

Preliminary Survey of Gram-Negative Bacterial Pathogens from Commonly Caught Fish Species (*Oreochromis niloticus*, *Cyprinus carpio* and *Clarias gariepinus*) in Lake Hayiq, Ethiopia

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Abstract

A cross-sectional study was conducted from October 2016 to May 2017 on *Oreochromis niloticus*, *Cyprinus carpio* and *Clarias gariepinus* fish species at Lake Hayiq, North East Ethiopia with the objective of isolation and identification of major gram-negative bacterial pathogens of commonly caught fish species from Lake Hayik. A total of 98 live fishes (49 *Oreochromis niloticus*, 30 *Cyprinus carpio* and 19 *Clarias gariepinus*) were collected and transported to the laboratory. From the three fish species a total of 384 organ samples (96 skins, 98 gills, 94 intestines, 33 swim bladders, 19 kidneys, and 44 livers) were taken. A systematic random sampling technique was used to select the fish samples after drawn from the water. Among 384 fish organ samples 116 (30.2%) gram negative bacterial isolates were found: 9 (2.3%) *Aeromonas* species, 24 (6.3%) *Pseudomonas* species, 6 (1.6%) *Enterobacter* species, 29 (7.6%) *Escherichia* species, 4 (1.6%) *Edwardsiella* species, 11 (2.9%) *Klebsiella* species, 5 (1.3%) *Proteus* species, 8 (2.1%) *Vibrio* species, 14 (3.6%) *Flavobacter* species and 6 (1.6%) *Salmonella* species were found. Isolation among organs: *Pseudomonas* species, skin (33.3%), intestine (33.3%), and swim bladder (12.5%); *Escherichia* species: intestine (41.4%), skin (24.2%) and gill (24.2%) and *Klebsiella* species: liver (27.3%), gills (36.4%) were found. All the isolated bacterial species were gram-negative bacteria. From the three fish species, *Oreochromis niloticus* was the most affected fish species (48.2%) while *Clarias gariepinus* species were the least affected species (17.2%). In conclusion, majorities of those pathogens isolated and identified were very important for different fish disease outbreaks and also public health importance. But, very few and disintegrated studies with scanty data have been done whereas, it has been nowadays fishery is one of the main growth transformation plan for food security in Ethiopia. Therefore, it needs further integrated investigation on fish bacterial diseases.

Keywords Bacteria; *Clarias gariepinus*; *Cyprinus carpio*; *Oreochromis niloticus*; Lake Hayiq

Abbreviations Gram's (-): Gram-Negative Bacteria, CSA: Central Statistical Agency, IMViC: Indole, Methyl Red, Voges-Proskauer, and Citrate Utilization Tests, SPSS: Statistical Packaging for Social Science; VP: Voges Proskauer.

Introduction

Ethiopia is a country in the horn of Africa endowed with numerous aquatic resources, including over 20 natural lakes, 12 large river basins, over 75 wetlands, and 15 reservoirs. Micro and macro-dam construction and river impoundment have created innumerable large and small water bodies. Both inland capture fisheries and aquaculture activities are concentrated around the many lakes and rivers in the Rift Valley, as well as around the Blue Nile, which supplies water to the country's largest water body, Lake Tana [1].

In Ethiopia above 200 fish species have been identified; among those fish species, the most common are *Oreochromis niloticus*, *Clarias gariepinus*, *Barbus* species and *Latesniloticus*. The country has high potential of fish production in Lakes such as Tana (25%), Ziway and Langano (19%), Chamo (18%) and Abbaya (12%) from the total bulk

of caught exists but the annual production may raise to 65,000 tons per year [2].

The fish sector makes a vital contribution to the food and nutritional security of 200 million Africans and it provides income for over 10 million people engaged in fish production, processing and trade. However, fish diseases are very important aspects of modern fish farming given the enormous impact on profitability and also cause of human diseases in many areas of the world [3]. Even in the past 50 years, there has been a significant increase in the number of emerging infectious diseases originated from aquatic life. These represent 72% of zoonosis all over the world [4]. The diseases caused by these agents demonstrate several virulent characteristics which show the link between human, animal and wildlife health. It needs a broader understanding of the ecological settings that play an important role in providing the opportunity for these pathogens to emerge, re-emerge and transmit to another host [4].

