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Isolation and characterisation of potential bacterial pathogens from *Oreochromis niloticus* (Linnaeus, 1758) of Lake Hawassa, Ethiopia

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Abstract

The aim of the current study was to isolate and identify potential bacterial pathogens from Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) in Lake Hawassa, Ethiopia. The study was conducted during January to December 2021. A total of 360 Nile tilapia were sampled from Lake Hawassa and subjected to bacteriological investigations. The results showed 64.2% prevalence of bacterial infection in Nile tilapia from the lake. Potential bacterial pathogens such as *Aeromonas hydrophila, Escherichia coli, Edwardsiella tarda, Vibrio* sp,, *Bacillus* sp., *Staphylococcus* sp., *Salmonella* sp., *Flavobacterum* sp., *Pseudomonas* sp. and *Shigella* sp. were isolated. The prevalence of infection in female fish (52.4%) was higher compared to male fish (47.6%). Comparatively higher prevalence was recorded in the smaller fish group than in the larger ones. The average bacterial loads recorded in various organs such as intestine, skin, gill, liver and kidney were, 4.86±0.56 x 105, 4.72±0.48 x 105, 4.14±0.53 x 105, 3.45±0.23 x 105 and 2.63±0.324 x 105 CFU ml^{-1} respectively. Significantly high bacterial loads were recorded from the intestine (5.81±0.01 x 105 CFU ml^{-1}) of fish during the summer season ($p<0.05$). The results of the study indicated that there is a risk of occurrence of disease outbreak under stressful situations. Moreover, the recovery of these bacteria in the fish which are potentially pathogenic to humans, suggests that if the fish are improperly handled, undercooked or consumed raw, may cause diseases to susceptible individuals.

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Introduction

Fish contributes around 20% to the average *per capita* animal protein intake for 3.2 billion people (FAO, 2018). Sixty percent of the developing countries derive 30% of their annual protein from fish (Abisoye *et al.,* 2011). It is especially critical for rural populations, who often have less diverse diets and lower food security rates (Thompson and Amoroso, 2014). However, disease outbreaks become a major constraint in fish production and economic development throughout the world. Fish production sectors lose about 50% due to diseases which is more severe in developing countries (Leung and Bates, 2013). Globally a loss of 6 billion dollars annually is encountered in fish production due to diseases. For instance, fish diseases are estimated to contribute to more than 30% of the overall production loss in China, India and Vietnam (Mohd-Aris et al., 2019). Infectious salmon anemia alone costs 2 billion dollars and causes 20000 workers to lose their jobs in Chile (Leung and Bates, 2013). Sea lice infections of salmon in Norway led to a loss estimated at >US$ 400 million in 2011 (Abolofia *et al*., 2017). Due to these considerable economic risks, disease outbreaks represent one of the main obstacles to the sustainable growth of aquaculture (Subasinghe *et al*., 2019).

Microorganisms that cause diseases in fish include bacteria, fungi, algae, protozoa and viruses (Ajayi and Okoh, 2014). Bacteria are the most important disease-causing organism in the wild and farmed fish (El-Adawy *et al*., 2020). They are usually present in the environment, in the surrounding water, on the fish surface, or in their internal

organs. Different species of bacteria can cause different types of symptoms of diseases in fish. Common symptoms include swimming abnormally, bloody scales at the base of the fins, open sores on the side of the body and near the fins, and reddened and frayed fins (Cipriano and Holt, 2005).

Fish are usually susceptible to bacterial disease under unfavourable conditions such as poor water quality, parasitic infections, poor nutrition, or temperature extremes (Snieszko, 1975). Stress decreases the immunity of fish and leads to decreased resistance to diseases and as a result opportunistic bacteria that are found in aquatic environments cause outbreaks of fish diseases (OlivaTeles, 2012). Bacterial species responsible for disease outbreaks in different fish species belong to the genera *Aeromonas, Edwardsiella, Vibrio, Pseudomonas, Streptococcus, Salmonella* and *Shigella* among others (Menanteau-Ledouble *et al*., 2016; Fadel *et al.,* 2018; Pandey *et al*., 2021; Colussi *et al.,* 2022). Several of these bacteria also have zoonotic potential, and can be transmitted to humans, posing potential threat to human health.Therefore, this research focused on the isolation, characterisation and identification of potential pathogenic bacterial species from Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) in Lake Hawassa, Ethiopia.

