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Assessment on the microbial safety status of fish products at fish retail areas in Addis Ababa, Ethiopia

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Abstract
Fish is rich in quality protein with high bioavailability of essential amino acids, essential fatty acids, and minerals. However, improper fish handling can result in contamination and potentially making it harmful for consumption. This study planned to assess the microbial safety status of fish sold at fish shops and supermarkets in Addis Ababa. A total of 56 samples (38 from fish shops and 18 from supermarkets) were collected. Determination of total viable count and coliforms count was performed by the standard plate count method. Characterization of the bacterial isolates was also done using biochemical techniques. The mean total viable count ranged from 5.77 ± 0.26 log CFU/g recorded from supermarket samples to 9.08 ± 0.043 log CFU/g from shop samples. Staphylococcus spp., Enterobacter spp., Proteus spp. and Bacillus spp. were identified from the specimen. The highest and least value of TVB-N were 76.00 ± 1.38 and 44.37± 1.35 mg/100g, respectively whereas, the highest and least value of pH were recorded approximately 7.24 ± 0.00 and 6.58 ± 0.02, respectively. Despite the absence of potential fish pathogens, this finding revealed the inadequacy of existing hygienic practices. Therefore, it is strongly recommended to cook fish products purchased from Addis Ababa fish markets sufficiently before consumption.

Keywords: Fish, perishable foods, Addis Ababa, food spoilage, microorganism, pathogenic bacteria

1. Introduction
Fish is one of the most perishable food products during handling and storage. Quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product. Raw fish is a highly perishable commodity compared to other fresh meat commodities and has a short lifetime even at refrigeration temperature (Lauzon *et al*., 2010) [1]. Spoilage is the degradation of food until it becomes unfit for human consumption. The most prevalent cause of food spoilage is microbial growth and residence in the food, which results in numerous undesirable metabolites being produced in the food that cause unwanted flavors and odors (Gram and Dalgaard, 2002) [2].

According to Parker (2000) [3], the current world food post-harvest and/or slaughter losses due to microbial spoilage estimates is about 25% of the total food produced. This is a big loss that can be reduced if the ecology of specific spoilage organisms is well understood and controlled. A spoiled food has lost the original nutritional value, texture, or flavor and can become harmful to people and unsuitable to eat. The microbial spoilage of food products constitutes an important economic problem, as it results in high economic losses for the food industry, especially under incorrect refrigeration conditions.

Spoilage of fish is mainly due to the activity of psychotropic gram-negative bacteria such as *Shewanella putrefaciens* and *Pseudomonas* species (Huss, 1995) [4], and other mesophilic groups *like Salmonella species*, *Staphylococcus species*, *Enterobacter species*, and *Escherichia coli* which can be introduced into foods during processing from the air, unclean hands, unsanitary utensils and equipment (Framatico *et al*., 2005) [5].

Compared to other foods, fish is unique as a substrate for microbial growth. This uniqueness stems from several important factors: a high post mortem pH in the flesh of fish (typically greater than 6.0) and high moisture content which favor growth of a wide range of microbes coupled with their poikilothermic nature (Herbert *et al*., 1971) [6], volatile nitrogen bases (ammonia, creatine, taurine, uric acid, carnosine, and histamine) which support post mortem bacteria growth (Jay *et al*., 2005) [7], the presence of non-protein-nitrogen (like free amino acids) in large quantities, and the presence of trimethylamine.

Microbiological and chemical methods are used to estimate the quality of fresh fish measure or evaluate parameters that change, disappear, or formed during the deterioration of fish (Huss, 1995) [4]. Fish spoilage essentially can be attributed to three main factors namely; microbial, enzymatic, or autolytic, and chemical spoilage (oxidative rancidity) of which microbiological contamination has been noted as the main cause of fish deterioration. There is no study on microbial identification of fish sold in Addis Ababa. Therefore, this study was carried out to identify pathogenic and spoilage bacteria and evaluate fish spoilage indicators to assess the quality and safety status of fish sold in Addis Ababa.

