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Open Access Journal of Microbiology & Biotechnology
ISSN: 2576-7771

Prevalence and Antimicrobial Profile of Bacterial Pathogens Isolated from Naturally Infected Fish in Lakes of Ethiopia

Dissasa G[1]*, Lemma B[2] and Mamo H[3]

[1]Institute of Biotechnology, Addis Ababa University, Ethiopia

[2]Department of Zoology, College of Natural and Computational Sciences, Addis Ababa University, Ethiopia

[3]Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, Ethiopia

Research Article

Volume 7 Issue 3

Received Date: August 15, 2022

Published Date: September 14, 2022

DOI: 10.23880/oajmb-16000238

***Corresponding author:** Guta Dissasa, Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia, Email: atomsad440@gmail.com

Abstract

Small scale fisheries play important role in providing food and livelihoods for households in Ethiopia. However, prevalence of bacterial fish infection and their antimicrobial resistance were increasing all over the world serving as important limitations in fish production. A cross sectional study was carried out to identify bacterial pathogens infecting common carp and Nile tilapia and to establish the antibiotic susceptibility of fish bacteria in Ethiopia. A total of 42 fish samples from two lakes (Hawassa and Ziway) were aseptically collected and bacteria isolated from the kidney, liver and intestine. The isolates were identified by their morphological characteristics, biochemical tests and sequencing of 16S rRNA gene. Nine well known fish pathogens were identified at the lakes with prevalence rate of *A. hydrophila* (11.9 %), *A. sobria* (7.14%), *A.veronii* (21.43%), *E. tarda* (16.67%), *P. hunanensis* (7.14%), *P. putida* (9.5%), *P. mirabilis* (19.05%), *S. flexneri* (11.9%) and *S. maltophilia* (11.9%). Six (6) more prevalent and known potential pathogens were evaluated for antibiotic susceptibility by using Kirby Bauer disk diffusion essay. All isolates tested were resistant to at-least three (3) of the eight antibiotics evaluated. High levels of resistance were however expressed by majority (87.5%) of the pathogens against penicillin and vancomycin. These suggested maximum levels of acquired antibiotic resistance in fish bacteria from the study area. The resistant bacteria may transmit resistance genes to other bacterial fish pathogens and there is a possibility that these resistant bacteria may be transmitted to humans who consume or handle the carrier fish. Thus, use of antibiotics in fish farming in Ethiopia should be discouraged. It is advisable that fish are cooked properly before consumption, in order to kill bacteria that may be present.

Keywords: Antimicrobial Profile; Bacterial Pathogens; Common Carp; Naturally Infected Fish; Nile Tilapia

Abbreviations: XLD: Xylose Lysine Deoxycholate; TSB: Tryptone Soy Broth; SAB: Society of American Bacteriologists; dNTPs: Deoxynucleotide Triphosphate Mix; CLSI: Clinical Laboratory Standards Institute.

Introduction

Fishery is a rapidly growing industry providing huge amount of fish for human consumption worldwide [1]. Fish

plays an important role in the human diet with an ever growing need globally [2]. Pathogenic microorganisms are a serious threat to fish production in all over the world due to high economic importance of diseases they cause. Environmental dynamics in freshwater ecosystems are fundamental in the development of pathogenic fish bacteria and there has been a steady increase in the numbers of bacterial species associated with fish diseases [3]. Gram-negative bacteria like Aeromonas spp., Flavobacterium spp., Pseudomonas spp., Edwardsiella spp., Vibrio spp., Acinetobacter spp. and Plesiomonas shigelloides are a great threat to freshwater fish production [3-5]. Most of these bacterial fish pathogens are zoonotic with the potential to infect humans and some are serious [6,7].

Different mechanisms are used to produce large stocks of fish, but frequent disease outbreaks occur, and the use of antimicrobials to control those pathogens provides ideal conditions for the emergence of resistant bacterial strains and stimulates horizontal gene transfer. Major concerns surround the use of antimicrobial agents in fishery sectors, including the potential impacts these uses may have on the development of antimicrobial resistant pathogens in fish and the aquatic environment. The passage of antimicrobial resistance genes and resistant bacteria from aquatic to terrestrial animal husbandry and to the human environment can have detrimental effects on both human and animal health and on aquatic ecosystems [1]. Currently, some antimicrobial agents commonly used in aquaculture are only partially effective against fish pathogens due to the emergence of resistant bacteria [8]. Genetic determinants of antimicrobial resistance are commonly found on mobile genetic elements which are recognized as the primary source of antimicrobial resistance for important fish pathogens [8].

