ORIGINAL ARTICLE
Stamford Journal of Microbiology, 2025.
ISSN: 2074-5346 (Print); 2408-8846 (Online)
Vol. 15, Issue 1, p. 40-46
DOI: https://doi.org/10.3329/sjm.v15i1.86453

Comparative Microbiological Analysis of Marine Fish Collected from Local Markets and Supermarkets

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Received 09 August 2025/Accepted 02 September 2025

The present study investigated microbial contamination and antibiotic susceptibility of bacteria isolated from marine fish sold in supermarkets and local markets. Two fish species, Rupchanda (*Piaractus brachypomus*) and Red Poa (*Otolithoides pama*), along with environmental samples (ice, shopkeeper's hand swab, and air), were collected from both markets. Microbial loads were measured using conventional culture and biochemical methods, while antibiotic susceptibility was determined with the disc diffusion technique. All fish samples showed high bacterial contamination, with total viable counts ranging from 10^6 to 10^8 cfu/g. Supermarket samples had higher microbial loads than those from local markets. Coliforms were detected in all samples; *Pseudomonas* spp. and *Staphylococcus aureus* were isolated only from the supermarket Red Poa, whereas *Salmonella* spp. was common in local market fish. *Vibrio* spp. was detected in samples from both markets. Environmental samples were also collected, including ice samples from stores, hand swabs of shopkeepers, and air quality, which were also heavily contaminated. Antibiotic susceptibility testing revealed multidrug resistance, with limited effectiveness of common antibiotics such as ciprofloxacin, tetracycline, and gentamicin. These findings demonstrate that both supermarket and local market fish can serve as reservoirs of pathogenic and multidrug-resistant bacteria, posing significant public health risks and underscoring the need for enhanced hygiene practices and stricter monitoring in fish retail environments.

Keywords: Comparative analysis, Marine fish, Local markets, Supermarkets, Environmental sample, Antibiotic resistance

INTRODUCTION

Marine fish are considered a valuable source of high-quality protein, as they contain all the essential amino acids that are necessary for human health (1, 2). Marine fish oil, in particular, is a valuable source of omega-3 fatty acids, and it also contains necessary vitamins. This nutritional value promotes metabolism and helps prevent various diseases (3, 4). Fish is one of the most significant sources of animal protein and other essential nutrients, particularly in developing countries like Bangladesh. Bangladesh is also one of the world's top-ranked countries in fish production. In 2024, the fishery sector contributed 2.53% to national GDP, and in Bangladesh, around 60% of animal protein is supplied from fish (5). In Bangladesh, marine fish are available both in the local market and in supermarkets, which can be suitable for consumption by people (6, 7). However, it's important to ensure that the fish is fresh.

Microbiological quality is crucial for fish and fishery products. However, fish is a highly perishable product that is susceptible to spoilage as soon as it is caught (8). In the context of Bangladesh, the microbial contamination of food and consumer products is excessively common, occurring in disease outbreaks (9). The microbiological quality of fish is dependent upon many factors, including microbial contamination that can occur during harvesting, handling, packaging, processing, distribution, and also a lack of proper hygiene or sanitary practices. Environmental factors, such as water quality and human activities, sanitation of utensils and equipment, market infrastructure, transportation, and temperature abuse, also contribute to microbial contamination in fish. These conditions favour the growth of harmful pathogens, including bacteria, fungi, and parasites, which can lead to severe health issues for consumers (10, 11). Besides, the emergence of antibiotic-resistant bacteria in fish and seafood significantly exacerbates food safety issues. Inappropriate antimicrobial use in aquaculture leads to the development of zoonotic antibiotic-resistant strains such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), Carbapenem-Resistant *Escherichia coli* (CREC), Extended-Spectrum Beta-Lactamase (ESBL)-Producing *E. coli*, Resistant *Salmonella* spp. and Campylobacter strains, and Vancomycin-Resistant *Enterococci* spp. (VRE), which can be transmitted to humans through food consumption (12, 13)

Marine fish available in supermarkets and local markets can contain pathogenic bacteria such as coliform, *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Staphylococcus aureus*, which can pose health risks if consumed (14). These bacteria can cause foodborne illnesses and gastrointestinal symptoms,

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especially in immunocompromised individuals. Coliform bacteria indicate unsanitary conditions, while Pseudomonas spp. can cause spoilage and potential infections. *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. are known pathogens associated with foodborne illnesses, and *Staphylococcus aureus* can produce toxins that cause food poisoning (15, 16). Therefore, it is important to handle and store fish properly to prevent bacterial contamination.