2. Materials and Methods

2.1 Sample collection

A total of 56 fish samples were collected from Addis Ababa fish retailers as indicated in (Table 1). Samples were aseptically collected in polyethylene bags and labeled with date and place of collection. Iceboxes filled with ice were used to store the samples during transportation to the National Fishery and Aquatic Life Research Center (NFALRC) laboratory. All the samples were delivered to the laboratory in one hour.

Table 1: Sample description

Retail market	Code	Description	Number of samples
Supermarket	SO	Solomon supermarket	2
	BE	Berta supermarket	2
	SH	Shisolomon supermarket	2
	FI	Firdos supermarket	2
	SE	Seven-eleven supermarket	2
	SM	Save more supermarket	2
	FA	Fantu supermarket	2
	NE	Negash supermarket	2
	TI	Titi supermarket	2
	LO	Loyal supermarket	2
	AT	All mart supermarket	2
	SY	Safeway supermarket	2
	QE	Queens supermarket	2
	AD	Abadir supermarket	2
	AS	Addis supermarket	2
Fish shops	LB	Bole sub-city	3
	AL	Kaliti sub-city	3
	DA	Arada sub-city	3
	KA	Addis Ketema sub-city	3
	KF	Kolfe Keranio sub-city	3
	UG	Gulele sub-city	3
	NA	Nifasilk lafto sub-city	2
	KO	Kirkos sub-city	3
	KY	Yeka sub-city	3

2.2 Sample preparation

Samples from different branches of the same supermarket were combined into one sample. Similarly, samples from shops were combined into one sample based on the sub-cities where the shops were located. Then, the samples were ground using a sterilized mortar and 1g of each sample was aseptically introduced into 9ml of peptone water in a universal bottle, to give 10^{-1} dilution and three subsequent serial dilutions were prepared in test tubes by transferring 1 ml to 9 ml of peptone water as described by the methods of (Andrews, 1992) [8].

2.3 Total viable count (TVC)

Plate Count Agar (PCA) was used to determine the total microbial load of fish. Using separate sterile pipettes, decimal dilutions of 10^{-1} to 10^{-5} of food homogenate was prepared by transferring 1 ml of the previous dilution to 9 ml of sterile peptone water. The dilutions were mixed by shaking 25 times in 30 cm (1 ft) arc within 7 seconds. Then 1 ml of appropriate dilution was transferred into separate, duplicate, appropriately labeled, and sterilized Petri dishes. Then, plate count agar was cooled to 45 ± 1°C and 15-20 ml of the medium was added to each plate within 15 min of the original dilution. After that, the sample dilutions and agar medium were mixed thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on a flat level surface. Then, the agar was left to solidify for 15 minutes and the solidified Petri-dish was inverted and incubated promptly for 48 ± 2 h at 35°C. Finally, the number of colonies was counted and multiplied by the dilution factor to calculate the total colonies forming units per gram of sample (Andrews, 1992) [8].

2.4 Total coliforms count (TCC)

Violet red bile agar was used to determine total coliform bacteria according to APHA (1972) [9]. From the homogenate, 1 ml of serially diluted (10^{-1}, $10^{-2,}$ and 10^{-3}) sample was transferred into 9 ml sterilized peptone broth in test tubes. The dilutions were mixed by shaking 25 times in 30 cm (1 ft) arc within 7 seconds. Then 1 ml of appropriate dilution was transferred into separate, duplicate, appropriately marked, and sterilized Petri dishes. Then, 15-20 ml of the medium was added to each plate within 15 min of the original dilution. After that, the sample dilutions and agar medium were mixed thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on a flat level surface. Then, the agar was left to solidify for 15 minutes and the solidified petri-dish was inverted and incubated promptly for 24h at 32 °C. Finally, the number of colonies was counted and multiplied by the dilution factor to calculate the total colonies forming units per gram of sample.

2.5 Culturing of samples

Enterobacter, *E. coli*, *staphylococcus*, and *pseudomonas* suspected samples were cultured on blood agar, whereas *salmonella* suspected samples were pre-enriched on buffered peptone water and both were incubated aerobically at 37ºC for 24 hrs.