The widespread occurrence of naturally resistant bacteria in the aquatic environment could contribute to the passage of antibiotic resistance genes to fish bacteria [9]. Besides, high levels of antimicrobial resistances have been reported in bacterial isolates from fish elsewhere [10-16]. However, no information is available on antimicrobial resistance for fish bacteria in Ethiopia amidst glaring reality for the impact of antimicrobial resistance and the increase in intensive aquaculture that could warrant antibiotic use. We, therefore, conducted this study to identify the bacteria infecting Nile tilapia and Common carp and to establish the antimicrobial profile of selected potential bacterial pathogens in Ethiopia.

Materials and Methods

Study Area and Design

This cross sectional study was conducted to collect naturally infected (Nile tilapia and Common carp) fish samples from two lakes (Lake Hawassa and Lake Ziway) of Ethiopia. Bacteria were isolated from the collected fish samples and identified by morphological characteristics, biochemical tests and 16S rRNA gene sequencing. Antibiotic susceptibility profiles of selected potential bacterial pathogens were determined by using the Kirby-Bauer disc diffusion essay [17].

Collection of Fish Samples

The collected live fish samples were inspected external abnormalities and a total of 42 naturally infected fish were sampled from two selected lakes with 22 Nile tilapia (13 from Lake Hawassa and 9 from Lake Ziway) and 20 Common carp (9 from Lake Hawassa and 11 from lake Ziway). The fish were euthanized by cervical dislocation in the fish's normal water without using anesthesia. Trained individuals practiced the technique using appropriate equipment. Cervical dislocation is among the methods recommended for fish sacrifice as it is relatively simple and effective for not big fish [18, 19].

As our fish were small to medium in size, they were killed by inserting a rod or 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. The samples were separately packed in sterile plastic bags and were shipped to Batu Fisheries and Other Aquatic Life Research Center located at Batu town (formerly Ziway), near Lake Ziway. The fish were dissected under aseptic conditions using a sterile dissecting scissor by following established protocol and standard operating procedures of bacteriology [20,21]. Tissue samples of kidney, intestine and liver were taken aseptically using sterile scalpel blade (forceps) and kept in sterile universal bottles of 100 ml capacities separately and homogenized in physiological saline solution.

Bacterial Isolation and Identification

Biological swabs were taken from physiological saline solution homogenized intestine, liver and kidney using sterile inoculating loops separately and inoculated on Nutrient Agar (HIMEDIA, India)and incubated at 37°C for 24 hours under aerobic condition. Each type culture colony was picked up and sub cultured on selective and differential media (Xylose Lysine Deoxycholate (XLD)) agar media (HIMEDIA, India) and incubated at 37°C for 24 hours. Suspected bacterial colonies were picked up and inoculated into Tryptone Soy Broth (TSB) (HIMEDIA, India) and incubated at 37°C for another 24 hours.

Morphological Observations of Isolates

For the identification of selected isolates, colony morphology such as colony form, elevation, margin, surface and pigmentation were studied. Colony color was noted by visual inspection and bacterial cell suspension using fresh culture was used for microscopic examination of isolates with simple staining and differential staining (Gram-staining) following Society of American Bacteriologists (SAB) procedure [20]. The morphologically identified isolates were stored at -20°C in 50% glycerol (Fine Chemical, Ethiopia) using cryovial tubes of 1.8ml (IMEC, China) for further biochemical identification.

Biochemical Studies of the Isolates

All isolates were identified biochemically by streaking bacterial colonies over TSB and incubated at 37°C for 24 hours and, then identified by using a range of biochemical tests performed according to respective manufacturer's instructions following Society of American Bacteriologists (SAB) [20] and Bergey's manual of determinative bacteriology procedure [21].

Molecular Characterization

Bacterial DNA Extraction: Each bacterial isolate was grown overnight in 10 ml of TSB broth at 37°C for 24 hours. Qiagen DNeasy DNA extraction protocol for bacterial cultures adapted from Qiagen DNeasy handbook, 2006 and stored at −20 °C till use.

PCR Amplification: It was performed in a DNA thermal cycler (Eppendorf, Hamburg, Germany). PCR reactions were performed in a final volume of 25 µl containing 20 ng of DNA, 0.1–0.3 µl of each primer (rD1 and fD1), and 1 µl of Hot Star Taq Master Mix containing MgCl2, Hot Star Taq DNA polymerase and deoxynucleotide triphosphate mix (dNTPs). PCR conditions were as follows: 95 °C for 15 min, 30 cycles at 95 °C for 45 s, 55 °C for 45 s, 72 °C for 1 min, followed by a final elongation at 72 °C for 7 min. The PCR products (8 µl) were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide using 1 µM Tris-Acetate-EDTA buffer at 100v for 1 hour and were visualized by UV transillumination [22].

