits compared to fish from super markets (2.7×10^5 cfu g^{-1}). Begum *et al.* (2010) conducted another study and found the highest TVBC in super shops compared to local markets. Sewan (1970) reported that processed food and food products are considered to be spoiled when TVBC reaches at 10^6 g^{-1} or more. This TVBC value is commensurate with our findings.

The load of *E. coli* in different organs ranged between 1×10^4 and 8.4×10^5 cfu g^{-1} or 4.00 and 5.92 log cfu g^{-1} which exceeded the limit of 1.0×10^2 cfu g^{-1}. The highest number of *E. coli* was found in the fish sample collected from local fish market (Savar Bazar) and the lowest was recorded in fish from Prince Bazar super shop. The highest *E. coli* in fish from super shops and local markets were found 6.5×10^5 cfu g^{-1} and 8.4×10^5 cfu g^{-1} respectively (Table 1). The data postulates that gill is the top *E. coli* carrier in both super shops and local market except Prince Bazar (Figure 2). Another study reported that the highest number of *E. coli* was found in local markets (9.5×10^7 cfu g^{-1}) and the lowest in super shops (2.2×10^4 cfu g^{-1}) (Kapute *et al.* 2012). Similar results were found in our study. The highest number of *E. coli* in local market may be due to lack of proper hygienic condition. Mandal *et al.* (2009) conducted another study in which they investigated the abundance of coliform bacteria in Nile tilapia sampled from different sources. In their study the highest density of total *E. coli* was measured in the intestine ($1.45\pm0.19\times10^3$ cfu g^{-1}). In our study, the highest *E. coli* measured in the intestine of local fish market and super shops were 2.7×10^5 and 2×10^4 to 2.2×10^5 respectively. Intestine of our fish samples were more contaminated with *E. coli* than those studied by Mandal *et al.* (2009). Although *E. coli* is an inoffensive inhabitant of the gastrointestinal tract, but it also has the pathogenic capacity to cause significant diarrheal and extra intestinal diseases (Croxen *et al.* 2013). So we have to be more careful about this enteric pathogen.

Salmonella is a highly pathogenic genus of bacteria. *Salmonella* food poisoning is only occasionally involved with fish and fisheries (Varnam and Evans 1991). In our study we observed *Salmonella* qualitatively and it was absent in all the fish samples of super shops but observed in gills of fishes collected from local market. According to ICMSF (1986) *Salmonella* spp. should not be present in fish and fishery products. Therefore, it can be said that environment of the local market was not good enough to maintain the health of fish. Begum *et al.* (2010) found that fish samples of local markets were more contaminated with *Salmonella* spp. than fish samples of super shops. Another study showed that load of *Salmonella* spp. in Tilapia samples from super shops and local markets were 2.4×10^3 cfu g^{-1} and 9.6×10^6 cfu g^{-1} respectively (Kapute *et al.* 2012). So, the results of our study highlighted that the environment of super shops is much better than local fish markets to resist the propagation of *Salmonella* spp.

Vibrio is another genus of gastrointestinal microorganism (Al-Harbi and Uddin 2004). Fish infected with *Vibrio* may cause infection to consumers (Begum *et al.* 2010). In our study, the load of *Vibrio* spp. in different parts of fish sample from different super shops and local fish market ranged from 1×10^4 to 9×10^4 cfu g^{-1} or 4.00 to 4.95 log cfu g^{-1} (Table 1). The highest number found in local fish market and the lowest number in super shop Agora (Figure 4). Our data revealed that *Vibrio* is abundant in gill and less in muscle (Figure 4). According to Kapute *et al.* (2012) load of *Vibrio* associated with Tilapia samples collected from super markets and local markets were enumerated as 8.8×10^3 cfu g^{-1} and 4.7×10^7 cfu g^{-1}. Another comparison between samples of local market and super shops were done by Begum *et al.* (2010) and they observed *Vib-*

rio spp. qualitatively. Similarly their study also represented that fish from local markets were more contaminated with *Vibrio* spp. than super shops. International Association of Microbiological Societies recommended that fresh and frozen fish should be free of *Vibrio*. But in our study revealed that microbial quality of both local market and super shops were not so good due to the presence of *Vibrio* spp.

The load of *Shigella* spp. in different organs ranged from 3×10^4 to 7×10^4 cfu g^{-1} or 4.30 to 4.85 log cfu g^{-1} (Table 1). In case of *Shigella* spp. 1×10^2 cfu g^{-1} is the acceptable limit (ICMSF 1986). In our study, values are higher than the acceptable limit. The highest number was found in super shops. Gill was the top carrier and muscle was the lowest carrier of the bacteria (Figure 3). This is the only exceptional organism that was found most in super shops samples. It indicates the lack of proper hygiene in super shops.