This study aims to perform a comparative analysis of microbial load, Environmental contamination, and antibiotic resistance patterns in marine fish (Rupchanda and Red Poa) collected from local markets and supermarkets in Dhaka city, to evaluate differences in food safety risks.

MATERIALS AND METHODS

Sample collection: The two most common and popular marine fishes, Rupchanda (*Piaractus brachypomus*), and Red Poa (*Otolithoides pama*) were collected from the random local markets and supermarkets in Dhaka city. To analyze their microbial etiology and antimicrobial resistance pattern, as well as environmental samples (hand swab, Air, and ice) were collected from both the supermarket and the local fish market between January 07, 2025, and February 24, 2025. We collected three specimens from three types of body parts for each fish. Together, these parts provide a comprehensive overview of microbial distribution throughout the fish, covering both internal and external contamination routes. After collecting all the specimens, the samples were immediately transported to the laboratory of the Department of Microbiology at Stamford University Bangladesh, using an aseptic zipper plastic bag. The specimens were stored in an insulated icebox to prevent any changes in quality due to microbial degradation.

Sample preparation and inoculation procedure: Fish specimens were sectioned into three distinct parts: head, body, and tail. From each portion, 10 grams of tissue were aseptically collected. Each 10-gram sample was then transferred into 90 mL of sterile normal saline and homogenized thoroughly. The resulting homogenates were subjected to a 10-fold serial dilution up to 10^{-3} using sterile normal saline. From the 10^{-3} dilution, 100 µl of each sample was inoculated onto various culture media. The samples were evenly spread using a sterile spreader until completely absorbed by the media surface.

Enumeration of isolates: From the 10^{-3} dilution, 0.1 ml of each homogenized sample was aseptically spread onto four types of selective and differential media: Nutrient Agar (NA) for total viable bacteria (TVB), MacConkey agar for total coliforms (TCC), Sabouraud Dextrose Agar (SDA) for fungi, and Mannitol Salt Agar (MSA) for *Staphylococcus* spp. enumeration. Plates for TVB and *Staphylococcus* spp. were incubated at 37 °C for 24 hours, while mFC agar plates were incubated at 44.5 °C for 24 hours. Fungal plates (SDA) were incubated at 25 °C for 48 hours (17). To isolate *Escherichia coli* and *Klebsiella* spp., 0.1 ml of each sample was spread onto MacConkey agar and incubated at 37 °C for 18–24 hours. *E. coli* colonies were identified by their dry pink appearance, while *Klebsiella* spp. showed moist pink colonies. For the enrichment of *Salmonella* spp. and *Shigella* spp., 1 ml of the homogenized sample was inoculated into 9 ml of selenite cystine broth. Similarly, enrichment of *Vibrio* spp. was performed by inoculating 1 ml into 9 ml of alkaline peptone water. All enrichment broths were incubated at 37 °C for 6 hours (18). Subsequently, 0.1 ml from each 10^{-1} to 10^{-3} dilution of the enriched broths was spread onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates. These were incubated at 37 °C for 24 hours, and characteristic colonies were then enumerated.

Biochemical tests: All presumptive bacterial isolates were further identified through a series of biochemical tests, selected based on the suspected organisms. These tests included the Triple Sugar Iron (TSI) test, indole test, Methyl Red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, motility test, oxidase test, catalase test, and tube coagulase test. Gram's staining technique was also performed for the morphological identification.

Antibiotic susceptibility testing: All bacterial isolates were subjected to antibiotic susceptibility testing to determine their resistance profiles against ten commonly used antibacterial agents: ampicillin (10 µg), cefixime, doxycycline, ceftazidime, azithromycin, vancomycin, ciprofloxacin (5 µg), chloramphenicol (10 µg), co-trimoxazole (25 µg), and gentamicin (10 µg). A single colony was picked and suspended in sterile normal saline to achieve a 0.5 McFarland standard. Using a sterile cotton swab, the bacterial suspension was evenly spread onto Mueller-Hinton agar plates, following the standardized protocol recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines 2021 (19-21).