16S rRNA Sequencing: Sequencing the 16S rRNA gene has been extensively used in the understanding of bacterial evolution and phylogeny and it is regarded as an essential tool in bacterial systematic and identification of new species. Sequencing of the amplicon was performed using the universal bacterial primers rD1 (5′-CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC-3′) and fD1 (5′-CGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG-3′). The 16S rRNA was sequenced using Sanger sequencing at Base Clear DNA research laboratory, Leiden, The Netherlands). For sequencing of amplified 16S rRNA directly, four identical 100µl amplification reactions were performed on each sample, with the resultant material being pooled and purified. A 500ng amount of template (16S rRNA) was combined with 10 ng of primer, 2µl of Sequence buffer, and water to 10µl. This sample was held at 98°C for 7 min and cooled to room temperature for 1 min, and then the labeling reaction was performed at room temperature (37°C) for 5 min. Chain elongation was terminated with sample loading buffer and sequencing was performed on buffer-gradient gels [23].

Antibiotic Susceptibility Testing of Selected Bacteria: Antibiotic susceptibility testing was carried out following Kirby-Bauer disc diffusion method on Mueller Hinton agar (HIMEDIA, India) as described by Hudzicki [17]. There are no registered antibiotic formulations for use in fishery sectors in Ethiopia, and therefore, the choice was guided by different classes of drugs reported elsewhere to treat diseases in aquaculture facilities [24]. Moreover, these drugs are commonly used in veterinary and human medicine therapy in Ethiopia. A total of six (6) bacterial pathogens (*A. hydrophila, A.veronii, E. tarda, P. mirabilis*, *S. flexneri* and *S. maltophilia*) were tested due to their high prevalence in the study area and they were considered to represent the isolated gram-negative bacteria, a group to which major bacterial fish pathogens belongs. Eight (8) commercially available antibiotic disks (Oxoid UK) were used in the following concentrations: ampicillin (10µg), Chloramphenicol (30µg), erythromycin (15µg), gentamicin (10µg), oxacillin (1µg), penicillin (10µg), tetracycline (30µg) and vancomycin (10µg).

The isolates were plated on Xylose Lysine Desoxycholate (XLD) agar media (HIMEDIA, India) and incubated at 37°C for 24 hours. Colonies were picked and emulsified in 0.85% Sodium chloride to create a suspension matching 0.5 McFarland standards. The bacterial suspension was, then, spread onto the surface of the Mueller Hinton agar (HI MEDIA, India) to make confluent growth. Antibiotic discs were immediately placed on the surface of the agar plate using forceps and incubated aerobically at 37°C for 18 hours. Inhibition zones for various isolates were measured and interpreted as sensitive or resistant according to the Clinical Laboratory Standards Institute (CLSI) [25].

Data Analysis: Bacterial infection status of the different fish sample types was determined and the proportion of infected samples/isolates was compared between various categories using the Chi-squared test. Bacterial species prevalence in the two fish species, examined tissue samples and lakes was compared using one-way analysis of variance (ANOVA). Statistical analysis was performed using IBM SPSS software version 25 (IBM, Chicago, USA) and $p<0.05$ was considered the level of statistical significance. The overall antibiotic response of each antibiotic was calculated as the number of bacteria resistant or sensitive to antibiotics over total number of bacteria isolates tested.

Results

Caught Naturally Infected Fish

A total of 42 naturally infected fish samples of the two species Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) were obtained from the two lakes. Out of the 42 naturally infected fish samples, 20 and 22 fish were collected from Lake Ziway and Lake Hawassa respectively with Nile tilapia (22) and common carps (20). Totally 126 (3 from each) tissue samples were obtained from the naturally diseased fish of which 38.88% tissue samples were positive for gram-negative bacterial infection. The body weights and lengths of Nile tilapia and common carp range between 16cm (139gm) to 23cm (200gm) and 18cm (378gm) to 22cm (462gm) respectively.

Clinical Examination

The clinical examination of the collected naturally infected Nile tilapia (*Oreochromis niloticus*) and Common carp (*Cyprinus carpio*) showed different external abnormalities like skin ulceration and hemorrhages all over the fish body especially at fins and tails (Figure 1).