2.6 Sub-culturing of the cultured samples

After 24 hours incubation period, the cultured samples on blood agar were again sub-cultured on another blood agar and incubated aerobically at 37ºC for 24 hours and those pre-enriched samples were then enriched on Rappaport-Vassiliadis (RVS) media and incubated aerobically at 42 ºC for 24 hours. After an hour incubation period, salmonella suspected colonies were sub-cultured on xylose lysine desoxycholate (XLD) media and incubated aerobically at 37 ºC for 48 hours. Then, suspected *salmonella* colonies were sub-cultured on urea media and incubated aerobically at 37 ºC for 24 hours.

2.7 Isolation, Characterization, and Identification of Bacteria

After repeated culturing, all were isolated and again sub-cultured on nutrient agar to get pure colonies for further studies. Then biochemical tests such as catalase, oxidase,

citrate utilization, indole production, methyl-red and Voges-Proskauer, urease, fermentation, motility, growth on MacConkey, triple sugar iron was done according to (Holt *et al*., 1994) [10].

2.8 Gram staining

Gram staining was performed for all isolated colonies according to the standard procedure. A smear of bacterial cells was prepared on a clean glass slide by a gentle heat fixation. The heat-fixed smear was flooded with crystal violet solution for one minute. Smear was washed with water followed by adding mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and rinsed with water. Finally, safranin was used as counterstains for 60-80 seconds and washed with water. Then, the gram reaction, shape, arrangement, and size of the cells were examined under the microscope with an oil immersion objective lens (x100).

2.9 Determination of Total Volatile Basic-Nitrogen (TVB-N)

Fish extracts for determination of Total Volatile Bases Nitrogen (TVB-N) were prepared by homogenizing 100 g of fish sample with 200ml of 7.5% (w/v) aqueous Trichloroacetic Acid (TCA) solution in a laboratory homogenizer for 1 min at high speed. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant liquid was then filtered through what man No. 1 filter paper. Then, TVB-N was measured by steam-distillation of the TCA-fish extract using the method of Malle and Tao (1987) [11]. Then, 25ml of the filtrate was added to a Kjeldahl-type distillation tube, followed by 5 ml of 10% (w/v) aqueous NaOH solution. Then steam-distillation was performed using a vertical steam distillation unit and the distillate was received into a beaker containing 10 ml of 4% (w/v) aqueous boric acid and 0.04 ml of methyl red and bromocresol green indicator solution. Then distillation was continued until a final volume of 50 ml was obtained in the beaker (40 ml of distillate). The boric acid solution was turned to green when alkalinized by the distilled TVB-N. The titration was allowed to run against an aqueous 0.1N sulfuric acid solution. Complete neutralization was obtained when the color turned pink on the addition of a further drop of sulfuric acid. The quantity of TVB-N in mg was determined from the added volume of sulfuric acid.

$$\text{TVB} - \text{N mg}/100\text{g} = \frac{(14\text{mg/mol x A x B x 300})}{25\text{ml}}$$

Where; A = ml of sulphuric acid and B = normality of sulphuric acid.

2.10 Measurement of pH

Before measurement, the pH meter was calibrated using pH 4 and 7 buffers. Ten grams of fish sample was homogenized in 50 ml distilled water in the ratio of 1:10 (w/v) using a laboratory warring blender. The pH of the fish specimens was measured according to (Naveena *et al*., 2001) [12].

2.11 Statistical analysis

Log-changed microbial populations, pH, and TVB-N were analyzed with one-way analysis of variance (ANOVA) and descriptive statistics. For the assessment of fish samples, log-transformed values obtained from triplicate samples were averaged before the analysis. Means and standard errors (SE) of three replications were used and differences were considered significant at $p < 0.05$.

3. Results and Discussion

The result of TVC, TCC, TVB-N, and pH measurements for fish shops and supermarkets are respectively given in (Table 2), and (Table 3). The presented values indicate the mean ± standard deviation of triplicate measurements.