RESULTS

Table 1 presents microbial counts (in colony-forming units per gram, cfu/g) in different body parts (head, body, and tail) of two fish species: Rupchanda (Pomfret) and Red Poa. Microorganisms analyzed include total viable bacteria, total coliforms, fungi, *Pseudomonas* spp., *Salmonella* spp., *Shigella* spp., *Citrobacter* spp. *Vibrio* spp., and *Staphylococcus aureus*. Values of '0' indicate no detectable growth. In our present study, microbial loads were assessed in two marine fish species (Rupchanda and Red Poa) collected from both supermarket and local market sources. The total viable bacteria count (TVBC) was observed within 10^7 to 10^8 cfu/g in all specimens collected from the supermarket. On the other hand, TVBC was observed within a range of 10^6 to 10^7 cfu/g in all specimens collected from local markets. Among local market specimens, the highest count of TVBC was found in Red Poa tail ($2.8x10^7$ cfu/g), and the lowest count ($9.9x10^6$ cfu/g) was also found in Red Poa body. On the other hand, in the supermarket specimen, the highest counts of TVBC were found in the Red Poa body (9.9×10^8 cfu/g), while the lowest count was in the tail (1.2×10^7 cfu/g). Local market specimens exhibit comparatively lower TVBC than supermarkets. It indicated the poor handling, transportation, and storage conditions of supermarket fish.

Total coliform (TM) was present in all the specimens of the two fish, collected from both local markets and supermarkets. The highest coliform load, 1.5×10^7 cfu/g, was found in the tail of local market Red Poa, while the lowest, 2.1×10^5 cfu/g, was observed in the body of local market Rupchanda. In the case of the supermarket, the high coliform count is 1.5×10^7 cfu/g in the tail of Red Poa, and the lowest is 1.5×10^5 cfu/g in the tail of Rupchanda fish. Fungal and *Shigella* spp. Microbial growth was absent in all samples. In Rupchanda, *Pseudomonas* spp. and *Staphylococcus aureus* were absent from both the supermarket and the local market sources. But in the case of Red Poa, *Pseudomonas* spp. and *Staphylococcus aureus* were present in the supermarket, whereas, surprisingly, *Pseudomonas* spp. and *Staphylococcus aureus* microbial growth were absent in the local market Red Poa. In case of *Salmonella* spp. The highest microbial count was observed in the local market, Red Poa body, 1.1×10^6 cfu/g, and in the supermarket, the highest microbial count was 5.8×10^3 cfu/g. *Vibrio* spp. present in supermarket red Poa and local market Rupchanda, where microbial count was $4.8x10^5$ cfu/g(head), $4.1x10^5$ cfu/g(body), 5.2×10^5 cfu/g (tail) and $4.0x10^5$ cfu/g(head), 1.0×10^5 cfu/g (tail), respectively. On the other hand, *Vibrio* spp. were absent from supermarket Rupchanda and local market Red Poa.

Table 2 represents the biochemical test results of the isolated microorganisms. The obtained profiles revealed distinct metabolic characteristics that facilitated differentiation at the genus and species levels. Variations in carbohydrate utilization, enzyme activity, and other

physiological traits were consistent with standard identification keys. These biochemical traits provided confirmatory evidence supporting the preliminary identification of the isolates.

Table 1: Prevalence of pathogenic microorganisms in collected marine fish samples from both the supermarket and local market.