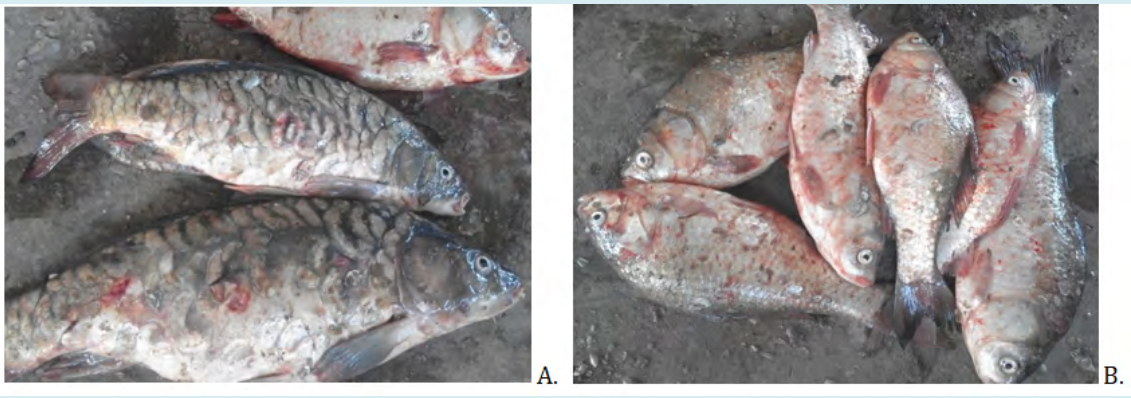

Figure 1: Selected naturally infected (A) carps and (B) Nile tilapia showing hemorrhage and skin ulceration.

Morphology of the Isolates

The morphological characteristics of the bacterial isolates were determined including Gram staining, bacterial colony's and bacterial cell's morphology. Colonies of the isolated isolates were found to be different in their form, elevation, margin, surface, colour and optical characteristics. On the basis of colony morphology, all strains were short rod and non- spore former (Figures 2 & 3).

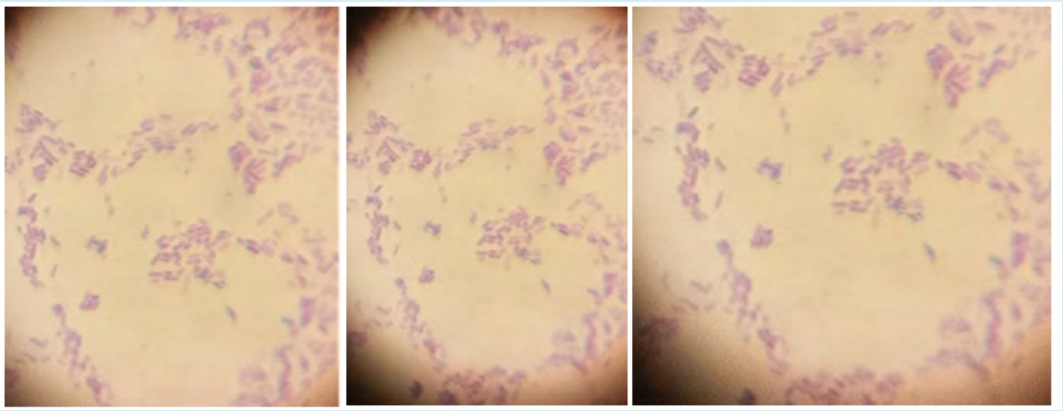

Figure 2: Selected microscopic morphology of gram-negative rods isolated from naturally infected fish.

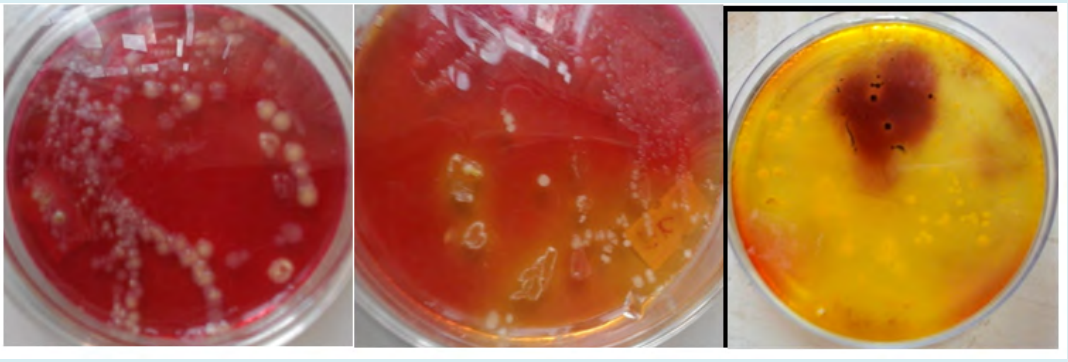

Figure 3: Selected colony morphology of gram-negative rods isolated from naturally infected fish on XLD agar.