Materials and methods

Description of the study area

The study was carried out in Lake Hawassa which is the smallest Rift Valley Lake in Ethiopia (Fig. 1). Lake Hawassa lies 275 km south of Addis Ababa, in the Main Ethiopian Rift, has a surface elevation of 1,680 m above sea level (6°33' - 7°33'N and 38°22' - 38°29'E) (Welcome, 1972). The surface area of the lake is about 90 km², 16 km long, 8 km wide with 1.3 billion m³ volume and mean depth of about 11 m. The mean monthly maximum atmospheric temperature varies between 24.5 and 30°C, while the mean minimum monthly air temperature ranges from 10.3 to 14.5°C and the mean annual rainfall is 1028 mm (Elizabeth Kebede *et al.,* 1994; Girma Tilahun and Ahlgren, 2010).

Study population and design

Every month, 30 naturally infected *O. niloticus* were sampled from the Lake Hawassa, forming a total of 360 samples during the study period. The sampled fishes were placed in ice box and immediately transported to the laboratory for analyses. In the laboratory, before dissection, each fish was observed macroscopically for clinical signs. Individual fish weight was recorded using digital weighing balance and their lengths were determined using a measuring board to record infestation in relation to length and weight of fish.

Total heterotrophic bacterial count

Out of the 30 fish collected every month, 10 fish were randomly selected and dissected aseptically and samples from skin, gill, intestine, liver and kidney were collected and homogenised in sterile saline solution using a mortar and pestle (Plumb *et al*., 1982). Then 0.1 ml of each sample was inoculated on nutrient agar plate, in triplicates by the spread plate technique for enumeration of total heterotrophic bacterial count. All inoculated plates were incubated in an inverted position for 24 h under aerobic conditions. The heterotrophic bacterial load were counted using colony counter and recorded as colony forming units per ml for different organs of fish.

CFU ml^{-1} = No. of colonies x Dilution factor/Volume of sample plated

Isolation and identification of bacteria

Samples from skin, gill, intestine, liver and kidney of fish were inoculated on different media such as Aeromonas medium base (AMB), Nutrient agar (NA), Salmonella-Shigella agar (SSA), Thiosulfate–citrate–bile salts–sucrose agar (TCBS), Trypticase soy agar (TSA), Xylose lysine deoxycholate agar (XLD) plates and incubated for 24 to 48 h. The morphological characteristics of bacterial colonies including colony shape, size, colour, elevation, edge and opacity were noted (Holt, 1982). Representative pure colonies were taken for identification of bacteria by Gram staining and biochemical tests were performed, that included catalase test, oxidase test, indole and hydrogen sulfide production, coagulase test,

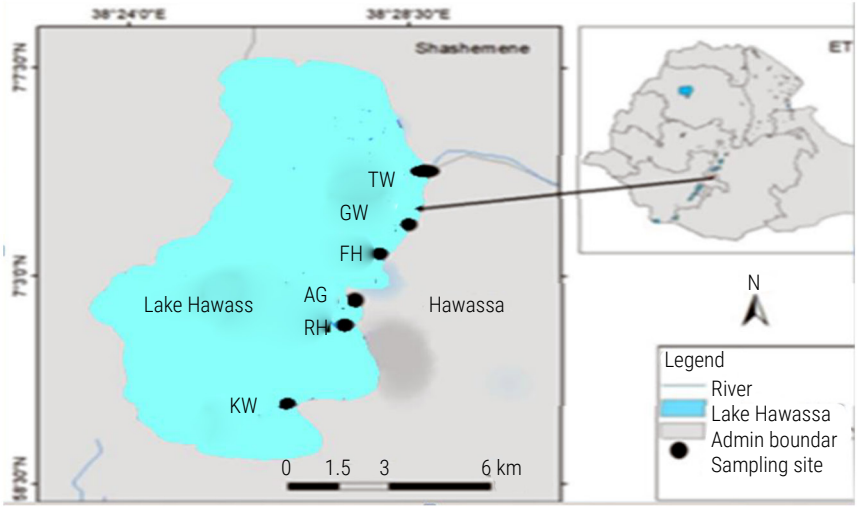

Fig. 1. Location map of sampling site in Lake Hawassa

methyl red, simmons citrate test and urease according to Bergey's Manual of Systematic Bacteriology (Bergey and Holt, 2000).