Bacterial diseases were considered the main cause of high mortalities and economic losses among fish and fish farms [5]. Even among bacterial pathogens, gram's (-) bacterial pathogens are the dominant pathogens which include *Escherichia coli*, *Shigella dysenteriae*, *Edwardsiella tarda*, *Vibrio cholera* and *Salmonella* [6,7]. The indigenous bacterial pathogens are found naturally living in the fish habitat, for example *Vibrio* species and *Aeromonas* species can contribute in different fish disease outbreaks [8].

In Ethiopia, both pathogenic and non-pathogenic bacteria can be isolated from different parts of fish, among pathogenic bacteria zoonosis *Edwardsiella tarda* has been isolated from apparently healthy fish of Lake Ziway and Tana [9]. The practice of consuming partially cooked fish meals, manual handling of fish and unhygienic practice during filleting would expose the public to higher risk of contracting the disease [10].

Even though, there is very good understanding on bacterial diseases are the main cause of high mortalities and economic losses fishes and fish farms and bacteria zoonosis in worldwide, in Ethiopia information is scant and inadequate except for few types of researches which have not been well documented. As far as the knowledge of researchers considered, there were no any research have been conducted in Lake Hayiq (Lugo). Therefore, this study aimed at isolation and identification of major gram's (-) bacteria which contribute for different fish diseases outbreaks as well as public health importance from commonly caught fish species; *Oreochromis niloticus* (Nile tilapia), *Clarias gariepinus* (cat fish) and *Cyprinus carpio* (common carp) in Lake Hayiq of Amhara region, Ethiopia.

Materials and Methods

Study area

The study was conducted on one of the high land Lakes of Ethiopia; Lake Hayiq (locally, Lugo) (Figure 1), which is located in South Wollo

Zone, Tehuledere district in North East, Ethiopia. It is located 440 kilometer north of Addis Ababa the capital of Ethiopia, situated at 11° 15'N latitude, 39° 57'E longitudes. It lays two kilometers East of Hayiq town [11]. It can be categorized as sub-humid tropical with an annual rainfall of 1173 mm with ambient temperature of 18.2°C. It is 6.7 kilometer long and 6 kilometer wide, with a surface area of 23 square kilometer. It has a maximum depth of 88.2 meter and is at an elevation of 2,030 meters above sea level. The Lake has no visible outlet and the water appearance looks clear and greenish [11].

Lake Hayiq (Lugo) provides a habitat to different fish species, water birds and aquatic organisms. It plays an economical role via tourism and fishery. The fish that inhabit Lake Lugo are *Oreochromis niloticus* (Nile tilapia), *Clarias gariepinus* (African catfish) and *Cyprinus carpio* (Common carp). The fish production potential of this lake is estimated to be 400 tons per year and the off take rate is 83% as reported by Federal Democratic Republic of Ethiopia [12].