Biochemical Examination of Bacteria

To further identify the morphologically identified bacteria, a range of biochemical tests (Indole, methyl red, Voges-Proskauer (VP), Citrate, H_2S gas, Urease, sugar fermentation and catalase test) were carried out. The results showed that different groups of isolates that belonged to 9 species were identified. These were *Aeromonas* spp. (*A. hydrophila, A. sobria* and *A.veronii*), *Edwardsiella (E) tarda*, *Pseudomonas* spp., (*P. hunanensis* and *P. putida*), *Proteus (P) mirabilis, Shigella (S) flexneri* and *Stenotrophomonas (S) maltophilia.* Each isolate were confirmed by molecular identification using 16S rRNA sequencing.

Analysis of 16S rRNA Gene

The nine identified isolates by morphological characteristics and biochemical tests were subjected to further identifyby16S rRNA (1-9) and sequenced by Sanger sequencing (BaseClear DNA research laboratory, Leiden, The Netherlands) and were compared to those available in the GenBank database (Tables 1, 2 & 4). The universal primers (rD1 and fD1) were used to amplify of 16S rRNA of bacterial isolates, with amplicon size ~1.4kbp (Figure 4). Compared with GenBank database, the nucleotide sequences of 16S rRNA gene could detect the isolates of bacteria in the level species, according to levels of homology of nucleotide ranging from 97.65-100% (Figure 5).

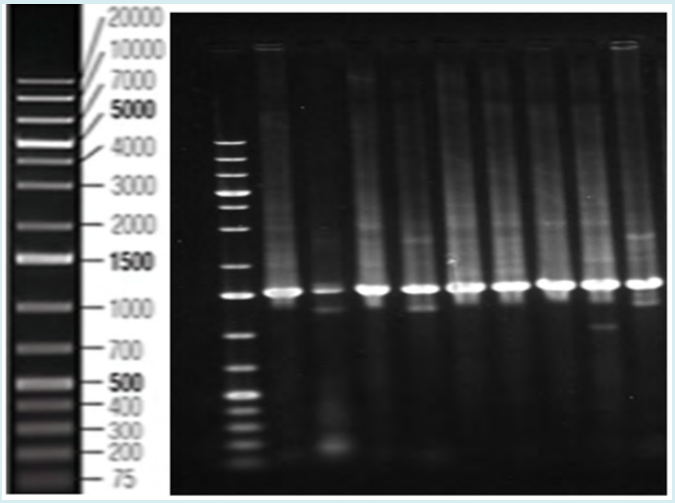

Figure 4: Agarose (1.5%) gel electrophoresis analysis of the PCR products from 16S rRNA gene of bacteria with universal primers (rD1 and fD1). Lane 1-9: Bacterial isolates.

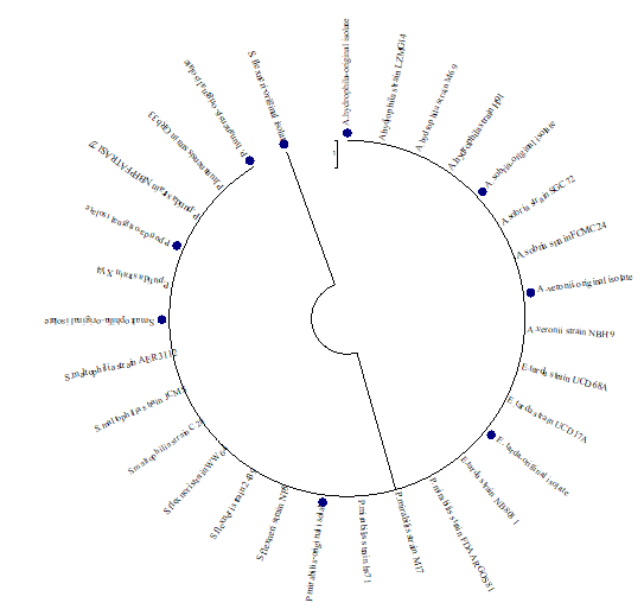

Figure 5: The phylogenetic relationships, based on 16S rRNA gene sequences, were constructed by the neighbor-joining method. Numerals at nodes indicate bootstrap percentages derived from 500 replications. The bacterial isolates from this study designated with bold dot. Accession numbers of 16S rRNA genes of the bacterial isolates *A. hydrophila*, *A. sobria*, *A. veronii, E. tarda, P. mirabilis, P. putida, P. hunanensis, S. flexneri* and *S. maltophilia* are KT998817, MT384381, KU525084, DQ884466, MK802107, MZ317275, MN177226, MW832309 and MT533800 respectively.