Data analysis

All the collected raw data were recorded in Microsoft excel and transferred to SPSS version 22 for descriptive analysis. The mean of bacterial loads were compared using ANOVA followed by Tukey's *post hoc* for multiple comparison.

Results

The details of morphometric parameters of fish samples are shown in Table 1. The weight of *O. niloticus* ranged between 50 and 285 g with mean weight of 154.72±41.57 g, while the total length varied between 12 and 29 cm with mean length of 19.83±2.43 cm. The minimum and maximum weight varied from 70 to 285 g for male fish with mean value of 182.88±23.84 g and 50 to 168 g for females with mean value of 115.74±26.74 g. The minimum and maximum length varied from 16 to 29 cm for male and 12 to 21 cm for female fish.

A total of 360 fish were collected and clinically examined. Naturally infected fish showed different clinical signs such as dark skin, haemorragic skin lesions, frayed fins, and body ulceration. Out of the 360 fish collected, 121 females (52.4%) and 110 males (47.6%) were found infected, with the overall prevalence of 64.2%. Fish size-wise analysis showed that 39.4% within 12-18 cm and 99 g average weight group, 35.5% within 19-21 cm and 162.1 g average weight group, 22% within 22-23 cm and 195.9 g average weight group and 3% within 24-29 cm and 232.5 g average weight group were infected with potential pathogenic bacteria. The month-wise analysis of prevalence is shown in Fig. 2. The highest prevalence rate (12.55%) was recorded during July, followed by August (11.7%) and September (10.4%), while the least (5.6%) was observed during December and January.

The total viable bacterial counts recorded in various tissues of *O. niloticus* are shown in Table 2. The counts represent the average of viable colonies which grew in triplicate agar plates for each sample. The bacterial counts in skin ranged from 4.20 x 10^5 and 5.42 x 10^5 CFU ml^{-1} with average of 4.72±0.48 x 10^5 CFU ml^{-1}. The highest count was recorded in the intestine during summer (5.81±0.01 x 10^5 CFU ml^{-1}), while the lowest was observed in kidney of Nile tilapia during spring (2.23±0.00 x 10^5 CFU ml^{-1}) season. Bacterial loads in the liver during winter and spring were not significantly different ($p>0.05$).

Based on morphological characteristics and conventional biochemical tests, the bacterial isolates belonging to 10 species were identified (Table 3 and 4). The highest prevalence was recorded for *Escherichia coli* (24.05%), followed by *Aeromonas hydrophila* (17.72%), *Pseudomonas* spp. (13.92%) and *Vibrio* spp. (12.65%). The highest number of bacterial occurrence was recorded from intestine (37.8%) followed by skin (28.1%) and gills (20.3%) (Table 5). The lowest occurrence was recorded from liver (3.9%). Isolates belonging to all the ten species were frequently encountered in the intestine compared to other organs, except *Flavobacterum* spp. which was not isolated from intestine. The results also showed the occurrence of *A. hydrophila and E. coli* in all the organs examined. *Edwardsiella tarda* was isolated only from the skin and intestine of Nile tilapia. The most frequently isolated bacterium from intestine of Nile tilapia was *E. coli* (12.6%), followed by *A. hydrophila* (6.3%).

Table 1. Morphometric data of *O. niloticus* sampled from Lake Hawassa

	Sex	No. of fish	Mean ± SD	Minimum	Maximum
Weight (g)	Female	151	115.74±26.74	50.00	168.00
	Male	209	182.88±23.84	70.00	285.00
	Total	360	154.72±41.57	50.00	285.00
Length (cm)	Female	151	17.73±1.93	12.00	21.00
	Male	209	21.35±1.42	16.00	29.00
	Total	360	19.83±2.43	12.00	29.00

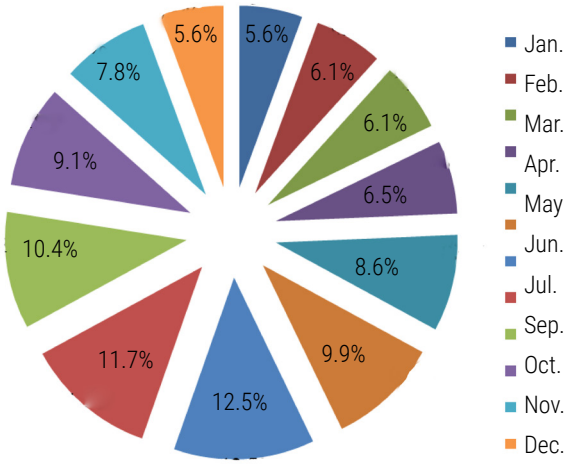

Fig. 2. Prevalence of infection by potential bacterial pathogens in *O. niloticus* from lake Hawassa

