Consumer health risk assessment of coliforms and associated bacterial pathogens in Nile tilapia (*Oreochromis niloticus*) from Lake Hawassa, Ethiopia

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Abstract

Fish is the most essential source of protein for humans and has high nutritional value. However, fish and fishery products have been documented as carriers of food-borne bacterial pathogens, and the presences of coliforms are used as indicator for the possible presence of these pathogens. Therefore, this study aimed to evaluate the loads of coliform and the prevalence of potential bacterial pathogens in Nile tilapia (*O. niloticus*), and the health risk to consumers from Lake Hawassa, Ethiopia. Freshly-caught *O. niloticus* (n=100) intestine, skin, muscle, and gills tissues were sampled. Out of the 400 samples, bacteria were isolated and identified from 293(73.25%) samples, and coliform loads were detected from 34(34%) muscle and intestine samples on suitable culture media. Bacterial pathogens belonging to 18 genera such as *Acinetobacter* (5.12%), *Aeromonas* (12.97%), *Bacillus* (10.92%), *Citrobacter* (2.05%), *Edwardsiella* (4.10%), *Enterobacter* (5.80%), *Enterococcus* (9.89%), *Escherichia* (6.48%), Klebsiella (1.36%), *Morganella* (5.80%), *Proteus* (4.44%), *Providencia* (5.80%), *Pseudomonas* (9.89%), *Salmonella* (4.10%), *Serratia* (1.71%), Shigella (3.07%) and Stenotrophomonas (6.48%) were isolated and identified. The highest number of bacteria (36.86%) was isolated from the intestinal sample, followed by the gills (25.94%), skins (23.89%), and muscle (13.31%), with statistically significant difference ($p<0.0001$). Overall, the least identified bacterium was Klebsiella (1.36%) followed by Serratia (1.71%), with statistically significant difference ($p<0.05$). The densities of fecal coliform (E. coli) (CFU/g) found in the intestines (4.21×105 CFU/g) were found to be higher than in the muscle samples (3.6×104 CFU/g) of *O. niloticus*. However, the densities of non-fecal coliform (CFU/g) found in the intestine (4.3×104 CFU/g) were found to be lower than the muscle (3.1×105 CFU/g) of *O. niloticus* samples. The potential bacterial pathogens isolated and identified are contaminants of fish in the study area, and could represent a risk to the health of consumers. The total coliform loads indicate that they were above the recommended level of the Centre for Food Safety standards. It was concluded that poor hygienic water conditions and overloading allowed the opportunistic bacterial contaminations to succeed, which caused a serious threat to consumers' health.

Keywords: Coliforms, Health risk, Nile tilapia (*Oreochromis niloticus*) pathogenic bacteria, Lake Hawassa

1. Introduction

Foodborne bacterial diseases are widespread and pose a significant threat to public health. As fish production and the consumption of fish products increase, the possibility of contracting infections from either handling or ingesting these products also increases [1, 2]. Fish is the most indispensable source of protein for individuals and has high nutritional value. On the other hand, the fish culturing raised issues of fish health due to close contact between the aquatic environment and the fish pathogens [3]. Fishery products are one of the world's essential protein sources whose quality and safety are threatened by bacterial diseases [4]. Overall, fish and fishery products have been continuously implicated in food-borne outbreaks, contributing to some 6-8% of total confirmed food-borne illness outbreaks over recent years [3]. This incidence is higher than the incidence of food-borne illness outbreaks associated with chicken and beef, which contributed to an average of 3.6% and 1.9% of total confirmed food-borne outbreaks in 2011 to 2017 [4], respectively. These fish and fish product outbreaks highlight the significant challenges to fish safety and the urgent need for better control strategies [5].

Pathogenic and potentially pathogenic bacteria associated with fish and fishery products include Aeromonas spp., Flavobacterium spp., Pseudomonas spp., Edwardsiella spp., Vibrio spp., *Acinetobacter* spp., and Plesiomonas shigelloides have been traced back to the consumption of fish. On the other hand, Worldwide total coliform bacteria are used as indicators of environmental and fecal contamination and hence, the possible presence of pathogenic organisms [6]. In Ethiopia, fish and fishery products are integral parts of the diet for many Ethiopians. Although fish plays an important role in the human diet, fish and fishery products have been documented as carriers of foodborne pathogens that are implicated in some serious bacterial infections, and total coliforms are indicators of the possible presence of these pathogens. The major purposes of this research were, therefore, to evaluate coliform loads and the prevalence of potential bacterial pathogens among *O. niloticus* from Lake Hawassa, Ethiopia.

