INCIDENCE OF ANTIBIOTIC RESISTANT BACTERIA IN FISH SOLD IN LOCAL MARKET IN DHAKA CITY

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Present study was designed to evaluate the microbial contamination in fish (Olive Barb, Bele, Pale-Carplet, Pabo Catfish, Olive, Tangra, Gizzard, Nola, Tatkini, Corica Soborna) samples were collected from the several local market of Dhaka city, Bangladesh. Most of the fish samples were found to be contaminated with huge number of micro-biota within the range of 10^5 to 10^6 CFU/g. The maximum total viable bacterial (TVB) load was estimated observed in Pale Carplet (*Amblypharyngodon mola*) (4.8×10^6 CFU/g) and the lowest count was found (1.5×10^5 CFU/g) in tangra (*Mystus* spp.) *Escherichia coli* and *Klebsiella* spp. were present in olive barb, bele, tangra and gizzard while *Salmonella* spp. and *Vibrio* spp. were present in olive barb, bele, tangra and gizzard fish samples. Moreover, most of the isolated bacteria exhibited resistance against maximum antibiotics like trimethoprime/sulfamethoxazole (25 µg), erythromycin (15 µg), amoxicillin (30 µg), Ceftriaxon (30 µg), ciprofloxacin (5 µg), streptomycin (10 µg), Ampicillin (10 µg). Microbial contamination in the fish samples especially those were resistant to drugs may pose a serious threat to public health.

Keywords: Food safety, Fish pathogen, Drug resistant, Consumer's risk.

INTRODUCTION

Animals and humans have traditionally relied heavily on fish and fish products as a source of nutrition. Being the second-highest source of foreign exchange earnings and employing 10% of Bangladesh's workforce directly or indirectly, the fisheries industry is extremely vital for the economy of Bangladesh (1-3). In addition, consumption of fish offers many health benefits alike expand life span of human being by preventing many diseases like breast cancer and heart diseases (1-4). However, the incidence of foodborne outbreaks brought on by microbial infection or intoxication associated with fish pathogens can pose a serious threat to public health (5-7).

Fish quality generally depends on the microbiological and chemical quality of environments where they grow up (6). Both opportunistic and pathogenic bacteria including *Staphylococcus aureus*, *Salmonella* spp., *E. coli*, fecal coliform and streptococci are directly responsible for different food borne outbreaks (8). As described in several studies that the overall environment of the local markets in Bangladesh is unhealthy. Due to the unhygienic processing and dirty storage condition the microbial contamination in fish is expanding (9, 10). However, in Bangladesh, limited research has been conducted on the microbiological quality of local fish, with a focus on identification of specific pathogens with their antibiotic resistance profiling. Therefore, present study was conducted to find out the overall microbial contamination in different fish samples as well as the drug susceptibility pattern of the isolated bacteria.

MATERIALS AND METHOD

Collection and preparation of samples: For the isolation of fish pathogens ten (10) fish samples (olive, barb, bele, pale-carpletpabo catfish, olive, tangra, gizzard, nola, tatkini, corica soborna) were collected randomly from different local markets of Dhaka city within the time frame from August, 2022 to November, 2022. Samples were collected aseptically early in the morning and taken in sterile sample collection box with ice and transported immediately to the laboratory for further analysis (11). An amount of 25 g of each fish sample was cut out using a sterile knife then sterile mortar was used to grind the fish into small pieces, which then mixed with approximately 250 ml of normal saline. Each sample were diluted up to 10^{-5} following the standard methods by adding a 1 ml aliquot of the crushed sample to 9 ml of normal saline. (11, 12, 13).

Microbiological analysis of each sample: A volume of 0.1 ml from 10^3 dilutions of each sample suspension was spread onto nutrient agar (NA) and Sabouraud dextrose agar (SDA) with appropriate incubation period (NA at 37ºC for 24 hours and SDA at 25ºC for 48 hours) for the enumeration of total viable bacteria (TVB) and fungal count (TF), respectively.

Isolation pathogenic microorganisms: For the isolation of coliform bacteria (*Escherichia coli, Klebsiella* spp.), *Pseudomonas* spp. and *Staphylococcus aureus*, 0.1 ml of each sample from 10^1 was spread over MacConkey (Oxoid Ltd., Basingstoke, Hampshire, England) agar, Pseudomonas agar (Oxoid Ltd., Basingstoke, Hampshire, England) and Mannitol Salt Agar (MSA) respectively. Further plates were incubated at 37ºC for 24 hours to observe the results (12-15).