Table 2. Season-wise total viable bacterial count in *O. niloticus* from Lake Hawassa (mean x10^5 CFU ml^{-1}±SD)

Season	Total viable bacterial counts				
	Skin	Gill	Intestine	Liver	Kidney
Summer	5.41±0.01^d	4.91±0.01^d	5.81±0.01^d	3.71±0.01^c	3.11±0.01^d
Autumn	4.91±0.01^c	4.21±0.01^c	4.51±0.00^b	3.10±0.00^a	2.70±0.00^c
Winter	4.34±0.10^b	4.03±0.01^b	4.71±0.01^c	3.50±0.00^b	2.50±0.00b
Spring	4.21±0.01^a	3.43±0.01^a	4.41±0.00^a	3.50±0.01^b	2.23±0.00a

Values bearing different superscript letters in the same column differ significantly ($p<0.05$)

Discussion

In the current study, the mean of morphometric parameters of the fish showed that male fish were higher in average body weight and total length compared to female fish. This might be due to the difference in growth pattern as male Nile tilapia grow faster than the female fish. Toshiharu *et al.* (2012) reported that male and female fish had significant differences ($p<0.001$) in body weight and total length and they opined that sexual maturation inhibits the size and growth rate of female fish. The prevalence of infection was found to be higher in female fish (57.5%) as compared to male fish (42.5%), during the present study. Similar to this finding, Rediet Wolde *et al.* (2022) reported that 34.6% of the female fish and 17.65%

Table 3. Morphological characteristics of bacterial isolates

Agar medium	Morphological characteristics of colony				Bacterial species
	Colour	Elevation	Optical feat	Cell shape	
NA	Whitish	Flat	Transparent	Rods	*Escherichia coli*
AMB	Yellowish	Convex	Opaque	Rods	*Aeromonas* sp.
TSA	Whitish	Raised	Opaque	Rods	*Pseudomonas* sp.
NA	Whitish	Raised	Opaque	Rods	*Bacillus* sp.
SSA	Colourless	Raised	Translucent	Rods	*Salmonella* sp.
SSA	Colourless	Convex	Transparent	Rods	*Shigella* sp.
TCBS	Green	Flat	Opaque	Short rods	*Vibrio* sp.
XLD	Pinkish	Raised	Translucent	Rods	*Edwardsiella* sp.
NA	Whitish	Convex	Semi-translucent	Cocci	*Staphylococcus* sp.
TSA	Yellow	Flat	Translucent	Rods	*Flavobacterum* sp.

NA: Nutrient agar, AMB: Aeromonas medium base, TSA: Trypticase soy agar, SSA: Salmonella-Shigella agar, TCBS: Thiosulphate citrate bile salts sucrose agar, , XLD: Xylose lysine deoxycholate agar

Table 4. Biochemical characteristics of bacterial isolates from Nile tilapia in Lake Hawassa

Isolate	Biochemical characteristics											
	GS	CT	UT	Coa	OT	SCT	MR	VP test	MT	SIM Medium		
										IN	MT	H_2S
A. hydrophila	-	+	+	+	+	+	-	+	+	+	+	-
E. coli	-	+	-	-	-	-	+	-	+	+	+	+
Vibrio spp.	-	+	-	-	+	+	-	+	+	-	+	-
Bacillus spp.	+	+	-	-	-	-	-	+	-	-	+	-
Pseudomonas spp.	-	+	-	-	+	+	-	-	-	-	-	-
Staphylococcus spp.	+	+	+	+	-	+	+	+	+	-	-	-
Salmonella spp.	-	+	-	-	-	+	+	-	+	-	+	+
Shigella spp.	-	+	-	-	-	-	+	-	-	+	-	-
E. tarda	-	+	-	+	-	-	+	-	-	+	+	+
Flavobacterum spp.	-	+	-	+	-	-	-	-	-	+	+	-