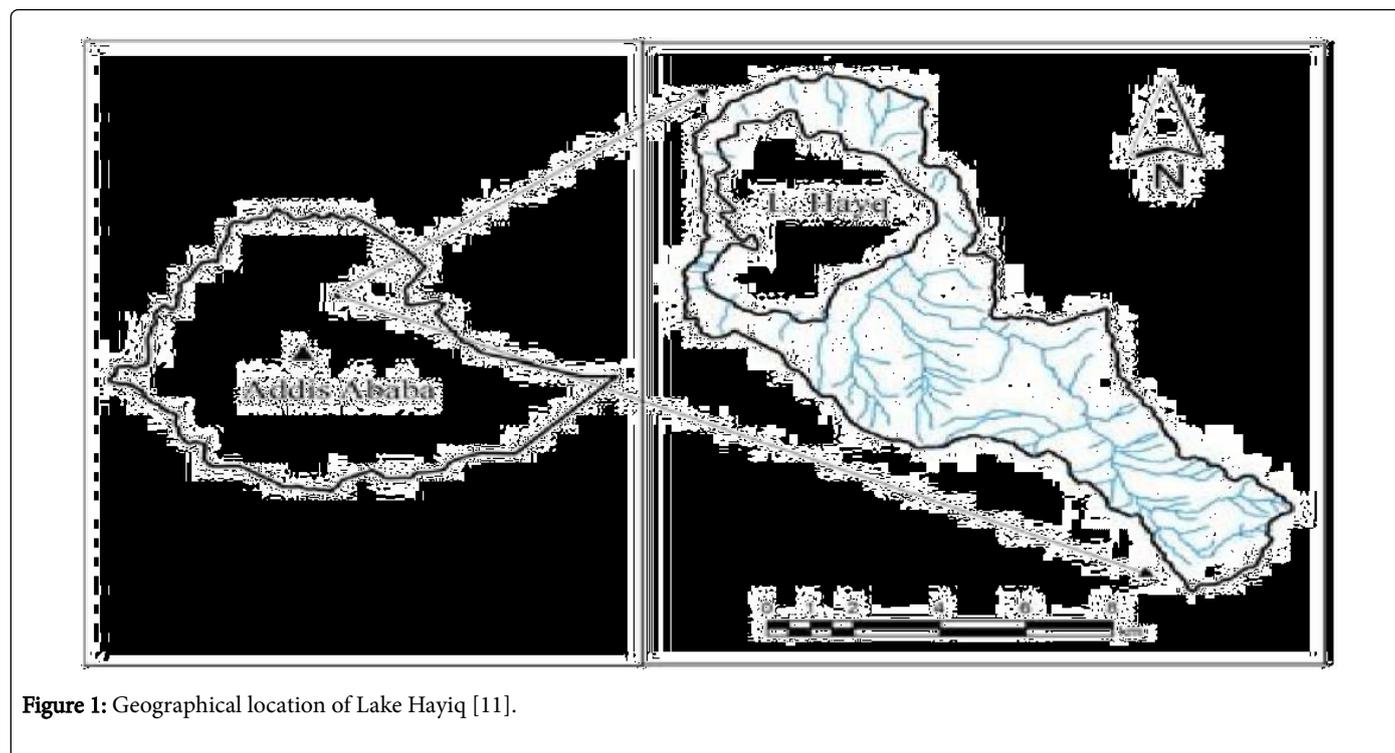


Figure 1: Geographical location of Lake Hayiq [11].

Study population and design

The study was a cross-sectional study design conducted from October 2016 to May 2017. A total of 98 live fishes (49 *Oreochromis niloticus*, 30 *Cyprinus carpio* and 19 *Clarias gariepinus*) were collected and transported to the laboratory. From the three fish species a total of 384 organ samples (96 skins, 98 gills, 94 intestines, 33 swim bladders, 19 kidneys, and 44 livers) were taken. All fishes were caught using

multi-mesh monofilament survey gillnet that is composed of six different randomly distributed mesh size-panels, ranging from 40 mm to 120 mm with 50 m total length and 2 m width. A systematic random sampling technique was used to select the fish samples after drawn from the water to transport them to the lab. Fish species were identified for their species type using methodologies applied by Nagelkerke [13].

Bacteriological samples collection

After transportation of the selected fish samples to Kombolcha animal health diagnostic and surveillance center, in microbiology laboratory, swab samples were first collected from skin and gill. Then, fishes were dissected aseptically and swab samples from intestine, swim bladder, kidney, and liver were also taken for bacterial isolation and identification. The swabs were directly inoculated onto blood agar media agar (Oxoid, UK) and inserted into nutrient broth media agar (Oxoid, UK) for bacterial enrichment for 24 hours in microbiology laboratory.