2. Materials and Methods

2.1 Study area

This study was conducted on fish samples collected from Lake Hawassa of Ethiopia, which is well-known for its common fish catches and supplies the national fish market. This lake is some 275 km to the south of Addis Ababa (Fig.1) [7].

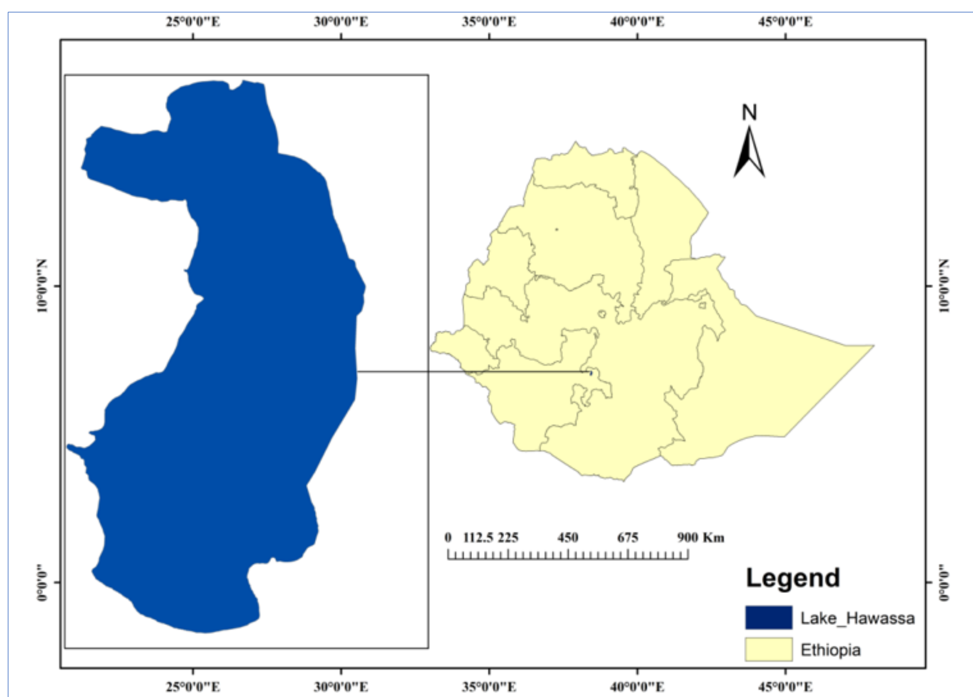

Fig 1: The study area: (Lake Hawassa)

2.2.Study design and sampling strategy

The study design was cross-sectional, and its period was from February 2024 to April 2025. For sampling, freshly-caught fish that are harvested for human consumption were used as a primary data [8]. Two sampling points were used for the collection of freshly caught fish. The sampling points are considered major fishing grounds along the shoreline. Accordingly, Amora Gedel and Haile resort areas were sites selected for the collection of freshly caught fish. The temperature of Lake Hawassa during sample collection was 26.3°C. Fish samples were collected by using the fishing boats and gears of local fishermen, and finally, fish were sampled by using stratified sampling techniques.

2.3. Fish samples

The freshly caught fish samples (n=100) were collected for bacteriological analysis from the fish harvested from the two sites. The samples were separately packed in sterile plastic bags and were immediately transported to the fishery laboratory of Addis Ababa University, College of Natural and Computational Science. The fish were euthanized by cervical dislocation in the fish's normal water without using anesthesia. Trained individuals practiced the technique using appropriate equipment. As our fish are small to medium in size, they were killed by inserting a thumb into the mouth, holding with the opposite hand, and displacing it dorsally. Death was recognized by cessation of movement, and confirmed by cessation of respiration (opercular movement) and cessation of heartbeat (palpation); and finally, by destruction of the brain.