GS: Gram stain, CT: Catalase test, UT: Urease test, Coa: Coagulase test, OT: Oxidase test, SCT: Simmon's citrate test, MR: Methyl red test, VP: Voges-Proskauer test, MT: Manitol test, SIM: Sulphur indole motility, IT: Indole test, MT: Motility test, H_2S; Hydrogen sulphide test, (+): Positive reaction, (-): Negative reaction

Table 5. Occurrence of bacterial isolates in various tissues of Nile tilapia from Lake Hawassa

Isolates	Skin No. (%)	Gill No. (%)	Intestine No. (%)	Liver No. (%)	Kidney No. (%)	Total	Prevalence (%)
E. coli	4 (5.1)	3 (3.8)	10 (12.6)	1 (1.3)	1 (1.3)	19	24.05
A. hydrophila	4 (5.1)	3 (3.8)	5 (6.3)	1 (1.3)	1 (1.3)	14	17.72
Pseudomonas spp.	4 (5.1)	5 (6.3)	2 (2.5)	0	0	11	13.92
Vibrio Spp.	3 (3.8)	2 (2.5)	5 (6.3)	0	0	10	12.65
Bacillus spp.	1 (1.3)	3 (3.8)	2 (2.5)	0	1 (1.3)	7	8.86
Staphylococcus spp.	1 (1.3)	1 (1.3)	2 (2.5)	1 (1.3)	0	5	6.32
Salmonella spp.	1 (1.3)	1 (1.3)	1 (1.3)	0	1 (1.3)	4	5.06
Flavobacterum spp.	2 (2.5)	1 (1.3)	0	0	1 (1.3)	4	5.06
Shigella spp.	1 (1.3)	0	2 (2.5)	0	0	3	3.79
E. *tarda*	1 (1.3)	0	1 (1.3)	0	0	2	2.53
Total	22 (28.1)	19 (20.3)	30 (37.8)	3 (3.9)	5 (6.5)	79	100

of the male fish were positive for *E. tarda* in Lake Bishophtu and Babogaya. Also, Fonkwa *et al*. (2022) reported that the females were found to be more infected (67.07%) than the males (58.94%).

Highest prevalence of bacterial infection (39.4%) was recorded in 12-18 cm length group and 99 g average body weight, which indicated that as the size of fish increases the infection rate decreases. In line with these findings, Begonesh *et al*. (2019) and Mzula *et al.* (2019) reported that smaller fishes were more prone to bacterial infection than larger ones. In the current study, it was observed that the percentage of prevalence increased gradually from January onwards and reached a peak in July. The prevalence remained almost the same for January, February, March and December. The outbreaks of fish diseases are seasonal and highly experienced in summer (Ibrahim *et al.,* 2008). Similar findings have been observed in the current study where high occurrences of bacteria were recorded during July (12.5%) and August (11.7%). The highest prevalence for the months of summer (July and August) compared

with the other months in the present study could be attributed to favourable temperature as well as to the run off to the lake during the rainy season in the study area. Mzula *et al.* (2019) also reported highest occurrence of infection during May and August.

In the present study, it was found that naturally infected Nile tilapia showed ulceration, corneal opacity, mucus secretion, frayed fins, hyperemia at the bases of fin, haemorrhagic skin lesions and dark skin. Consistent with the current study, Aishi-Hamom *et al.* (2020) reported clinical signs such as skin lesions, corneal opacity, excess mucus secretion, hemorrhages and frayed fins in infected fish. Thune *et al.* (1993) also reported hemorrhagic septicemia in fish due to bacterial infection, especially when fish are under stress. The present resulta are also in conformity with the findings of Kaleeswaran *et al.* (2012) and Noor El-Deen *et al.* (2014).

Callinan *et al.* (1995) reported seasonal occurrence of diseases in fishes due to climatic variations. In the present study, bacterial loads of fish tissues showed considerable variation among seasons. The distribution of heterotrophic bacteria were higher during summer compared to winter and spring. The aquatic environment that harbours fish may be the source of contaminants due to animal excreta and other environmental wastes, especially during the rainy season. The highest bacterial load detected during the rainy season might be attributed to discharges of wastewater to the lake through run off water. Ali *et al.* (2011) reported that the richness of the effluents in organic carbon exerted a specific enrichment effect on the microbial population. A similar finding was noticed by Wondimu *et al.* (2015) in Koka Reservoir. The results also showed that the bacterial load in the intestine of fish was higher than in other organs, which is in conformity with the reports of Shankar *et al*. (2009).