The highest number of bacteria found in muscles of local market fish that is highly risky to the consumers because only muscle is the edible part of a fish. But during handling and processing of fish, microorganisms may take entry into human body from the intestine and gill of the fish. The overall results of our study demonstrated that microbial condition of Tilapia from three selected super shops were good in comparison with local market as they showed lower counts in most microbial parameters. The examination also indicates that fish of local market has the chance to transfer various types of pathogens to the consumer.

Availability of enteric pathogenic bacteria species in the fish from both super shops and local markets may be a clear preface of cross contamination due to lack of sanitary standards and mishandling by the salesman (Mhango *et al.* 2010). This study echoed a report by Huss (1995) that the most important element in assuring final product quality of fresh fish between capture and delivery to the consumer is proper handling. Though fishes of super shops have better cold storage facilities, bacterial quality in super shop is better than local market (Begum *et al.* 2010).

The prevalence of microorganisms in fish products might be due to water pollution, fish feed quality, anthropogenic activities, mishandling, transportation and storage condition. In freshwater aquaculture, microbial load may be increased in water by different factors such as quality of the supplied water and environmental conditions.

5 | CONCLUSION

Based on the findings of our study, it can be concluded that fish obtained from both local market and super shops had compromised sanitary and hygiene conditions and not safe to consume since the examined microbial load are always higher than the recommended levels. Therefore, to improve the situation it is imperative to follow the conduct of handling of catch, icing, storage and hygienic measures (Begum *et al.* 2010). Besides, it is necessary to practice HACCAP steps to ensure food security of fish fishery products at local markets and super shops.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available within the article [and/or] its supplementary materials.

REFERENCES

Abbas MS (2014) Isolation of bacteria from fish. International Journal of Advanced Research in Biological Sciences 2(3): 274–279.

Al-Harbi AH, Uddin MN (2004) Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. Aquaculture 229(1–4): 37–44.

Apun K, Yusof AM, Jugang K (1999) Distribution of bacteria in tropical freshwater fish and ponds. International Journal of Environmental Health Research 9(4): 285–292.

Begum M, Ahmed AT, Das M, Parveen S (2010) A comparative microbiological assessment of five types of selected fishes collected from two different markets. Advances in Biological Research 4(5): 259–265.

Bitton G, Dutka BJ (1983) Bacterial and biochemical tests for assessing chemical toxicity in the aquatic environment: a review. Critical Reviews in Environmental Science and Technology 13(1): 51–67.

Cappuccino JG, Sherman N (2005) Microbiology: a laboratory manual. San Francisco: Pearson/Benjamin Cummings. 507pp.

Clarence SY, Nwinyi O, Chinedu SN (2009). Assessment of bacteriological quality of ready to eat food (meat pie) in Benin City metropolis, Nigeria. African Journal of Microbiology Research 3(6): 390–395.

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB (2013) Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews 26(4): 822–880.

Dashti AA, Jadaon MM, Abdulsamad AM, Dashti HM (2009) Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. Kuwait Medical Journal 41(2): 117–122.

DoF (2018) Yearbook of Fisheries Statistics of Bangladesh, 2017–18. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Ministry of Fisheries, 2018. Volume 35: p. 129.

Dorella FA, Pacheco LG, Oliveira SC, Miyoshi A, Azevedo V (2006) *Corynebacterium pseudotuberculosis*: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Veterinary Research 37(2): 201–218.

Eissa NM, El-Ghiet EA, Shaheen AA, Abbass A (2010) Characterization of *Pseudomonas* species isolated from tilapia "*Oreochromis niloticus*" in Qaroun and Wadi-El-Rayan lakes, Egypt. Global Veterinaria 5(2): 116–121.

El Deen AN, Dorgham-Sohad M, Hassan-Azza HM, Hakim AS (2014) Studies on *Aeromonas hydrophila* in cultured *Oreochromis niloticus* at Kafr El Sheikh Governorate, Egypt with reference to histopathological alterations in some vital organs. World Journal of Fish and Marine Sciences 6(3): 233–240.

El-Sayed AFM (2006) Intensive culture. In: Abdel-Fattah M. El-Sayed (Ed) Tilapia culture. London, Cap 5: 70–94.

Frazier WC, Westhoff DC (1978) Contamination, preservation and spoilage of fish and other seafoods. Food microbiology 4: 243–254.