Isolation of *Salmonella* spp*., Shigella* spp*. and *Vibrio* spp.: The *in-vitro* cultivation of the species of *Salmonella*, *Shigella* and *Vibrio* often appears difficult or with faulty results (false-negative) due to their viable but non-culturable (VBNC) attributes (16, 17). Therefore, enrichment technique was used prior to isolating these bacteria (12, 16, 17). Enrichment was performed for *Salmonella* spp. and *Shigella* spp. in the selenite cysteine broth (SCB). 1 ml of homogenized sample was transferred to SCB followed by incubation at 37ºC for 4 hours and serial dilutions were made up to 10^{-6}, and from 10^{-4} dilution 0.1 ml sample was spread onto *Salmonella Shigella* (SS) agar (Himedia, India) followed by the incubation at 37ºC for 24 hours. For the enrichment of *Vibrio* spp., 0.1 ml of the homogenized sample was transferred to alkaline peptone water (APW) and incubated at 37ºC for 4 hours and serially diluted to 10^{-6} and from 10^{-4} dilution, 0.1 ml was spread onto TCBS (Oxoid Ltd., Basingstoke, Hampshire, England) agar followed by the incubation at 37ºC for 24 hours (16, 17).

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Determination of antibiotic susceptibility pattern of the isolates: Isolates were tested for antibiotic susceptibility on Mueller-Hinton agar (Difco, Detroit, MI) against Trimethoprime/Sulfamethoxazole (25 μg), erythromycin (15 μg), amoxicillin (30 μg), Ceftriaxon (30 μg), ciprofloxacin (5 μg), streptomycin (10 μg), Ampicillin (10 μg), by modified Kirby-Bauer method (12, 14, 19). In 2 ml of Mueller-Hinton broth, a single colony was injected (compared with $10^{\wedge 5}$ cells of McFarland standard) and it was then incubated at 37ºC for 4 hours. The growing cultures turbidity was then adjusted to a 0.5 McFarland standard. After 15 minutes of dipping a sterile cotton swab into the adjusted solution, the surplus broth was removed by forcefully pushing and spinning the swab against the tube's interior above the fluid level. To obtain homogenous inoculums, the swab was then uniformly dispersed across the whole surface of the MHA plate. The plates were left to dry for 10 to 15 minutes. Using sterile forceps, antibiotic-impregnated discs were subsequently placed onto the inoculated plate surface. All the plates were incubated at 37ºC and examined the zone diameters to measure the susceptible, intermediate and resistant pattern of the isolates according to the CLSI guidelines, 2013 (12, 14, 19).

RESULTS AND DISCUSSION

Presence of microorganism in the of fish samples:
In the present study, 10 fish samples were studied to isolate fish pathogens, where all samples were found to exhibit huge load of total viable bacteria within a range of 10^5 to 10^6 CFU/g (Table 1). Total fungi count was always found to be lower and out of 10 samples only 4 samples were contaminated with fungi (range of 10^3 CFU/g which exceeded the standard microbial limit according to the International Commission on Microbiological Specifications for food (ICMSF) (26, 27). The biochemical identification of the isolates from fish samples were presented in Table 2.

In case of specific pathogens *E. coli* and *Klebsiella* spp. were found in 4 samples out of 10 and their count were up to 10^4 CFU/g. Presence of these groups of organisms indicated the faecal contamination though polluted water. Based on the results of the research, most of the local fish samples exceeded the IAMS (International Association of Microbiological Societies) limits for total coliform (100/g) and fecal coliform (11/g), which indicates that most of our fish markets sell low-quality fish (28).

The most alarming issue is *Salmonella* spp. was found in all fish samples collected from local market.

Pseudomonas spp. was found to be present in 6 samples within a range of 10^2 to 10^4 and *Aeromonas* spp. were found only in two samples. On the other hand, *Vibrio* spp. was predominant in most of the samples with in a range of 10^2 to 10^4 CFU/g (Table 1). Some the studies showed that they found the similar amount of microbial contamination in fish caused by the polluted external environment (6, 9).

The study by Nur *et al*., 2020, found that rawest fish samples were contaminated with TVB with the range of 10^5 to 10^6 CFU/g, *Pseudomonas* spp. was predominant (33). Similar results were reported by Hassan *et al*. 2013, Antony *et al*. 2002, and Novotny *et al*. 2004. A number of studies have also reported the presence of *S. aureus* (26, 29-30).

In the present study, fish samples exceed this limit for bacterial count, thereby, demonstrating a substantial risk on the public health. A period of time between harvesting and processing determines the quality of fish and fish products if these are not preserved properly. During this period, the quality of fish continues to deteriorate (30).

Moreover, aseptic handling and applying gutting as soon possible can prevent the growth of pathogenic bacteria and spoilage of fish (31, 32).

Antibiotic sensitivity pattern of bacteria:
Most of the isolates cultivated during the current investigation were found to be resistant against commonly used antibiotics (Table 3). The development of drug-resistance might be due to amazing genetic abilities of microbes, misuse of antibiotic and several epidemiologic influences (21-23). For fish borne disease outbreaks to be effectively managed, such resistance to drugs must be resolved (24, 25). Furthermore, with increased antibiotic resistance and their side effects, using herbal products to eliminate fish pathogens could be a safer, more cost-effective and more effective solution than antibiotics (24, 25).