Sampling source sites	Fish Samples	Sampling Area	Total Viable Bacteria (cfu/g)	Total Coliform (cfu/g)	Fungi (cfu/g)	*Pseudomonas* spp. (cfu/g)	*Salmonella* spp. (cfu/g)	*Shigella* spp. (cfu/g)	*Vibrio* spp. (cfu/g)	*Staphylococcus aureus* (cfu/g)
Supermarket	Rupchanda (*Piaractus brachypomus*)	Head	2.3×10^7	1.7×10^5	0	0	1.1×10^4	0	0	0
		Body	2.2×10^7	1.4×10^6	0	0	0	0	0	0
		Tail	1.2×10^7	1.5×10^5	0	0	2.0×10^4	0	0	0
	Red Poa (*Otolithoides pama*)	Head	4.0×10^7	2.9×10^6	0	1.5×10^4	0	0	4.8×10^5	4.0×10^5
		Body	9.9×10^8	1.2×10^7	0	1.0×10^3	0	0	4.1×10^5	1.2×10^5
		Tail	3.8×10^7	1.5×10^7	0	2.8×10^4	5.8×10^3	0	5.2×10^5	3.4×10^5
Local market	Rupchanda	Head	1.3×10^7	4.5×10^5	0	0	1.9×10^5	0	4.0×10^3	0
		Body	1.7×10^7	2.1×10^5	0	0	1.1×10^5	0	0	0
		Tail	2.3×10^7	3.4×10^5	0	0	2.0×10^5	0	1.0×10^3	0
	Red Poa	Head	1.3×10^7	8.4×10^5	0	0	0	0	0	0
		Body	9.9×10^6	8.0×10^5	0	0	1.1×10^6	0	0	0
		Tail	2.8×10^7	1.5×10^7	0	0	3.5×10^5	0	0	0

Table 2: Biochemical Identification of the pathogenic isolates.

Isolated Pathogenic Microorganisms	TSI				Indole test	MR test	VP test	Citrate test	Motility test	Oxidase test	Catalase test	Tube Coagulase test
	Slant	Butt	Gas	H_2S								
Escherichia coli	R/Y	Y	+	-	+	+	-	-	+	N/A	N/A	N/A
Klebsiella spp.	Y	Y	+	-	-	-	+	+	-	N/A	N/A	N/A
Citrobacter spp.	R/Y	Y	+	+/-	-	+	-	+/-	+	-	N/A	N/A
Pseudomonas spp.	N/R	N/R	-	-	N/R	N/R	N/R	+	N/R	+	N/A	N/A
Salmonella spp.	R	Y	-	+(Little)	-	+	-	-	+	N/A	N/A	N/A
Vibrio spp.	Y	Y	-	-	+	+	-	+	+	+	N/A	N/A
Staphylococcus aureus	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	+	+

Note: TSI, Triple Sugar Iron Test, Y, Yellow (Acid), R, Red (Alkaline), MR, Methyl red, VP, Voges Proskauer, Citrate, Motility, Oxidase, Catalase, and Coagulase tests. "+" = positive, "–" = negative, "N/A" = not applicable. "N/R" = non-reactive.

Table 3: Microbial load on environmental samples from both the supermarket and local market.

Sample Type		Supermarket	Local Market
Rupchanda	Ice	1.8×10^9	7.3×10^8
	Hand Swab of the shopkeeper	6.7×10^8	4.5×10^8
	Air	3+	3+
Red Poa	Ice	2.5×10^9	3.8×10^8
	Hand Swab of the shopkeeper	3.9×10^8	4.9×10^7
	Air	3+	3+

Note: 3+ = Highly Contaminated (<400 c.f.u.), 2+ = Moderately Contaminated (<200 c.f.u.), 1+ = Mildly Contaminated (<100 c.f.u.).

Table 3 provides information on the microbial load found in environmental samples. The samples were collected from two sources: Rupchanda and Red Poa. The microbial load is measured in terms of the total colony count. The environment is a significant source of microbial contamination in fish (22). In our study, we conducted microbial load analysis on the supermarket and local market environment (including ice, a shopkeeper's hand swab, and air) samples (Table 3). In the case of the supermarket sample, Rupchanda showed a total colony count for ice was $1.8x10^9$, indicating a high level of contamination. The shopkeeper's hand swab yielded a total colony count of $6.7x10^8$, also indicating a high level of contamination. The air sample was marked as 3+, which suggests a highly contaminated environment. Similarly, for Red Poa, the total colony count for ice was $2.5x10^9$ cfu/g, indicating a high level of contamination. The hand swab of the shopkeeper yielded a total colony count of $3.9x10^8$ cfu/g, indicating a high level of contamination. The air sample was marked as 3+, indicating a highly contaminated environment.