Ghosh K, Sen SK, Ray AK (2002) Characterization of *Bacilli* isolated from the gut of rohu, *Labeo rohita*, fingerlings and its significance in digestion. Journal of Applied Aquaculture 12(3): 33–42.

Gupta MV (1992) Socioeconomic impact and farmer's assessment of Nile tilapia (*Oreochromis niloticus*) culture in Bangladesh (Vol. 805). WorldFish.

Huss HH (Ed) (1995) Quality and quality changes in fresh fish No. 348. Food and Agriculture Organization of the United Nations.

ICMSF (1986) Microorganisms in foods. Sampling for microbiological analysis: principles and specific applications. International Commission on Microbiological Specification for Foods. Blackwell Scientific Publications 2(2): 197–202.

Islam SB, Habib MM (2013) Supply chain management in fishing industry: a case study. International Journal of Supply Chain Management 2(2): 40–50.

Joshi M, Deshpande JD (2010) Polymerase chain reaction: methods, principles and application. International Journal of Biomedical Research 2(1): 81–97.

Kapinga I, Mlaponi E, Kasozi N (2014) Effect of stocking density on the growth performance of sex reversed male Nile tilapia (*Oreochromis niloticus*) under pond conditions in Tanzania. World Journal of Fish and Marine Sciences 6(2): 156–161.

Kapute F, Likongwe J, Kang'ombe J, Kiiyukia C, Mpeketula P (2012) Quality assessment of fresh Lake Malawi tilapia (Chambo) collected from selected local and super markets in Malawi. Internet Journal of Food Safety 14: 112–120

Mandal SC, Hasan M, Rahman MS, Manik MH, Mahmud ZH, Islam MS (2009) Coliform bacteria in Nile tilapia, *Oreochromis niloticus* of shrimp-gher, pond and fish market. World Journal of Fish and Marine Sciences 1(3): 160–166.

Mhango M, Mpuchane SF, Mpuchane BA (2010) Incidence of indicator organisms, opportunistic and pathogenic bacteria in fish. African Journal of Food, Agriculture, Nutrition and Development 10(10): 4203–4218.

Mortuza MG, Al-Misned FA (2013) Length-weight relationships, condition factor and sex-ratio of Nile tilapia, *Oreochromis niloticus* in Wadi Hanifah, Riyadh, Saudi Arabia. World Journal of Zoology 8(1): 106–109.

Nilla SS, Khan MAR, Khan MMR, Ahsan DA, Mustafa MG (2012). Bacteriological quality of marketed mola fish, *Amblypharyngodon mola* from Dhaka metropolis. Bangladesh Journal of Zoology 40(1): 77–88.

Noor R, Acharjee M, Ahmed T, Das KK, Paul L, Munshi SK, Urmi NJ, Rahman F, Alam MZ (2013) Microbiological study of major sea fish available in local markets of Dhaka city, Bangladesh. The Journal of Microbiology, Biotechnology and Food Sciences 2(4): 2420–2430.

Okoro CC, Aboaba OO, Babajide OJ (2010) Quality assessment of a Nigerian marine fish, mullet (*Liza falcipinnis*) under different Storage Conditions. New York Science Journal 3(8): 21–28.

Rahman AKA (1985) Introduction of exotic fishes in Bangladesh. Fisheries Research and Training Centre (booklet). Department of Fisheries, Chandpur, Bangladesh.

Rehbein H, Oehlenschlager J (Eds) (2009) Fishery products: quality, safety and authenticity. John Wiley & Sons.

Shewan JM (1970) Bacteriological standards for fish and fishery products. Chemistry & Industry 6: 193–199.

Tsadik GG, Bart AN (2007). Effects of feeding, stocking density and water-flow rate on fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromis niloticus*. Aquaculture 275: 152–162.

Uddin J, Hossain K, Hossain S, Saha K, Jubyda FT, Haque R, Billah B, Talukder AA, Parvez AK, Dey SK (2019) Bacteriological assessments of foodborne pathogens in poultry meat at different super shops in Dhaka, Bangladesh. Italian Journal of Food Safety 8(1): 6720.

Varnam AH, Evans MG (1991) *Salmonella*, foodborne pathogens, an illustrated text. London: Wolfi.

Yücel N, Balci Ş (2010) Prevalence of *Listeria*, *Aeromonas*, and *Vibrio* species in fish used for human consumption in Turkey. Journal of Food Protection 73(2): 380–384.

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SS sample collection, laboratory works, data analysis, manuscript preparation;
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