The highest and significantly different bacterial load (5.81±0.01 x 10^5 CFU ml^{-1}) was recorded during summer from the intestine of the fish ($p<0.05$), while the lowest bacterial load (4.41±0.00 x 10^5 CFU ml^{-1}) was recorded during spring. In contrast to the present findings, El-Fadaly *et al.* (2019) reported highest bacterial loads (4.62 x 10^7 CFU g^{-1}) in fish intestine during spring and the lowest in autumn from Manzala Lake, and this contradictory observation with respect to season suggest that other intrinsic (parasite and host) or extrinsic (water physico-chemical characteristics) factors may also regulate the bacterial population.

Similarly, the highest (5.41±0.01 x 10^5 CFU ml^{-1}) and lowest (4.21±0.01 x 10^5 CFU ml^{-1}) bacterial loads from fish skin were recorded during summer and spring ($p<0.05$). The highest bacterial load in the skin of fish could be owing to constant exposure of fish skin to contaminated water (Apun *et al.*, 1999). The higher bacterial load recorded in gills could be attributed to the fact that the gill mucus is in constant contact with the environment, providing a portal of entry for several pathogens. Mwega *et al*. (2019) stated that the high proportion of infection in gills is due to the exposed nature of the organ to microbiota. A higher number of bacteria on the surface of the skin and gills indicate the level of lake pollution.

In the current study, bacterial species such as *A. hydrophila, E. coli, E. tarda, Pseudomonas* spp., *Vibrio* spp., *Bacillus* spp., *Staphylococcus* spp., *Flavobacterum* spp., *Salmonella* spp. and *Shigella* spp. were isolated from Nile tilapia in Lake Hawassa. The phenotypic and biochemical characteristics of all isolates recorded in the present study were in line with those reported in Bergey's manual of determinative bacteriology (Bergey and Holt, 2000; Austin and Austin, 2016).

A. hydrophila is one of the most opportunistic pathogens for freshwater fish and the main etiological agent in disease outbreaks and mortality of fish. Austin and Austin (2016) and Tooba *et al.* (2022) reported *A. hydrophila* from naturally infected Nile tilapia. In the present study, *A. hydrophila* showed a high frequency of occurrence of 17.7%. The prevalence of *A. hydrophila* in the present study was in conformity with Abd-El-Malek (2017), who reported 16% of the occurrence of *A. hydrophila* from wild Nile tilapia in Egypt. However, Jimoh and Jatau (2010) and Rodrigues *et al.* (2019) reported a higher prevalence of 41.7, 47 and 46.66% for *A. hydrophila* from fish of Nigeria and Brazil, respectively. The highest prevalence (6.3%) of *A. hydrophila* was observed in the intestine of Nile tilapia. Al-Harbi *et al.* (2005) indicated that the presence of bacteria in fish's digestive flora is normal. However, the outbreak of disease is related to the existence of stress factors based on the interaction between fish, pathogens and the aquatic environment. Also, Khalil and Mansour (1997) added that stress is often considered to be a contributing factor in the outbreaks of disease caused by these bacteria.

The present study showed the highest frequency of occurrence for *E. coli* (24.1%) from Nile tilapia in Lake Hawassa. Similar to the present results, Aynadis Tilahun and Aweke Engdawork (2020) reported a 24% occurrence of *E. coli* from Nile tilapia in Lake Hawassa. Also, Adanech Haile and Temesgen Getahun (2018) reported 12% occurrence of *E. coli* from fish in Lake Zeway. The presence of *E. coli* in the tissues of fish indicates faecal contamination of water from which the fish were harvested (Gerokomou *et al.,* 2011). The occurrence of *E. coli* in the intestine of Nile tilapia was higher than in the other organs during the study period. This could be attributed to the existence of bacteria as normal intestinal microflora of fish and may result in clinical infection if it spread to other organs (Hanson *et al.,* 2008). Bacteria often occur on the body or internal organs of fish indicating the extent of pollution of the aquatic ecosystem (Ibemenuga and Okeke, 2014).