Isolation and identification of gram's negative bacteria

Swabs and a loop full from enriched nutrient broth media were streaked directly Tryptic Soy Agar (TSA) plate (Himedia, India). After incubation only purified isolates were identified by through colony characterization and staining. The subjected colonies were re-streaked onto nutrient agar and blood agar. The pure cultures were used for studying cultural and biochemical characteristics. All tests were done according to the flow chart outlined by [14]. Biochemical tests; include Catalase test, Oxidase test, Triple sugar iron (TSI) (Himedia, India) test, XLD (xylose lysine deoxycholate) (Himedia, India) test, IMViC (Himedia, India) and Hydrogen sulfide production test were used for gram's negative bacteria characterization. Additionally, other sugar tests were also applied to build the isolated bacterial biochemical reaction profile so that it could aided for further bacterial identification at genus and species level.

Data management and analysis

All the collected raw data were recorded in Microsoft excel and transported to SPSS version 20 for data analysis. The data was analyzed using descriptive statistics and compared as percentages, frequencies and presented as Tables and graphs. Chi-square (X^2) test was applied gram's (-) bacterial isolate status between apparently healthy fishes and lesioned fishes.

Results

From a total of 98 live fishes, *Oreochromis niloticus* (49), *Cyprinus carpio* (30), and *Clarias gariepinus* (19), a total of 384 organ samples (96 skins, 98 gills, 94 intestines, 33 swim bladders, 19 kidneys, and 44 livers) were collected and sampled for this study. From 384 samples 116 isolates were processed for gram negative bacteria.

Bacterial genus and species identification

The most dominant gram's (-) bacterial species isolated among the selected fish species were *Escherichia coli* 29 (25%), *Pseudomonas* species 20 (20.7%) and *Flavobacteria* species 14 (12%) (Figure 2). Regarding gram's (-) bacterial examination of fresh water fish, present study displayed that *Oreochromis niloticus* species was the most infected species with gram's (-) bacterial isolates and *Clarias gariepinus* was the lowest infected species.