Prevalence of Bacterial Infections among the Examined Fish

The prevalence of isolated bacteria from 42 fish is represented in Table 1. Forty nine (49) isolates were identified as gram-negative bacteria from naturally infected fish (Nile tilapia and Common carps) samples. The most prevalent bacterial isolates were *A.veronii* (21.43%) followed by P. mirabilis (19.05%) and *E. tarda* (16.67%). *A. hydrophila*, *S. flexneri* and *S. maltophilia* have similar prevalence rate of 11.9%, while A. sobria and P. hunanensis were the least prevalent isolates (7.14%). The highest prevalence rate of the isolated bacteria from naturally infected Nile tilapia (Oreochromis niloticus) were *A. veronii* (14.28%), E. tarda (11.9%), *P. mirabilis* (11.9%), *S. maltophilia* (11.9%) and *A. hydrophila* (9.5%). *A. veronii*, *P. mirabilis* and *S. flexneri* have showed similar prevalence rate of 7.14% in naturally infected common carp among the isolated bacteria.

Table 1: Prevalence of bacterial infections among the examined Nile tilapia and Common carp fish from Lakes Hawassa and Ziway (N = 42).

	Lake Hawassa		Lake Ziway			
Bacteria spp	**carp**	**Tilapia**	**carp**	**tilapia**	**Total**	**P-value**
A. hydrophila	-	2(4.76%)	1(2.38%)	2(4.76%)	5(11.9%)	0.578
A. sobria	2(4.76%)	1(2.38%)	-	-	3(7.14%)	0.154
A. veronii	1(2.38%)	2(4.76%)	2(4.76%)	4(9.5%)	9(21.43%)	0.632
E. tarda	2(4.76%)	5(10.2%)	-	-	7(16.67%)	0.042*
P. hunanensis	-	-	-	3(7.14%)	3(7.14%)	0.037*
P. putida	-	-	3(7.14%)	1(2.38%)	4(9.5%)	0.062
P. mirabilis	1(2.38%)	2(4.76%)	2(4.76%)	3(7.14%)	8(19.05%)	0.632
S. flexneri	2(4.76%)	1(2.38%)	1(2.38%)	1(2.38%)	5(11.9%)	0.768
S. maltophilia	-	2(4.76%)	-	3(7.14%)	5(11.9%)	0.078
Total	8(19.05%)	15(35.7%)	9(21.43%)	17(40.47%)	42(100%)	<0.0001

Note: Not detected.

Except *P. hunanensis* and S. maltophilia, all other isolates were recovered from Nile tilapia (O. niloticus) and common carps (C. carpio). E. tarda was isolated from 5 Nile tilapia and 2 common carps with overall prevalence of 16.67%. The prevalence of E. tarda and P. hunanensis were significantly ($p < 0.05$) vary with the occurrence of any other species among naturally infected fish from both Lakes. A. veronii was isolated from 6 tilapia and 3 carps with overall prevalence of 21.43% which was higher than the occurrence of any other species among naturally infected fish from each Lake (Table 1).

The highest numbers of isolates were isolated from Lake Ziway (53.1%) and relatively the less number of isolates (46.9%) isolated from Lake Hawassa. The prevalence of bacterial infection in the two Lakes was statistically significant ($p<0.0001$). Except *P. hunanensis* and *P. putida,* all the others bacterial pathogens (85.7%) were isolated from Lake Hawassa fish samples. Similarly, all of the species were also isolated from Lake Ziway fish samples except *E. tarda* and *A. sobria*. On the other hand, 65.31% of the 9 species were detected in both Lakes fish samples (Table 1).

Occurrence of Isolated Bacteria in the Tissue Samples

The highest number of bacteria occurrence was isolated from the kidney (36.73%) followed by the intestine (32.65%) and liver (30.61%) (Table 2). The most frequently isolated bacterium from the examined tissue samples of naturally infected fish was *A. veronii* with similar (8.16%) frequency both in the intestine and kidney (Table 2). *E. tarda* and *P. mirabilis* are the most frequently isolated bacteria from the liver (14.3%). The least frequently isolated bacteria from the examined tissue samples of fish were *A. sobria* and *P. hunanensis* (6.1%) (Table 2).

Isolated bacteria	Intestine, n (%)	Kidney, n (%)	Liver, n (%)	Total, n (%)	P-value
A. hydrophila	2 (4.1%)	2 (4.1%)	1 (2.0%)	5 (10.2%)	0.714
A. sobria	1 (2.0%)	-	2 (4.1%)	3 (6.1%)	0.064
A. veronii	4 (8.16%)	4 (8.16%)	1 (2.0%)	9 (18.4%)	0.541
E. tarda	3 (6.1%)	1 (2.0%)	3 (6.1%)	7 (14.3%)	0.296
P. hunanensis	-	2 (4.1%)	1 (2.0%)	3 (6.1%)	0.562
P. putida	1 (2.0%)	2 (4.1%)	1 (2.0%)	4 (8.2%)	0.274
P. mirabilis	2 (4.1%)	3 (6.1%)	3 (6.1%)	8 (16.3%)	0.742
S.flexneri	1 (2.0%)	3 (6.1%)	1 (2.0%)	5 (10.2%)	0.536
S. maltophilia	2 (4.1%)	1 (2.0%)	2 (4.1%)	5 (10.2%)	0.391
Total	16 (32.65%)	18 (36.73%)	15 (30.61%)	49 (100%)	0.694

Table 2: Occurrence of isolated bacteria from examined tissue samples of Nile tilapia and common carp fish caught from Lakes Hawassa and Ziway (N = 49).