On the other hand, in the case of the local market sample, Rupchanda showed a total colony count for ice was $7.3x10^{8,}$ indicating a highly contaminated status. The hand swab of the shopkeeper had a total colony count of $4.5x10^8$ cfu/g, also indicating a highly contaminated status. The air sample received a rating of 3+, which indicates more than 400 cfu/g. For Red Poa, the ice sample had a total colony count of $3.8x10^8$, indicating a high contamination rate. The hand swab of the shopkeeper had a total colony count of $4.9x10^7$cfu/g, also indicating a high contamination rate. The air sample received a rating of 3+, which signifies a highly contaminated status.

In our study, six different bacteria (*E. coli*, *Klebsiella* spp., *Vibrio* spp., *Salmonella* spp., *Pseudomonas* spp., *Staphylococcus aureus*) isolates were presumptively identified and selected to determine their antibiotic resistance patterns against ten different antibiotics. This result is shown in Figures 1-6, respectively. Upon antibiogram profiling, we found that *E. coli* isolated from local market fish samples exhibited high resistance to ceftazidime, azithromycin, vancomycin, ciprofloxacin, co-trimoxazole, and gentamicin compared to isolates from supermarket samples. In the case of *Klebsiella* spp. from supermarket fish, we found higher resistance to all tested antibiotics except ceftazidime, whereas local samples showed high resistance similar to *Vibrio* spp. Supermarket samples are highly resistant compared to the local market sample.

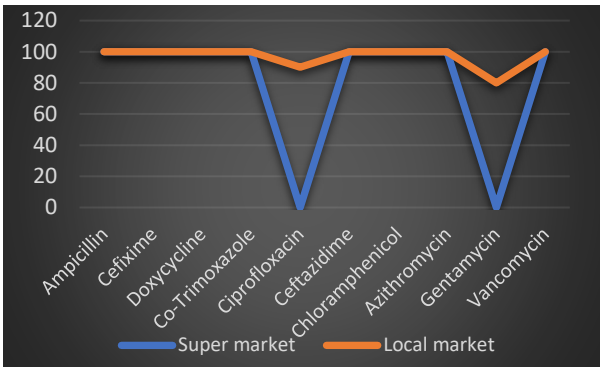

Figure 1: Antibiotic resistance pattern of *E. coli.*

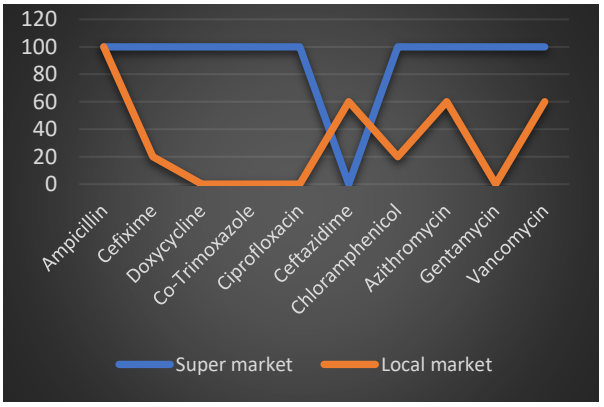

Figure 2: Antibiotic resistance pattern of *Klebsiella* spp.

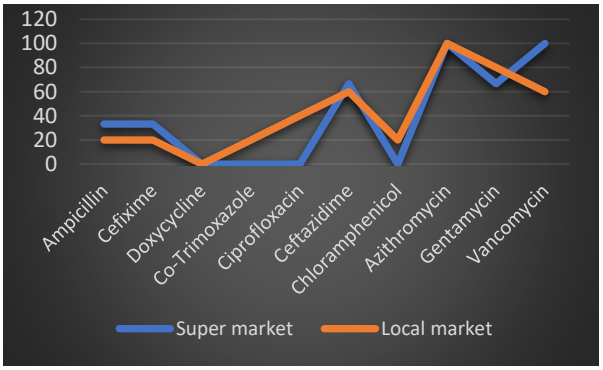

Figure 3: Antibiotic resistance pattern of *Salmonella* spp.