2.4. Fish sample preparation

In the laboratory, the collected fish were dissected under aseptic conditions using sterile dissecting scissors, and the muscle, intestines, skin, and gills were taken separately using sterile forceps by following established protocol [9, 10] and standard operating procedures of bacteriology [11]. About 10g of the obtained tissue samples were aseptically transferred to sterile bottles (100ml), and homogenized with 50 ml physiological saline using the Knife Mills Grind mix GM 200 homogenizer at the rate of 8,000 rpm for 3 hours [12]. From the homogenized samples, 1 ml was drawn using sterile pipettes and further homogenized in a clean, dry, sterile beaker containing 9 ml of distilled water to have a 1:10 dilution. Finally, the samples were stored at 4°C for bacteriological analysis. Bacterial isolation was started immediately, within 12 hours after tissue samples were prepared.

2.5. Bacteria culture and colony morphology

All bacteriological experiments were performed following Bergey's manual of determinative bacteriology [13] and the respective manufacturer's instructions under complete aseptic conditions. All incubations were made for 24 hours at 37°C. First, sample suspensions were streaked across the nutrient agar medium (Oxoid, England) and incubated under aerobic conditions. Then, specific colonies were picked up and inoculated on selective and differential media, Eosin methylene blue (EMB) agar and Xylose lysine Deoxycholate (XLD) agar (HIMEDIA, India), for gram-negative and MRS and BHI agar for gram-positive bacteria, and incubated further. Suspected bacterial colonies were picked up, inoculated into Tryptone soy broth (HIMEDIA, India), and incubated as before. Colony morphology, like form, elevation, margin, surface, and pigmentation, was examined. Colony color was determined by

visual inspection of bacterial cell suspensions using fresh culture. The colonies were screened microscopically using Gram staining. The morphologically presumptively identified isolates were stored at -20°C in 25% glycerol (Fine Chemical, Ethiopia) using 1.8 ml cryovial tube (IMEC, China) for further identification.

2.6. Biochemical characterization

For taxonomic identification, the isolates were subjected to a series of biochemical tests, which include methyl red test, Voges-Proskauer test, Indole test, citrate utilization test, Urease test, catalase test, H2S production, and carbohydrate fermentation tests. The isolate differed in H2S production, methyl red test, motility test, Bile esculin agar test, Voges-Proskauer test, and other tests. Morphological and biochemical characteristics, including the colony characters of the 18 isolates, were not identical, which proved that the structure and functions of the isolates were different.

All the media used in the tests were from HIMEDIA, India. The tests began with inoculating the respective media with 24-hour-old pure culture colonies. All incubations were at 37°C for 24 hours, and expected color changes were confirmed by test positivity. Briefly, Indole production was tested by inoculating 10ml of Dev tryptophan broth, incubating and adding 2-3 drops of indole reagent. Methyl red (MR) test was conducted by inoculating 10 ml of MR Voges-Proskauer (MR-VP) medium, incubating, and adding 2-3 drops of 0.05% MR. Voges-Proskauer (VP) test was done by inoculating 10 ml of MR-VP medium, incubating, and adding 2-3 drops of 5% α-nephtol followed by 40% KOH and shaking and leaving it open for an hour. Bile esculin agar test was done by inoculating the medium with a pure culture, incubating aerobically, and observing for blackening of the medium around the colonies. The motility test was done by the stab technique, where a sterile needle inoculates a semi-solid agar medium.

For the catalase enzyme test, a small amount of bacterial colony was transferred to a clean glass slide using a sterile loop, and a drop of hydrogen peroxide was added, and the formation of bubbles was checked for. For the citrate utilization test, Simmons citrate agar slant was inoculated and incubated. The hydrogen sulfide or triple sugar iron (TSI) test was done by inoculating the TSI by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant, and incubating. Similarly, urea production was tested using Christensen's Urea Agar slant. Sugar fermentation test was conducted using sugar broth medium prepared by mixing 1g peptone, 0.3g meat extract, 0.5g table salt, 0.5g sugar, and 0.008g phenol indicator per 100 ml distilled water. Three tubes having three different sugars (glucose, sucrose, lactose) in the broth medium were inoculated and incubated.