Antibiotic Sensitivity Testing

Results of sensitivity testing for the eight (8) tested antibiotics on the six (6) bacterial pathogens are indicated in Table 3. The result showed that, except *S. maltophilia* all the others pathogens were sensitive to vancomycin. Majority (87.5%) of the pathogens were resistant to penicillin and vancomycin. The majority of the pathogens tested were resistant to most of the antibiotics evaluated, suggesting maximum levels of acquired antibiotic resistance in fish bacteria from the study area (Table 3).

Bacterial strains	Antibiotics and its susceptibility patterns							
	Amp	Chl	Ery	Gen	Oxa	Pen	Tet	Van
A. hydrophila	R	S	S	S	I	R	R	R
A. veronii	R	R	S	S	R	R	R	R
E. tarda	S	S	I	S	R	R	S	R
P. mirabilis	S	S	R	S	S	S	R	R
S. flexneri	R	R	R	S	S	R	R	R
S. maltophilia	R	I	S	R	R	R	S	R

Table 3: Antibiotic sensitivity profiles of the bacterial pathogens, based on CLSI's inhibition zones interpretive criterion.
Note: Amp: Ampicillin, Chl: Chloramphenicol, Ery: Erythromycin, Gen: Gentamicin, Oxa: Oxacillin, Pen: Penicillin, Tet: Tetracycline,

Van: Vancomycin, R: Resistance, S: Sensitive.

Discussion

Regardless of the fact that the prevalence of bacterial fish infection and antimicrobial resistance were increasing all over the world by serving as important limitations in the production and sustainability of fishery industry; still very less determination is observed towards focusing on this aspect of the field. In present study, the two fish species were found infected with different groups of bacterial pathogens which indicate that these species are equally susceptible for these bacterial diseases. Nine (9) species of bacterial pathogens were isolated and identified from naturally diseased caught fish in Lake Hawassa and Lake Ziway of Ethiopia. The isolated bacteria were identified as *Aeromonas* spp. (*A. hydrophila, A. sobria* and *A.veronii*), *E. tarda*, *P. mirabilis, Pseudomonas* spp. (*P. hunanensis* and *P. putida*), *S. flexneri* and *S. maltophilia.* Most of the bacterial pathogens isolated from the fish were originated from the lake Ziway (53.1%) and relatively less numbers of the isolates were isolated from Lake Hawassa (46.9%). The prevalence of bacterial infection in the two Lakes was statistically significant ($p<0.0001$).

Overall, the most dominant bacteria were *A.veronii* (18.4%), *P. mirabilis* (16.3%) and *E. tarda* (14.3%).This is in line with the finding of Zorrilla I, et al. [26] who reported the dominance of *Aeromonas* spp., *E.tarda* and *Pseudomonas* spp., in freshwater fish pathogens and causing diseases in different tropical freshwater fishes. Similar findings have been also reported from Egypt, Pakistan and Uganda in previous studies [27-30]. Bacteria are the leading causative agents of diseases in freshwater fishes all over the world [26]. More severe disease conditions in fish industry are mostly caused by Gram-negative bacteria [16]. An investigation made on bacterial pathogens of fish presented pathogenic and zoonotic bacteria such as *Aeromonas* spp (*A. hydrophila*); *Pseudomonas* sp. (*P. fluorescens* and *P. putida*) and *K. oxytoca* recovered from naturally infected Nile tilapia in El-Serw aquaculture fish farm of Egypt [24]. The author also reported that, the highest numbers of *Aeromonas* isolates commonly infect Nile tilapia than *Pseudomonas* isolates. A study on naturally infected fish in fish farms of district Kasur, Punjab Pakistan, found significantly higher potential pathogenic *A. hydrophila* in Nile tilapia (*Oreochromis niloticus*) [28].