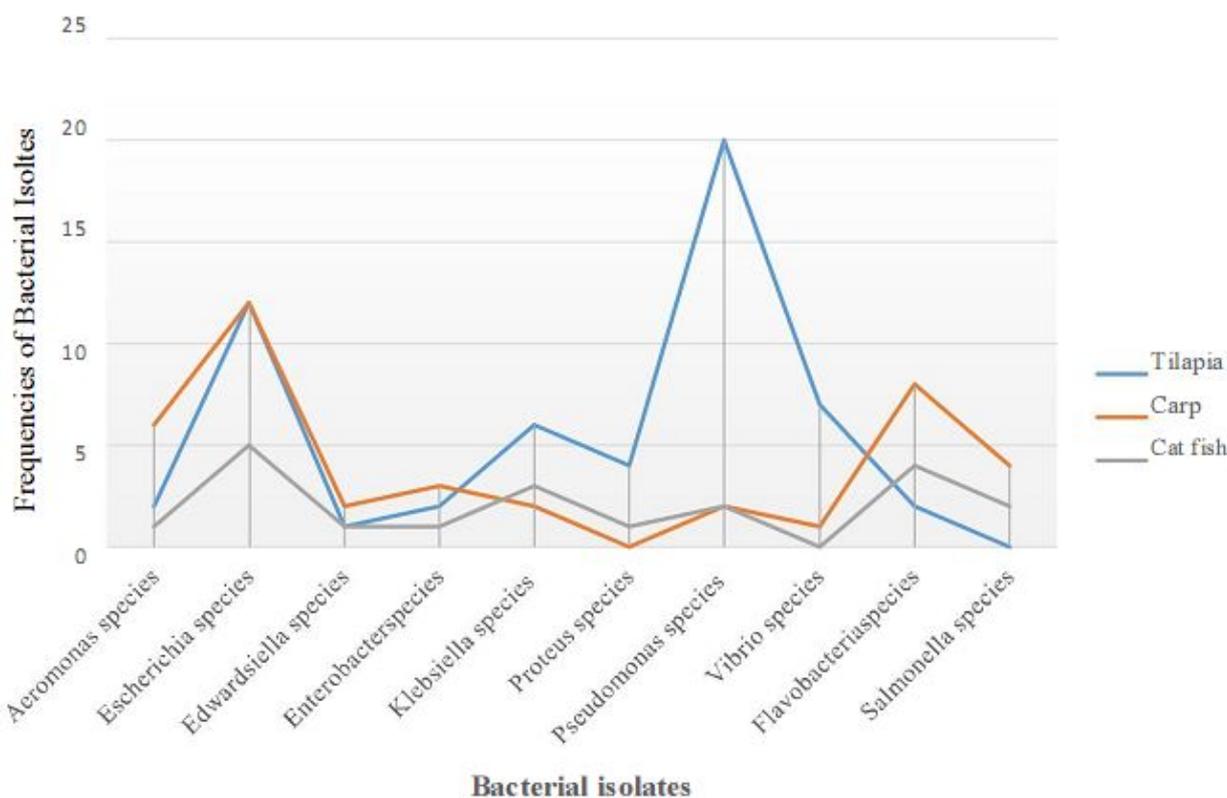


Figure 2: Distribution and frequencies of gram's (-) bacteria among fish species; *Oreochromis niloticus*, *Cyprinus carpio* and *Clarias gariepinus*.

Distribution and frequencies of gram's (-) bacteria from skin, gills, intestine, kidney, liver, and swim bladder

The distribution of gram's (-) bacterial isolates from different organs described with in Table 1. Out of these isolates the most dominant

species from intestine, skin and gill were *Escherichia coli* and *Pseudomonas* species was the 2nd most isolated species from skin and intestine. Kidneys were the list infected organ from which only 4 (3.5%) isolates were found.

Isolated gram's (-) bacteria	Organs						
	Skin	Gill	Intestine	Swim bladder	Kidneys	Liver	Total
<i>Aeromonas species.</i>	1	2	5	Nil	1	Nil	9
<i>Escherichia species</i>	7	7	12	1	Nil	2	29
<i>Edwardsiella species</i>	3	Nil	1		Nil	Nil	4
<i>Enterobacter species</i>	1	4	Nil	1	Nil	Nil	6
<i>Klebsiella species.</i>	4	3	Nil	1	Nil	3	11
<i>Proteus species.</i>	2	1	1	Nil	Nil	1	5
<i>Pseudomonas species</i>	8	3	8	3	1	1	24
<i>Vibrio species</i>	1	5	1	Nil	Nil	1	8
<i>Flavobacteria species</i>	5	3	3	1	2	Nil	14
<i>Salmonella species</i>	1	2	3	Nil	Nil	Nil	6
Total	33	30	34	7	4	8	116

Table 1: Distribution and frequency of gram's (-) bacteria isolated from skin, gills, intestine, kidney, liver and swim bladder at Lake Hayiq.

Gram's (-) bacterial isolated from different age and sex groups

From 384 sampled fish 332 (86.5%) were adults and 52 (13.5%) juveniles; whereas in sex group 195 (50.8%) were males and 189 (49.2%) were females (Table 2).

Isolated bacteria	Age group					Sex group			
	Total (N)	Adult	Percent (%)	juvenile	Percent (%)	Male	Percent (%)	Female	Percent (%)
<i>Aeromonas species</i>	9	8	88.8	1	11.1	5	55.5	4	44.4
<i>Escherichia species</i>	29	23	79.3	6	20.7	17	58.6	12	41.3
<i>Edwardsiella species</i>	4	4	100	Nil	Nil	4	100	Nil	Nil
<i>Enterobacterspecies</i>	6	3	50	3	50	2	33.3	4	66.6
<i>Klebsiella species</i>	11	8	72.7	3	27.27	6	54.54	5	45.45
<i>Proteus species</i>	5	5	100	Nil	Nil	5	100	Nil	Nil
<i>Pseudomonas species</i>	24	19	79.1	5	20.8	10	41.6	14	58.3
<i>Vibrio species</i>	8	7	87.5	1	12.5	5	62.5	3	37.5
<i>Flavobacteria species</i>	14	13	92.8	1	7.1	3	21.4	11	78.5
<i>Salmonella species</i>	6	6	100	Nil	Nil	3	50	3	50
Total	116	96	82.75	20	17.25	60	51.72	56	48.28

Table 2: Distribution of Gram's (-) bacterial isolates among age and sex group at Lake Hayiq.