2.7. Determination of total coliform

The Colony Forming Unit (CFU) method was used for the determination of each coliform [14]. The Buffered Peptone water (BPW) was used as a reagent, and Chromocult Coliform Agar (CCA) was used as a culture medium. This method counts colonies units formed from appropriate serial decimal dilutions of the physiological saline homogenates of the original (muscle and intestine) samples. 1 mL of the homogenized muscle and intestine samples was added to a tube containing 9 mL of Buffered Peptone water (BPW), and this resulted in a dilution of 10-1 each. Using separate sterile pipettes, decimal dilutions of 10−2, 10-3, 10-4, 10-5 and 10-6 were prepared by transferring 1mL of the previous dilutions to 9 mL of diluents (peptone water). 20 mL of the molten sterilized PCA medium (agar cooled to 44°C-47°C) was poured into each Petri dish and 1 mL of the diluted muscle and intestine samples was inoculated into sterile Petri plates separately.

After complete solidification, the prepared plates were inverted and incubated at different temperatures according to the targeted bacteria (37°C for 24 hours for non-fecal Coliform; 44°C for 24 hours for fecal coliform (E. coli)). The counts for each plate were expressed as a colony-forming unit of the suspension (CFU/g) [15]. The number of colony-forming unit per gram in the sample was calculated from the number of colonies obtained on the PCA plate from selected dilutions. It was assumed that each visible colony was the result of multiplication of a single cell on the agar surface [16]. Identification of colonies and appropriate biochemical tests were done, and the isolates were identified by comparing their morphological and biochemical characteristics.

2.8. Data Analysis

Bacterial species prevalence in the fish tissue samples of the lake was compared using one-way analysis of variance (ANOVA). Statistical analysis was performed using IBM SPSS software version 26 (IBM, Chicago, USA), and $p<0.05$ was considered the level of statistical significance. The coliform loads were calculated as the number of colonies counted multiplied by the final dilution factor divided by volume of sample taken.

3. Result

3.1. Total number of samples

100 freshly-caught fish specimens of *O. niloticus* were collected from Lake Hawassa of Ethiopia. Skin, gills, muscle, and Intestinal tissue samples were taken from the fish, making the number of tissue samples for bacteriological analyses 400.

3.2. Morphology of the isolates

The morphological characteristics of the bacterial isolates were determined using Gram staining, bacterial colony formations, and morphology of bacterial cells. Colonies of the isolates were found to be different in their form, elevation, margin, surface, colour, and optical characteristics. Based on cell morphology, all strains of gram-negative bacteria were short rods and non-spore formers, while the strains of gram-positive bacteria were rod-shaped and spore-formers.

3.3. Biochemical identification

The results showed that from the 400 samples, 293(73.25%) bacteria were detected, and 61 (15.25%) and 232 (58%) were confirmed as gram-positive and gram-negative bacteria,

respectively. Different groups of isolates that belonged to 18 genera were identified (Table 1).

3.4. Bacteria distribution by freshly-caught fish tissue

The highest number of isolates (36.86%) were isolated from the intestines of the fishes; followed by the gills (25.94%), skins (23.89%), and muscle (13.31%). The most frequently isolated bacterium from freshly-caught fish was the genus Aeromonas, with the prevalence of 3.97% in the intestine and 4.44% in the skin (Table 1). The two gram-positive bacteria, Bacillus (4.78%) and Enterococcus (3.75%), are the most frequent isolates from the gills. Similarly, Pseudomonas and Enterobacter are most frequent isolates from the skin, having a similar prevalence to Bacillus (2.73%). The least frequently isolated bacteria from freshly-caught fish were Klebsiella (1.36%), followed by Serratia (1.71%), with a statistically significant difference ($p<0.05$).