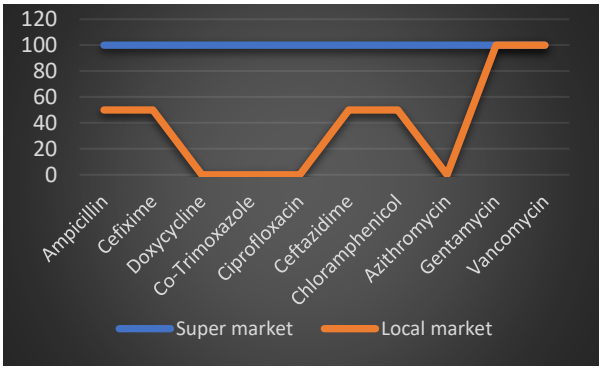

Figure 4: Antibiotic resistance pattern of *Vibrio* spp.

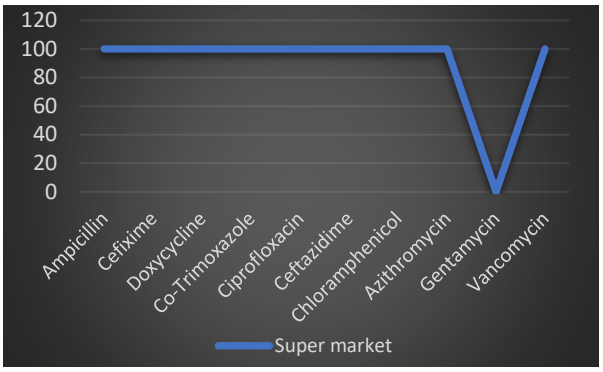

Figure 5: Antibiotic resistance pattern of *Pseudomonas* spp.

Salmonella spp. Showed high resistance to azithromycin and vancomycin across both sources, with no significant difference between supermarket and local market samples. In the case of *Pseudomonas* spp. and *Staphylococcus aureus* found in supermarket fish specimens, and not found in Local market fish specimens. Whereas *Pseudomonas* spp. and *Staphylococcus* spp. Shows 100% resistance against all antibiotics except gentamicin, doxycycline, azithromycin, and chloramphenicol.

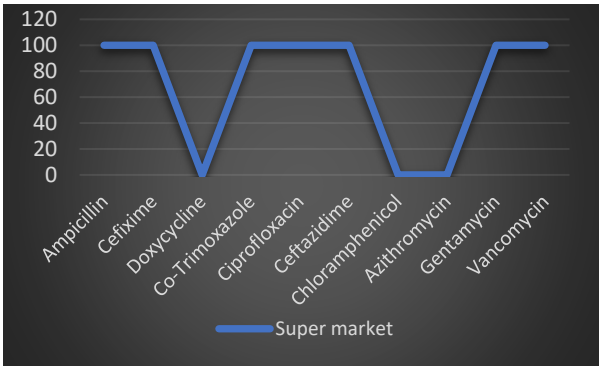

Figure 6: Antibiotic resistance pattern of *Staphylococcus aureus.*

DISCUSSION

Fishery products are significant carriers of foodborne pathogens (23). In our study, in most cases, the bacterial count was always higher in the supermarket than in the local market (Table 1). Fish specimens from the supermarket exhibited the highest Total Viable Bacterial Counts (TVBC), whereas local market fish showed comparatively lower microbial count. Food products or processed foods have been considered spoiled when their TVBC content reaches 10^6 cfu/g or higher (24). The TVBC of all specimens from both the supermarket and local market was found to be beyond the acceptable limit. *Salmonella* spp. is a highly pathogenic genus of bacteria. *Salmonella* spp. is not a usual part of the fish microbial spectrum, but the fish can be asymptomatic carriers, causing possible food poisoning (25). Asymptomatic carriers are infected hosts that can transmit the infection to others, subsequently acting as a silent harbor of the contamination (26). In our study, we observed *Salmonella* spp. showed an elevated count in the local market, both fish, whereas in the supermarket, it showed a low count, and some of these specimens showed no growth. Therefore, we can say that the lack of proper storage conditions and poor environmental hygiene in the local market make it difficult to maintain the physiological status of fish. On the other hand, supermarkets provide a better environment and storage conditions than local markets, which protect *Salmonella* spp. The presence of *Salmonella* spp. in our sample is concerning because they have strong survival strategies against environmental stress, including tolerance to high temperature, as described in a previous study (27). Coliform bacteria are indicators of fecal contamination, which indicate the possibility of any health risks due to the pathogenic bacteria (28). In our study, we observed that coliform found in supermarkets has a higher load than in the local market, but there is not much difference. Water and ice play crucial roles in the processing and quality maintenance of frozen fish (29). Coliform bacteria are present in the supermarket, which may be contaminated during fish processing and handling can also occur from water used for washing or icing. *Vibrio*-infected fish can lead to infection in the consumer. In our study, *Vibrio* spp. Showed a huge count in the Red Poa from the supermarket, whereas the same samples of the local market showed no growth. Interestingly, in Rupchanda, *Vibrio* spp. were present in the local market but absent in the supermarket. It indicates the microbial quality of both the super and local markets is not acceptable in microbiological terms. Similarly, in the case of *Pseudomonas* spp. and *Staphylococcus aureus*, they were surprisingly present at high rates in the supermarket, while they were absent in the local market. *Pseudomonas* spp. in fish samples may reflect possibilities of human pathogens and indicators of food quality as spoilage organisms (30). The presence of these organisms in the supermarket sample may indicate poor storage conditions, a lack of hygiene during processing and handling, whereas none were found in the local market, possibly due to a shorter holding time.