Table 1: Microbial analysis of fish samples (CFU/g).

Sample	TVB	TF	*E. coli*	*Klebsiella* spp.	*Staphylococcus* spp.	*Pseudomonas* spp.	*Vibrio* spp.	*Salmonella* spp.
Olive Barb	1.2×10^6	2.6×10^3	3.0×10^2	1.1×10^2	4.5×10^3	4.6×10^3	+	+
Bele	3.6×10^6	4.2×10^3	2.2×10^2	0	9.3×10^3	2.6×10^3	+	+
Pale Carplet	4.8×10^6	3.0×10^3	0	3.6×10^2	6.5×10^2	9.8×10^2	-	-
Pabo Catfish	1.2×10^6	3.5×10^3	0	1.4×10^2	1.2×10^3	2.5×10^3	-	-
Olive	1.5×10^5	0	0	0	7.5×10^3	0	-	-
Tangra	1.5×10^5	0	3.0×10^2	0	1.0×10^3	3.0×10^3	+	+
Gizzard	3.0×10^5	0	3.0×10^2	0	6.0×10^3	0	+	+
Nola	1.7×10^5	0	0	TNTC	1.7×10^3	TNTC	-	-
Tatkini	2.0×10^6	0	0	6.0×10^3	8.6×10^3	2.2×10^3	-	-
Corica Soborna	6.0×10^5	0	0	0	3.5×10^4	TNTC	-	-

Note: IAMS limits (100/g) for total coliform and (11/g) for fecal coliform.

Table 2: Confirmative biochemical tests for the isolates.

Assumed Organism	TSI			H₂S reaction	Indole test	MR test	VP test	Citrate test	Catalase	Oxidase test
	Slant	Butt	Gas							
Escherichia coli	Y	Y	+	-	-	-	-	+	+	-
Klebsiella spp.	Y	Y	+	-	-	-	+	+	+	-
Pseudomonas spp.	R	R	-	-	-	-	-	+	-	-
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+
Salmonella spp.	R	Y	-	+	-	+	-	-	+	-

Note: TSI, Triple Sugar Iron Test; Y, Yellow (Acid); R, Red (Alkaline); MR, Methyl Red; VP, Voges-Proskauer.

Table 3. Antibiotic sensitivity pattern of isolates collected from fish samples.

Antibiotics name	*E. coli* (n-4)		*Klebsiella* spp. (n-4)		*Pseudomonas* spp. (n-10)		*Staphylococcus* spp. (n-10)		*Salmonella* spp. (n-4)		*Vibrio* spp. (n-4)	
	R	S	R	S	R	S	R	S	R	S	R	S
Penicillin	75%	25%	25%	75%	80%	20%	70%	30%	75%	25%	75%	25%
Oxacillin	75%	25%	25%	75%	ND	ND	ND	ND	ND	ND	ND	ND
Ampicillin	50%	50%	50%	50%	70%	30%	50%	50%	75%	25%	25%	75%
Amoxicillin	50%	50%	50%	50%	50%	50%	80%	20%	ND	ND	ND	ND
Chloramphenicol	50%	50%	50%	50%	70%	30%	80%	20%	ND	ND	ND	ND
Azithromycin	25%	75%	50%	50%	50%	50%	80%	20%	25%	75%	25%	75%
Erythromycin	25%	75%	50%	50%	60%	40%	50%	50%	25%	75%	50%	50%
Imipenem	25%	75%	50%	50%	40%	60%	30%	70%	75%	25%	75%	25%
Vancomycin	25%	75%	25%	75%	40%	60%	40%	60%	25%	75%	75%	25%
Tetracycline	25%	75%	25%	75%	10%	90%	30%	70%	75%	25%	75%	25%
Streptomycin	25%	75%	25%	75%	10%	90%	20%	80%	75%	25%	ND	ND
Gentamycin	25%	75%	0%	100%	10%	90%	10%	90%	75%	25%	25%	75%

Note: ND, Not Done.

CONCLUSIONS

The current study indicates that the experimental fish sold from the local market did not meet the requirements as most microbial loads were found to exceed the limit values. The presence of multidrug resistance traits among bacterial isolates has also accelerated the threat to public health. Given these results, this study recommended adherence to appropriate guidelines in order to preserve the microbiological quality of fish. To overcome this situation, it is necessary to improve practices for handling, freezing, post-harvest procedures, and storage, including cleaning and hygiene measures, must be applied to the catch. Additionally, appropriate training programs for fish farmers on fish management should be organized to reduce the risk of cross-contamination.

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