Table 1: Distribution of bacteria in the Skin, Gills, Muscle and Intestine of freshly-caught *O. niloticus* from Lake Hawassa (N = 293)

Isolated bacteria	Intestine, n (%)	Skin, n (%)	Gills, n (%)	Muscle, n (%)	Total, n (%)	P-value
Acinetobacter	9(3.07)	4 (1.36)	2(0.68)	0(0)	15(5.12)	0.579
Aeromonas	14(4.78%)	13(4.44%)	8(2.73)	3(1.02)	38(12.97)	0.210
Bacillus	10(3.41)	8(2.73)	14(4.78)	0(0)	32(10.92)	0.621
Citrobacter	4 (1.36)	0(0)	2(0.68)	0(0)	6(2.05)	0.327
Edwardsiella	4 (1.36)	2(0.68)	4(1.36)	2(0.68)	12(4.10)	0.392
Enterobacter	6(2.05)	8(2.73)	3(1.02)	0(0)	17(5.80)	0.731
Enterococcus	11(3.75)	7(2.39)	11(3.75)	0(0)	29(9.89)	0.597
Escherichia	8(2.73)	2(0.68)	5(1.71)	4(1.36)	19(6.48)	0.067
Klebsiella	1(.36)	2(0.68)	1(.36)	0(0)	4 (1.36)	0.048*
Morganella	5(1.71)	2(0.68)	4 (1.36)	6(2.05)	17(5.80)	0.194
Proteus	2(0.68)	4(1.36)	4 (1.36)	3(1.02)	13(4.44)	0.064
Providencia	11(3.75%)	3(1.02)	3(1.08)	0(0)	17(5.80)	0.617
Pseudomonas	10(3.41)	8(2.73)	6(2.05)	5(1.71)	29(9.89)	0.414
Salmonella	4(1.36)	1(0.40)	2(0.68)	5(1.71)	12(4.10)	0.244
Serratia	3(1.08)	0(0)	2(0.68)	0(0)	5(1.71)	0.042*
Shigella	1(.34)	3(1.02)	1(.34)	4(1.36)	9(3.07)	0.394
Stenotrophomonas	5(1.71)	3(1.02)	4(1.36)	7(2.39)	19(6.48)	0.630
Total	**108(36.86)**	**70(23.89)**	**76(25.94)**	**39(13.31)**	**293(100)**	**P<0.0001**

3.5. Density (CFU/g) of fecal coliform (E. coli)

Bacterial growth is the main cause of fish spoilage and is a public health concern; therefore, the total bacterial density was used as a general index of fish quality. In this study, the densities of E. coli (CFU/g) found in the intestines (4.21×105 CFU/g) were found to be higher than muscle samples (3.6×104 CFU/g) of *O. niloticus* (Table 2)

Table 2: *E. coli* load in Intestine and Muscle tissue Samples of *O. niloticus*

Samples source	Min. *E. coli* Count	Max. *E. coli* Count	Ave. *E. coli* count
Muscle	8×10^2 CFU/g	7×10^4 CFU/g	3.6×10^4 CFU/g
Intestine	4×10^4 CFU/g	2.2×10^7 CFU/g	4.21×10^5 CFU/g

Note: Min. = Minimum; Max. = Maximum; Ave. = Average

3.6. Density (CFU/g) of non-fecal coliform

The densities of non-fecal coliform (CFU/g) found in the intestine (4.3×10^4 CFU/g) was found to be lower than muscle (3.1×10^5 CFU/g) samples of *O. niloticus* (Table 3).

Table 3: Non-fecal coliform load in Intestine and Muscle tissue Samples of *O. niloticus*

Samples type	Min. non-fecal coliform Count	Max. non-fecal coliform Count	Ave. non-fecal coliform count
Muscle	2.7×10^5 CFU/g	6.2×10^5 CFU/g	3.1×10^5 CFU/g
Intestine	3×10^4 CFU/g	8.7×10^4 CFU/g	4.3×10^4 CFU/g

Note: Min. = Minimum; Max. = Maximum; Ave. = Average

4. Discussion

Evaluating the coliform loads and detecting the prevalent etiological agents in fish and fishery products is necessary to assess their health risks to consumers. The current study presented evaluation levels of coliform and identification of potential bacterial pathogens prevalent in *O. niloticus* fish tissue samples collected from Lake Hawassa of Ethiopia. A total of 18 different genera of bacteria of Gram-negative bacteria (*Acinetobacter, Aeromonas, Citrobacter, Edwardsiella, Enterobacter, Escherichia, Klebsiella, Morganella, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Shigella and Stenotrophomonas*) and Gram-positive bacteria (Bacillus and Enterococcus) were isolated from 293 (73.25%) of the evaluated tissue samples. The genera of Aeromonas, Bacillus, and Pseudomonas were the majority of the isolates, covering 33.78% of the whole isolates in this study. The predominance of these genera can be attributed to their ubiquitous nature in freshwater fish [17].