Vibrio spp. showed occurrence at the rate of 12.65% which was higher than the occurrence of *Vibrio* spp. reported by Nabiyu Kassa and Mareshet Adugana (2021) at a rate of 6.6%, but lower than the occurrence of *Vibrio* spp. of 17% as reported by Traore *et al.* (2014) in Nile tilapia of reservoirs in Ouagadougou, Burkina Faso. The highest occurrence of *Vibrio* spp. was found in the intestine of Nile tilapia (6.5%). Runfit *et al.* (2014) found that the bacterium attached to the fish intestinal eiphtelialium and formed micro-colonies. Similar to the present findings, Tesfaye Shimels *et al.* (2018) and Nur Anwar *et al.* (2012) reported *Vibrio* species from Nile tilapia of Lake Hayqe and Lake Tana, respectively.

E. tarda infects both cultured as well as wild fish with worldwide distribution and an opportunistic pathogen in other animals including humans. Marked variations in the occurrence rate of this bacterium from different species of fish has been recorded by different authors (Rashid *et al.,* 1994; Nur Anwar *et al.*, 2012). *E. tarda* showed the lowest frequency of occurrence of 2.5% during the present study. Similar to the present results, Nur Anwar et al. (2012) and Adanech Haile and Temesgen Getahun (2018) reported 4 and 2% occurrence of *E. tarda* from Lake Tana and Lake Ziway, respectively. A similar result was reported by Galal et al. (2005)

from Egypt, which was 3.5%. In the present study, *E. tarda* was recorded only from the skin and intestine. Bedaso Kebede and Habtamu Tilahun (2016) reported *E. tarda* most frequently from the liver (6.5%) followed by the intestine (2.4%) and kidney (0.8%) with significant differences among organs from Lake Zeway and Lake Langano. Mohanty and Sahoo (2007) stated that *Edwardsiella* is a Gram-negative bacterium that could be found in the intestine of healthy fish. The variation in the occurrence of *E. tarda* in fish could be attributed water quality.

Salmonella spp. are among the most important causes of human gastrointestinal disease worldwide. The presence of *Salmonella* spp. indicates faecal contamination of water from which the fishes were sampled. In the present study, *Salmonella* spp. were recorded from the skin, gill, intestine and kidney of *O. niloticus*. The occurrence of *Salmonella* spp. was 5% and the results go in line with that reported by Ertas *et al.* (2015) from fish samples in Turkey. However, Esther (2022) reported a 24% prevalence of *Salmonella* species in Nile tilapia from Tanzania. Adams and Tobaias (1999) have demonstrated that fish and fish products are only occasionally associated with *Salmonella* and that filter-feeding shellfish harvested from polluted waters have been identified as higher-risk products.

The presence of *Staphylococcus, Shigella* and *Salmonella* species in fish indicates faecal and environmental pollution (Sichewo *et al.,* 2013; Gufe, 2019). Therefore, they are most likely to cause food-borne diseases for fish consumers (Sichewo *et al.,* 2013). The frequency of occurrence of *Shigella* spp. in the present study was 5.1%, which was found to be lower than the observation by Onyango *et al.* (2009) who reported 14.3% occurrence of *Shigella* spp. in fish from the Winam Gulf of Lake Victoria, Kenya. The percentage occurrence of *Pseudomonas* spp., in the present study was 13.9%, which was lower than the report by Abd-El-Naser *et al.* (2021) recorded 23.3% of occurrence in fish marketed in Sohag Governorate, Egypt. The occurrence, of *Staphylococcus* spp. recorded was 6.5%, which was found to be higher than the report by Ali (2017) who observed 1.75% occurrence of *Staphylococcus* spp. in fresh Indian mackerel (Rastrelliger kanagurta) in Unguja Island.

The occurrence of *Bacillus* spp. during the current study was 8.8%, which was found to be higher than the result reported by Nebiyu Kassa and Mareshet Aduga (2021) from Ethiopia, but much lower than the findings that reported 80% occurrence of *Bacillus* spp. from pond water fish in Trinidad, South America (Newaj-Fyzu *et al.,* 2008). *Bacillus* spp. have great potential to be used as disease inhibitors (Kesacordi-Watson *et al.,* 2008). They are used as dietary supplements for the improvement of human and animal health (Lee *et al.,* 2019). However, some *Bacillus* species are also known producers of toxins in their human and animal hosts (Elshaghabee *et al.,* 2017).

Bacteria loads of tissues of Nile tilapia in Lake Hawassa generally followed the order; intestine>skin>gills>liver>kidney. The occurrence of potential bacterial pathogens in Nile tilapia of Lake Hawassa indicates human health risks from fish handling and by consuming improperly cooked fish harvested from this lake.

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