In environmental samples, high microbial load is present in both the super and local market environments, including ice, air, and shopkeeper hand swab samples, which may act as a major source of contamination in fish. The presence of pathogenic microorganisms might be the result of environmental contamination. This highlights the importance of maintaining proper hygiene and sanitation practices to prevent contamination and ensure food safety. Ice and water are the processing materials, and should also maintain the standard quality for the processing of any fish (31). After analyzing the antibiotic resistance patterns, we found that all isolated microorganisms exhibited superbug resistance. Indiscriminate use of antibiotics in fish farms for preservation leads to antimicrobial resistance. Further, resistant bacteria can be transferred into our body through food, which leads to a threat to public health and serious

health hazards, also limiting treatment choices for foodborne diseases. The resistance of bacteria against commonly available antibiotics has also been reported to increase the complications of effective medication (32). To prevent resistance, effective antibiotics need to be improved, alongside preventing the overuse of antibiotics (33). Scientists now consider other options and come up with new sensitive drugs to overcome such conditions (34). The findings revealed no substantial differences between the expensive fish obtained from supermarkets and those collected from local markets. Nonetheless, fish samples from local markets exhibited comparatively superior quality, likely due to their more frequent turnover and regular sale.

The supermarket claimed that their product processing and handling are much better than the local market, and it is safer to consume. But our study showed that supermarket fish exhibit a higher microbial load compared to the local market. So, we can say that there is no significant difference in the quality of fish obtained from the super and local markets. Also, environmental samples collected from both markets showed a high microbial load. Additionally, in our study, among the ten tested antibiotics, ciprofloxacin, tetracycline, amoxicillin, erythromycin, and gentamicin, which are commonly used antibiotics, were found to be resistant against the isolated bacteria. It indicates the government has an urgent need to apply food safety management systems such as Hazard Analysis Critical Control Point (HACCP), Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP), and Sanitation Standard Operating Procedures (SSOP) in both markets. The overuse and misuse of antibiotics for fish preservation has led to the development of multidrug-resistant (MDR) bacteria.

CONCLUSIONS

The results of the current study confirmed that both local and supermarket marine fish are contaminated by microorganisms. Besides, Environmental samples showed high contamination, and isolated pathogens showed superbug resistance against antibiotics. Overall, this result indicates that fish from both local and supermarket environments, as well as those with poor hygiene conditions, are not safe to consume. In addition, applying HACCP, GMP, GHP, and the SSOP approach is necessary to ensure food security for fish products at local and supermarket. The study has several limitations. Therefore, surveillance is important for monitoring microbial contamination in both market fish products. And it helps to track the spread of pathogens, identify potential outbreaks, and evaluate the effectiveness of safety and security approaches (35). One limitation is the small sample size and the molecular detection of this bacterium. Future research should focus on a large and more diverse sample. In addition, future studies should conduct a comparative study between raw and cooked fish samples to identify whether cooking can reduce microbial load.

CONFLICTS OF INTEREST

The authors have declared that no competing interests exist.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Microbiology, Stamford University Bangladesh.

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