Table 2: Rate of bacterial contamination, TVB-N and pH values in fish shops

Shop ID	Parameters			
	TVC	TCC	pH	TVB-N (mg N/100g)
LB	8.40 ± 0.03^{b}	6.30 ± 0.03^{a}	6.66 ± 0.05^{c}	61.70 ± 0.84^{f}
AL	6.75 ± 0.20^{cde}	3.63 ± 0.25^{d}	6.79 ± 0.02b^{c}	66.20 ± 0.46^{d}
DA	6.53 ± 0.10^{de}	4.20 ± 0.03^{c}	6.99 ± 0.04^{a}	70.65 ± 0.49^{a}
KA	9.08 ± 0.04^{a}	5.33 ± 0.12^{b}	6.68 ± 0.02b^{c}	63.60 ± 0.58^{e}
KF	6.95 ± 0.03^{cd}	0.00 ± 0.00^{f}	7.14 ± 0.07^{a}	69.42 ± 0.45^{ab}
UG	7.15 ± 0.03^{c}	3.58 ± 0.16^{d}	7.01 ± 0.06^{a}	66.70 ± 0.23^{cd}
NA	6.45 ± 0.03^{e}	1.40 ± 0.12b^{c}	6.88 ± 0.02^{c}	56.05 ± 0.61^{g}
KO	6.00 ± 0.23^{f}	3.85 ± 0.26^{cd}	6.82 ± 0.02^{b}	48.70 ± 0.46^{h}
KY	6.95 ± 0.26^{cd}	0.00 ± 0.00^{f}	7.07 ± 0.08^{a}	68.22 ± 0.23^{bc}
%CV	13.67	68.33	2.75	10.81

TPC = Total plate count, TCC = Total coliform count, pH = power of hydrogen, TVB- N = Total volatile basic nitrogen. Mean values in the above table indicate the result of triplicate experiments which were reported as mean ± SE. Means in the same column with the same treatment categories assigned by different letters are significantly different as judged by LSD at $P \leq 0.05$.

Total viable count (TVC)

A high bacterial load was recorded in samples from fish shops (Table 2) with a total viable count ranging from 6.00 ± 0.23 to 9.08 ± 0.04 CFU/g. Similarly, samples from supermarkets (Table 3) showed a high load of bacterial count ranging from 5.77 ± 0.26 CFU/g to 7.00 ± 0.00 CFU/g, respectively in QE and FA supermarkets. Similarly,

Table 3: Rate of bacterial contamination, TVB-N and pH values in supermarkets

Supermarket ID	Parameters			
	TVC	TCC	pH	TVB-N (mg N/100g)
SO	6.40 ± 0.29^{abcd}	4.85 ± 0.03^{ab}	6.58 ± 0.02^{i}	59.17 ± 1.82^{e}
BE	6.30 ± 0.29^{abcd}	3.85 ± 0.03^{e}	6.87± 0.04^{ef}	71.40 ± .81^{ab}
SH	6.60 ± 0.00^{abc}	4.65 ± 0.03^{bc}	6.86 ±0.02^{ef}	65.17 ± 2.4^{d}
FI	5.90 ± 0.29^{cd}	4.50 ± 0.00^{cd}	6.87 ± 0.01^{ef}	69.47 ± 1.13^{bcd}
SE	6.20 ± 0.29^{bcd}	2.45 ±0.03^{h}	7.13 ± 0.01^{b}	59.10 ± 1.33^{e}
SM	6.50 ± 0.00^{abcd}	2.80 ± 0.00^{g}	7.08 ± 0.29^{b}	72.50± 1.79^{ab}
FA	7.00 ± 0.00^{a}	4.25 ± 0.03^{d}	6.80 ± 0.03^{h}	59.07 ± 1.76^{e}
NE	6.20 ± 0.29^{bcd}	2.70 ± 0.00^{gh}	6.90 ± 0.01^{de}	73.00 ± 2.54^{ab}
TI	6.80 ± 0.16^{ab}	5.00 ± 0.00^{a}	6.75 ± 0.01^{h}	65.60 ± 0.92^{cd}
LO	6.20 ± 0.29^{bcd}	4.70 ± 0.00^{bc}	6.83 ± 0.01^{fg}	51.47 ± 1.99^{f}
AT	6.35 ± 0.32^{abcd}	4.30± 0.03^{d}	7.24 ± 0.00^{a}	50.40 ± 1.21^{f}
SY	6.05 ± 0.26^{bcd}	3.10 0.00^{f}	6.97 ± 0.01^{c}	73.00 ± 1.15^{ab}
QE	5.77 ± 0.26^{d}	4.25 ± 0.03^{d}	6.94 ± 0.01^{cd}	70.27 ± 1.36^{bc}
AD	6.45 ± 0.03^{abcd}	3.80 ± 0.00^{e}	6.81 ± 0.04^{fg}	76.00 ± 1.39^{a}
AS	6.55 ± 0.03^{abc}	2.75 ± 0.03^{g}	7.10 ± 0.01^{b}	44.37± 1.36^{h}
%CV	7.08	22.46	2.41	15.17