The proportions of isolates from the intestines of *O. niloticus* (36.86%) were significantly higher than those of the muscle (13.31%), skins (23.89%) or gills (25.94%). But, there were slight variations concerning the individual bacterial genus. For instance, the number of isolates from intestines and skins was almost similar concerning Aeromonas, which was the most prevalent bacterium isolated. The least prevalent in this study, Klebsiella, was similarly isolated from the intestines and gills in equal proportion. In most freshwater fish, higher bacterial load was recovered from the intestine than from other organs like skin and gills [6]. There is evidence that dense microbial populations occur within the intestinal contents, with numbers of bacteria much higher than those in the surrounding water, like the current finding, indicating that the intestines provide favorable ecological niches for these organisms [17]. Microenvironment dynamics along the gut of various fish species may influence the composition and abundance of the bacterial flora [18, 19].

It is known that fish possess distinct intestinal microbiota, but feeding habits, trophic level, species type (attribute to complexity of the fish digestive system) and the environmental conditions (salinity of the habitats and the bacterial load in the water) are the most influential factors which change the intestinal microbiota composition and abundance [20, 21, 22]. This may explain the observed differences in the distribution of bacteria from fish tissue samples in the study Lake. While bacteria in water can influence the microbial flora associated with fish, the reverse is also worth consideration. Although contamination of fish products with potential bacterial pathogens like Shigella spp. and Salmonella spp. is commonly from the environment, their incidence in ready-to-eat fish products could be due to unhygienic handling [23, 24]. Citrobacter, Enterobacter, and Klebsiella are non-fecal coliforms indigenous to general the environment and frequently present in fish, but most of these bacteria are considered non-pathogenic environmental strains and serve as indicators of contamination of foods and water.

The common bacterial genera detected from *O. niloticus* indicate that the fish were susceptible to major potential bacterial pathogens. Different studies reported the occurrence of Aeromonas, Edwardsiella, Pseudomonas, Citrobacter spp. and Klebsiella spp. in *O. niloticus* [25, 26, 27, 28, 29, 30]. Similar studies indicated the prevalence of pathogenic and zoonotic bacteria such as Edwardsiella, Salmonella, Escherichia, Staphylococcus, Vibrio, and Aeromonas in fish sampled from various parts of Ethiopia [31, 32]. A more recent molecular study that analyzed the diversity of microbiota in different sections of the *O. niloticus* gut found more diversity in Lake Chamo than in Lake Hawassa [33]. A study conducted on major bacterial pathogens of freshly-caught fish, processed fish, and their natural source habitat /water revealed the occurrence of Edwardsiella, Salmonella, Escherichia, *Acinetobacter* spp, Vibrio, Shigella, and Enterobacter spp., in rift valley lakes of Ethiopia [34, 35].

A seminar paper reviewed the occurrence and Distribution of Bacterial Pathogens from Commonly Catched Fish Species in Lake Tana of Amhara Region, North West Ethiopia presented pathogenic and zoonotic bacteria such as Edwardsiella, Shigella, Salmonella, Escherichia, Vibrio and Aeromonas which were isolated from *O. niloticus* in this study. Detection of the potential zoonotic human pathogens (*Aeromonas, Edwardsiella, Pseudomonas, Acinetobacter spp., Salmonella spp., Proteus, Shigella, Enterobacter spp., and E. coli*) in *O. niloticus* in this study, commonly used as food serves as a warning sign for the passage of these pathogens to human through infected and contaminated fish products [36, 37].