Association of Gram's (-) bacterial isolates with organs lesion recorded

During this study fishes sampled were observed for any lesion and abnormality before the samples were taken. From the total sampled organs 329 (85.7%) were with-out any observed lesions and 55 (14.3%)

were with different lesions like ulceration, hemorrhage, reddening and discoloration. The occurrences of Gram's (-) bacterial isolates in apparently healthy fish and fish with lesion were identified (Table 3).

The lesions observed and Gram's (-) bacterial isolates were found to be significantly associated ($X^2=22.58$, $P=0.007$).

Fish status (observed lesion)							
Isolated bacteria	Total	With lesion	Percent (%)	Without lesion	Percent (%)	X^2	P value
<i>Aeromonas species</i>	9	Nil	Nil	9	9.1	22.58	0.007
<i>Escherichia species</i>	29	2	11.1	27	27.5		
<i>Edwardsiella species</i>	4	2	11.1	2	2.04		
<i>Enterobacterspecies</i>	6	2	11.1	4	4.08		
<i>Klebsiella species</i>	11	4	22.2	7	7.1		
<i>Proteus species</i>	5	Nil	Nil	5	5.1		
<i>Pseudomonas species</i>	24	1	5.5	23	23.4		
<i>Vibrio species</i>	8	Nil	Nil	8	8.16		
<i>Flavobacteria species</i>	14	5	27.7	9	9.1		
<i>Salmonella species</i>	6	2	11.1	4	4.08		
Total	116	18	15.52	98	84.48		

Table 3: Gram's (-) Bacterial isolates from fish with lesion and without lesion at Lake Hayiq.

Discussions

The major diseases associated with fish are parasites, bacteria, viruses and toxic algae that reduces fish production by affecting the normal physiology of fish and if left uncontrolled, it can results in mass mortalities or in some cases, can be serve as source of infection for human and other vertebrates that consumed fish [15]. Bacterial infection of fish constitutes a huge menace for aquaculture, leading to disastrous economic loss [16].

In the present investigation *Aeromonas species*, *Escherichia species*, *Edwardsiella species*, *Enterobacter species*, *Klebsiella species*, *Proteus species*, *Vibrio species* and *Flavobacteria species* were isolated from *Oreochromis niloticus* fish. This result is in agreement with several studies which reported the isolation of *Edwardsiella species*, *Escherichia species* and *Klebsiella species* from *Oreochromis niloticus* fish species [17] on Lake Ziway and isolation of *Aeromonas species* from different freshwater fish species [18]. Similarly Anwar et al. [19] also reported *A. hydrophilia*, *E. coli*, *E. tarda*, *K. pneumoniae*, *proteus species*, *Vibrio species* *Y. enterocolitica*, *Y. rukckeri* and *A. aeromona Salmonicida* from *Oreochromis niloticus* from fish species in Lake Tana. Many authors [20,21] stated that *Aeromonas species* are important fresh water fish pathogenic bacteria, causing septicemic infections and associated with economic losses in fresh water fish culture worldwide.

In this study *Pseudomonas species* were isolated from gills and intestines of *Oreochromis niloticus* fish. There is a similar report from previous investigation in Sudan which reported the presence of *Pseudomonas species* in gills and intestines of *Oreochromis niloticus* fish [22]. This result is also in agreement with the Tripathy et al. [23] as

reported the presence of *Pseudomonas species* from different parts of a number of fresh water fish species. In the present investigation *Escherichia species* was isolated from skin, gills, swim bladder, liver and intestines of *Oreochromis niloticus* fish. This is in agreement with previous study in Sudan by Hnadi [22] who reported the presence of *Escherichia species* in gills and intestine of *Oreochromis niloticus* fish. *Escherichia species* were also reported from intestines and gills of different freshwater fish species [24,25]. But, it disagree with those findings that it was only these report which identified *E. coli* from Liver. These findings might be due to septicemic infection on sampled fishes.