Mean values in the above table indicate the result of triplicate experiments which were reported as mean ± SE. Means in the same column with the same treatment categories assigned by different letters are significantly different as judged by LSD at $P \leq 0.05$.

The finding indicates a high load of bacteria with a considerable difference ($p<0.05$) between most of the fish shops. In other studies, a similarly high load of bacterial count has been reported from different fresh fish samples. For instance, Pao *et al.*, (2008) [13] reported a total viable count ranging from 3.84 to 8.23 log CFU/g while Eizenberga *et al.*, (2015) [14] recorded a total viable count ranging up to 7.57 log CFU/g. Based on the International Commission for Microbiological Specifications for Foods (ICMSF, 1998) [15], which suggests acceptance of samples that contain a total viable count below 7 log cfu/g, 37.5% of the fish samples from shops fail to meet the initial requirement. On the other hand, all samples from supermarkets recorded ≤ 7 log cfu/g TVC, which is acceptable according to ICMSF. High load of total bacterial load could be an indicator of a possible risk of spoilage and/or safety that could further affect the health of consumers since all the different bacteria has a varying effect (Gram *et al.*, 2000) [16]. This phenomenon could occur as a result of unhygienic handling practices along the supply chains of the fish.

3.2 Total Coliforms Count (TCC)

Samples from fish shops (Table 2) showed relatively lower TCC than fish samples from supermarkets. Coliforms count of samples from supermarkets (Table 3) was in a range between 2.45 ± 0.03 to 5.00 ± 0.00 CFU/g. In samples from some fish shops, no TCC was recorded while the remaining shops showed high TCC that reach up to 6.30 ± 0.03 CFU/g. Among the samples from fish shops, total coliforms were not recorded in two shops and the remaining shops had shown considerably different ($p<0.05$) count of TCC. In contrast to samples from fish shops, all samples from supermarkets recorded a range of TCC. The presence of coliforms in the majority of the samples could be due to environmental contamination such as washing water, abuse of temperature, and relative humidity during handling and storage of the fish. A previous study by Ananchaipattana *et al.*, (2012) [17] also observed the presence of coliforms in all samples from both supermarkets and open markets in Thailand. The absence of coliforms in two of the fish shops, while all samples from supermarkets showed counts of coliforms, could not be only because of the proper handling at the fish shops like some other supermarkets, but it could be due to good handling practice throughout the supply chain.

3.3 Total Volatile Base-Nitrogen (TVB-N)

A high level of TVB-N was recorded in both supermarket and fish shop samples. The recorded TVB-N content in supermarkets was in a range from 44.37± 1.36 to 76.00 ± 1.39. Comparably, samples from fish shops showed TVB-N content ranging from 48.70 ± 0.46 to 70.65 ± 0.49. TVB-N is an indication for the level of bacterial decomposition since it is produced by the action of bacteria on the fish flesh. Hence the observed high level of TVB-N, 63.48 and 64 mg /100 g in fish shops and supermarkets respectively is related to the presence of a high total viable count in both sampling locations.

Significant variations were also observed within the different fish shops and supermarkets indicating variation between the different samples which could be due to the difference in species, storage period, or environmental factors. The recorded TVB-N level is by far higher than a previous finding by Chytiri *et al.*, (2003) [18] who found a maximum level of 26.06 mg/ 100g in rainbow trout samples stored at the refrigerated condition for 18 days. In contrast, a study by Jianadasa *et al.*, (2014) [19] observed a highly varying range of TVB-N ranging from 16 to 234 mg/ 100 g in Yellowfin tuna and 16 to 1021 mg/100 g in sailfish samples collected from the retail market in Sri Lanka. The observed variation between the different studies could be due to the variation of the species and environmental factors.