In the present study, the highest numbers of bacterial pathogens (36.73%) were recovered from the kidney of the two naturally infected fish. Our findings are in line with Meron D, et al. [31] who found that, significantly higher potential pathogenic bacteria were recovered from kidneys than in liver samples of naturally infected marine fish and variations were found among the fish species. The author also reported that *A. veronii* was most frequently isolated from the kidney of naturally infected fish Nile tilapia and Common carp. This finding indicated that, Nile tilapia were relatively more susceptible to the potential bacterial pathogens than carp. This is in line with the finding of Meron D, et al. [31] who found that; Nile tilapia was very sensitive to different potential bacterial pathogens especially gram negative bacteria *Aeromonas* spp. and *pseudomonas* spp. *P. hunanensis* was described as the novel species of the genus *Pseudomonas* commonly isolated from soil samples subjected to long-term manganese pollution [32], but in this study it was isolated from naturally infected Nile tilapia. This finding suggests that, the naturally infected fish might have been exposed to biological pollutants which have been diluted or neutralized from the environment.

Among the identified bacteria, the most prevalent potential pathogens (*A. hydrophila, A.veronii, E. tarda, P. hunanensis, P. mirabilis*, *S. flexneri* and *S. maltophilia*) were evaluated for their antibiotic susceptibility profiles. The majority of the pathogens evaluated were resistant to most of the antibiotics evaluated. *A. veronii* and *S. flexneri* were resistant against 75% of the antibiotics evaluated. All the bacterial isolates showed multiple resistances to various drugs tested. Except *S. maltophilia* all the others pathogens were sensitive to vancomycin and this are in agreement with the findings of Gufe, et al. [11]. The majority (87.5%) of the pathogens were resistant to penicillin and vancomycin. The susceptibility levels of each pathogen were relatively low. Similar findings have been reported in Egypt in previous studies Ayoub HF [29].

This result is in line with the finding of Wamala SP, et al. [30] who investigated the occurrence and antibiotic susceptibility profiles of fish bacteria infecting *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda and found the prevalence of *Aeromonas* spp., (*A. hydrophila* and *A. sobria*) and *E. tarda* in naturally infected Nile tilapia (*Oreochromis niloticus*). The author also evaluated the antibiotic susceptibility profiles of these bacteria and obtained similar results with our findings. Antimicrobial resistance is a worldwide public health concern that has drawn attention in the recent time. Existence of antibiotic resistance amongst these bacterial strains may have public health concern in fish consumers and handlers. Fishery sectors are a rapidly growing industry providing fish food for human consumption worldwide [9]. However, fish farming is confronted with acute problem of disease like bacterial, fungal and viral disease [27].

The use of antimicrobials has become a customary practice to control those pathogens that provides ideal conditions for the emergence of resistant bacterial strains and stimulates horizontal gene transfer [1]. The passage of antimicrobial resistance genes and resistant bacteria from

aquatic to terrestrial animal husbandry and to the human environment can have detrimental effects on both human and animal health and on aquatic ecosystems [1]. Generally, findings from the present study indicate a high frequency of bacterial pathogens isolation among collected naturally infected fish samples and good evidence of resistance in the antimicrobial susceptibility profiles of evaluated pathogens.

Conclusion

This study identified the well-known bacterial pathogens infecting Nile tilapia and Common carp and evaluated the antibiotic susceptibility profiles of selected bacterial pathogens in Ethiopia. Bacterial fish pathogens are prevalent in Ethiopia and could partly be responsible for the fish-borne diseases and observed reduction in fish production in the country. Fish bacteria in Ethiopia are highly resistant to a number of antibiotics and accelerate the emergence and spread of antimicrobial resistance in the aquatic environments from where most of the fish have been harvested for human consumption. This information facilitates formulation of effective strategies for control and development of vaccine for the prevention of bacterial fish diseases. Focus on alternative control strategies for bacterial infections in fish farms without the use of antibiotic should be encouraged. Characterization of the isolated bacteria including pathogenicity studies is urgently needed so as to clearly understand their implication on fish and human health in the country. This is the first study from Ethiopia to report comprehensive data on the prevalence and antimicrobial susceptibility profiles of major bacterial fish pathogens.

Acknowledgement

The authors are grateful to Addis Ababa University the Office of the Director for Research for their financially supported and to the fishermen in the two study Lakes for their technical assistance during sample collection.

Author Contributions

GD conceived and designed the experiments, collected samples, performed the laboratory works, analyzed the data and drafted the manuscript. BL and HM conceived and designed the experiments, critically comment and revised the manuscript.

Competing interests

The authors declare that there is no competing interest.

Consent for publication

This is not applicable.

Data Availability

All data are within the manuscript.

Ethics approval

The study has got ethical approval by the College of Natural and Computational Sciences Institutional Review Board (IRB), Addis Ababa University. The authors confirm that all methods were performed in accordance with the relevant guidelines and regulations. Specifically, permission for sample collection was obtained from Batu Fisheries and Other Aquatic Life Research Center as per the local legislation.

Funding

The Office of the Director for Research, Addis Ababa University, financially supported this study.

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