In this study the dominant isolates from *Cyprinus carpio* were *Escherichia species*, *Aeromonas species* and *Flavobacterias species*. These findings might be the first report from Ethiopian, this is because very limited *Cyprinus spp*s distribution among fish producing Lakes that indicated no study done on these fish species in Ethiopia. But from different countries, it was possible to find bacterial isolates like *Aeromonas hydrophila* which causes Bacterial Haemorrhagic Septicemic disease Disease of *Cyprinus carpio* [26]. Additionally, similar report from Eman et al. [27] indicated *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas species* are common bacterial isolates from *Cyprinus carpio*. But on *proteus species* our finding stood in opposite to the finding of Eman et al. [27] who reported *Proteus vulgaris* from kidney of *Cyprinus carpio*.

Isolation and identification of *Pseudomonas species* from *Clarias gariepinus* are in agreement with those reports of Maha et al. [28,29] from the gill and skin. Others isolates also include *Aeromonas species*, *Escherichia species*, *Edwardsiella species*, *Enterobacterspecies*, *Klebsiella species*, *Proteus species*, *Flavobacterias species*, *Pseudomonas*

species and Salmonella species. There was no isolation of vibrio species unlike other fish (*Oreochromis niloticus* and *Cyprinus carpio*) [28,29]. In the present study the isolation and identification of Edwardsiella species indicated Edwardsiellosis. Edwardsiella infection in fish usually occurs under imbalanced environmental conditions such as high water temperature, poor water quality, and high organic content. The present study displayed that *Oreochromis niloticus* species was the most infected species with bacterial isolates and *Clarias gariepinus* was the lowest infected species. This may be due to that *Oreochromis niloticus* species were genetically suitable to be infected by investigated bacterial isolates. *Clarias gariepinus* species may be more immunologically protected from that infection.

Among the organs sampled the highest bacterial isolate were from intestine, Skin and gills whereas small number of bacteria were isolated from kidney and swim bladder. The chance of infection of each organ might vary has a chance if contact with bacteria. However, other conditions like environmental conditions and level of exposure (access) to infection can vary the level of infection with bacteria. In many diseases situation age is a determinant factor due to the level of immunity. In this study both adult and juvenile age group were considered. Adults harbor more frequently than juvenile groups.

The association between organs and isolates was statistically significant ($P < 0.05$). Even though, the result indicates high number of isolates were from apparently healthy fishes, many bacteria of fresh water fish which considered as a normal flora of water; they become pathogenic when the fish are stressed due to change of environmental conditions [30]. This indicates that each organ have a chance of infection if the lesions exposed for septicemic infection. However, other conditions like environmental conditions and level of exposure (access) to infection can vary the level of infection with bacteria.

Conclusion

This study demonstrated that the dominant fish Gram's (-) bacterial pathogens which are commonly isolated from fresh water fish also isolated and identified from fish species of in Lake Hayiq, Ethiopia. Majorities of those pathogens isolated and identified were very important for different fish disease outbreaks and also public health importance. It was found that the dominant bacterial pathogens especially Edwardsiella species, Aeromonas species, Escherichia species Klebsiella species, pseudomonas species and Salmonella species isolation can be indicators of public health importance. Additionally, the findings have shown that the presence of opportunistic fish pathogens especially in kidney, Liver indicates that risk of the occurrence of disease outbreak at any time when the fish are exposed to stress. Generally, it needs further integrated investigation on fish diseases, water quality, other associated factors and management fresh fishery on these lakes. Additionally, there should be awareness for the fisher men, handler and consumer in order to prevent public health importance bacterial infections from fish.

Declarations

Ethics approval and consent to participate

The authors followed internationally recommended fishing guidelines and procedure during fish catching as well as fish dissecting procedure for sample collection.

Consent for publication

Not applicable

Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that there is no financial or non-financial competing interest from anybody or institute. We also want to assure that we did not receive any technical assistant in developing the research concept or preparation of the manuscript.

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Authors' contributions

ST carried out the conception of the research concept and designed the methodology, data analysis and interpretation and preparation of the manuscript for publication. MK and ZA carried out the laboratory work, sample collection, and revision of the manuscript. MC and BB carried out funding and editing of the manuscript for publication. All authors read and approved the final manuscript.

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