Table 4. Biochemical characterization of bacteria isolated from Fish

Retail market	Sample ID	Catalase	Oxidase	Motility	Growth on MacConkey	Citrate	Indole	MR/ VP	Urease	Identification
Fish shops	AL	+	-	+	-	-	-	+/-	+	*Proteus* spp.
		+	-	+	-	-	-	+/-	-	*Staphylococcus* spp.
		+	-	-	-	-	-	+/-	+	*Proteus* spp.
		+	-	-	-	-	-	+/-	-	*Proteus* spp.
	KA	+	-	-	-	-	-	+/-	+	*Proteus* spp.
	KF	+	-	-	-	-	-	+/-	+	*Proteus* spp.
	UG	+	-	-	-	-	-	+/-	+	*Staphylococcus* spp.
	KO	+	-	-	-	-	-	+/-	+	*Staphylococcus* spp.
	DA	+	-	-	-	-	-	+/-	+	*Proteus* spp.
	NA	+	-	-	-	-	-	+/-	-	*Proteus* spp.
		+	-	+	-	-	-	+/-	+	*Proteus* spp.
	LB	+	-	-	-	-	-	+/-	-	*Proteus* spp.
		+	-	-	-	-	-	+/-	-	*Staphylococcus* spp.
		+	-	-	-	+	-	+/-	-	*Staphylococcus* spp.
	KY	+	-	-	-	+	-	+/-	-	*Staphylococcus* spp.
Supermarkets	SO	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.
	BE	+	-		-	+	-	-/+	-	*Enterobacter* spp.
	SH	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.
	FI	+	-	+	-	+	-	+/-	-	*Bacillus* spp.
	SE	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.
	SM	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.
	FA	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.
	NE	+	-	+	-	+	-	-/+	-	*Enterobacter* spp.
	TI	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.
	LO	+	-	+	-	+	-	-/+	-	*Enterobacter* spp.
	AT	+	-	+	-	+	-	-/+	-	*Enterobacter* spp.
	SY	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.
	QE	+	-	-	-	+	-	+/-	-	*Bacillus* spp.
	AD	+	-	-	-	+	-	+/-	-	*Bacillus* spp.
	AS	+	-	-	-	+	-	-/+	-	*Enterobacter* spp.

3.4 Biochemical Characterization of the Bacterial Isolates

Biochemical characterization result for both fish shops and supermarket samples of the bacterial isolates is given in (Table 4). As can be seen in the table, the most prevalently observed bacterial isolates in fish shops were *proteus* spp. followed by *Staphylococcus* and *Bacillus* species respectively with 58%, 38.7%, and 3.2% occurrence. On the contrary, *Enterobacter* spp. and *Bacillus* spp. were the only isolates from samples of supermarkets with an average occurrence of 80% and 20% respectively. A previous study by Ananchaipattana *et al.*, (2012) [17] identified *Bacillus* spp. in 39% of fish samples from open markets and 30% of samples from supermarkets in Thailand.

4. Conclusion

The present study indicates the bacteriological quality of fish sold at supermarkets and fish shops in Addis Ababa. Based on information obtained from this study, fish from supermarkets are less contaminated by bacteria than fish from fish shops. *Protease* spp. and *Staphylococcus* spp. were found as the most prevalent species in fish shops while *Enterobacter* spp. were predominantly found in fish samples from supermarkets. The presence of these microbial isolates could be due to inadequate hygienic practice, poor handling, transportation, and conditions of storage. The bacteriological quality can be improved by implementing hygienic handling practices across the supply chain particularly during transportation and ensuring an uninterrupted cold chain from catch to table. The cold chain is not efficiently maintained across the fish supply chain in Ethiopia due to several challenges such as lack of basic inputs like ice and refrigerated trucks. Despite the existing challenges, responsible government authorities should make stringent control over the quality and safety of fish in the market. Existing national and international standards should be implemented by the retailers and wholesalers, and this should be checked by the regulatory bodies. The finding of this study can be used to understand the scale of food safety status in the Addis Ababa fish marketing area.

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