Among the bacteria found in this study, Shigella spp., Proteus spp., Salmonella spp., and Pseudomonas spp., which are potential zoonotic pathogens were detected in hand gloves, protective gowns and face-shields of hospital staff working in Nigeria [38] and the potential human pathogens (Citrobacter spp., Enterobacter spp., E. coli, Klebsiella spp., Proteus spp., Providencia spp., Morganella sp., Salmonella spp., Shigella spp., and Pseudomonas spp.) were isolated from clinical samples of patients in different part of Ethiopia [39]. By extension, this indicates that wastes coming from settlements (hospitals, latrine, household, etc., wastes) in the watersheds of the study lake contaminate the lake waters through runoffs and consequently the fishes in them.

In Egypt, investigations of the major bacterial pathogens infecting different fish species was conducted at El-Serw fish farm indicating that Aeromonas spp., Pseudomonas spp. and Klebsiella were the common infecting agents of *O. niloticus* [40]. The same author also reported that the highest number of Aeromonas spp commonly infect *O. niloticus*. The availability of pathogenic bacteria isolates in fish may be attributed to the environmental conditions and preservation processes used by fish handlers that promote the survival and spread of these pathogenic bacteria. The high number of bacterial isolates from the fresh caught fish could be due to contamination of the fish habitats. It has been reported that proper fish harvesting methods can significantly reduce bacterial isolates present on the fish skin and other fish surfaces, hence reducing bacterial contamination, and fish processing controls microbial growth and reduces fish contamination compared with unprocessed raw fish [41].

The current results of coliform loads were not compatible with the standards of the Centre for Food Safety (CFS) [42] and the APHA and WHO [43] and thus pose a significant public health risk. This study indicate that a low mean value of E. coli and high mean value of non-fecal coliforms load was found in fish muscle samples sampled from lake Hawassa. This might be due to the positive impact of water purity on the bacteria load during fish processing.

The result of this study showed lower coliforms load compared with the results of Wendwesen et al. [44] who reported that there was 4.63×106 CFU/g in frozen raw fish samples in Arba Minch town, Ethiopia. However, the result of this study is in line with the finding of Dhanapal et al. [45] which showed the loads of coliform in raw fish found to be 4.9×104 CFU/g. Bacterial load in the intestine of freshwater fish product in Saudi Arabia was reported as 6.8×106 to 7.5×107 CFU/g [46] which is incomparable to the present study. But a study conducted in Bangladesh showed similar coliform loads with the current study as E. coli (1.45 ± 0.19×103CFU/g) and non-fecal coliform (3x104 CFU/g)[29]in fishery products. A similar result was also obtained elsewhere in the world [47].

Detection of E. coli in muscle and intestines of fish samples could be due to unhygienic handling during processing and indicate recent fecal contamination of the fish. Moreover, the fact that the detection of highest load of E. coli during this study reflects the level of pollution of the water by warm-blooded animal. Escherichia coli can have a long-term survival and can multiply depending on fish and water temperatures [48-49]. The Center for Food Safety organization has set minimum standards for the recovery of microorganisms from foods of various origins. When compared with that standard, the recovery rate in the current study result was higher, and this could be due to the absence of hygienic practices and strict follow-up of this sector by the concerned authorities. According to Centre for Food Safety (CFS) [53] guidelines, <20 CFU/g is satisfactory, 20-102 intermediate or borderline and >102 unacceptable for human consumption. The average microbial load (coliform count) of the muscle and intestine samples of the fish was in an unacceptable range.

This work will serve as an initial step to establish a baseline dataset of the loads of coliform and bacterial pathogens associated with wild freshwater fish in Ethiopia. But, it has certain notable limitations. It neither quantified the detected bacteria except coliforms, nor molecularly identified them, and no antibiotic susceptibility test was done for the identified bacteria. The results would have been more robust if samples from different fish species and Lakes have been included. Moreover, the study did not assess seasonal patterns of both the loads of coliform and fish microbiota.

5. Conclusion

Majority of the collected *O. niloticus* were dominated by zoonotic bacterial pathogens mostly associated with food-borne illnesses. Muscle and intestine of the *O. niloticus* tissue samples were also found to contain higher coliform loads which were above the recommended level of the Centre for food safety standards. This has implications for the consumer health and adherence to simple hygienic steps is advisable. In this respects, rapid detection and identification of bacterial fish pathogens using different molecular techniques is helpful to develop preventive measures and control the spread of pathogens in order to decrease the health risk of consumers.

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