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Original Research Article

The Bacterial Flora on Selected Organs of *Oreochromis Niloticus* in Lake Chivero, Zimbabwe

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Abstract: Fish can harbour and spread pathogenic bacteria that form the natural micro-flora of the environment, but the flora may be altered by man's activities such as sewage effluent disposal. The aim of this study was to isolate, identify and enumerate bacteria associated with the different organs of *Oreochromis niloticus* in Lake Chivero, Zimbabwe and compare their diversity and prevalence. Bacteria were isolated, identified and enumerated from the gills, intestine, kidney, liver, muscle and skin of thirty (30) fish using the culture-based technique on selective and non-selective media. A total of twelve (12) bacteria were isolated, namely *Acinetobacter* spp., *Aeromonas* spp., *Bacillus subtilis*, *Citrobacter* spp., *Edwardsiella tarda*, *Enterobacter* spp., *Escherichia coli* including *E. coli* O157:H7, *Klebsiella* spp., *Listeria ivanovii*, *Shigella* spp., *Staphylococcus* spp., and an unidentified isolate. *Staphylococcus* spp. were most prevalent on primarily all organs with prevalence rates ranging from 53.3% to 100%. *Escherichia coli* O157:H7 was least prevalent and was not detected on intestine, liver and kidney. *Staphylococcus* spp. was the most abundant isolate ($1.46 \times 10^5 \pm 4.014 \times 10^4$ cfu/g) on the skin whereas *E. coli, L. ivanovii* and *Shigella* spp. were absent on the kidney. Fish pathogenic bacteria (*Aeromonas* spp. and *Edwardsiella tarda*) did not vary significantly in their distribution between the organs ($p > 0.05$), though high counts were recorded on the gills. Opportunistic pathogens of fish and humans, were significantly high on external organs mainly the skin and gills. The high bacterial infection levels indicate that the fish are heavily stressed and this may negatively impact the populations of *O. niloticus* in Lake Chivero. The several human pathogenic bacteria isolated render *O. niloticus* unfit for consumption according to the CODEX Alimentarius standards and pose health risks to consumers.

Keywords: Oreochromis Niloticus Chivero, Bacteria.

INTRODUCTION

Fish are one of the largest groups in the animal kingdom and they are present in various aquatic environments. These aquatic environments range from freshwater to marine environments and they present several habitats and niches for different fish groups. Also present in these environments are complex groups of protistan and metazoan organisms as well as fungal, bacterial and viral microorganisms that interact with fish as symbionts, commensals, parasites or pathogens [1]. Some of the organisms have been shown to be beneficial offering movement, digestion, eicosapentaenoic acid production, neuramindase and histamine production [2]. Microorganisms mainly bacteria play a role in the production of vitamin B 12 in the intestine of carp [3].

Freshwaters are the habitat of about 40% of the fish species [1]. The properties of natural water do not generally support the growth of microorganisms as they are void of habitats such as soil, plants or animals which are mainly natural habitats of microorganisms. Natural waters are oligotrophic and resident aquatic microorganisms are adapted to these conditions through biofilm formation. Microbes mainly occupy two main habitats in water: the air-water interphase while others sediment. Typical freshwaters were initially thought to be dominated by coliforms and *Proteus* bacteria as well as *Flavobacterium* and *Achromobacter spp.* [1]. However, molecular techniques mainly 16S rRNA sequencing have revealed more phyla of bacteria in water including alpha-, delta-, epsilon- and beta- proteobacteria, *Actinobacteria*, *Cytophaga* and cyanobacteria [4]. The significance of the microbial flora in the aquatic environment includes photosynthesis, sulphate reduction and metabolic activity in the nitrogen cycle [5]. Anthropogenic

activities introduce bacteria that are not natural in aquatic systems such as *Pseudomonas, Bacillus, Vibrio* and members of the Enterobacterioceae family.

According to Austin [6], the bacterial flora of fish is mainly determined by the water and feed and it comprises gram positive and gram negative bacteria. Also the surface tissues (the skin and gills) usually have a higher bacterial count than the internal organs. The digestive tract comprises a diversity of bacteria that are crucial in nutrition production for the fish. Internal organs such as liver, spleen and kidney are also found to be infected with bacteria even in healthy fish. Tissue such as the muscle is believed to be sterile while eyes of healthy fish are devoid of bacteria.

Studies on fish bacteriology have been carried out mainly focusing on human health implications associated with the fish bacterial flora after harvesting [7, 8]. A significant number of bacteria have been shown to cause disease in fish. Bacteria such as *Flexibacter, Flavobacterium, Edwardsiella, Yersinia* and *Aeromonas* spp, *Vibrio* spp, *Mycobacterium* spp, some members of the Enterobacteriaceae family such as *Proteus* spp and *Serratia* spp, and *Pasteurella* spp have been isolated from different organs of fish [9]. Bacteria associated with fish can be categorized into three groups: (i) indeginous bacteria (*Clostrodium botulinium*, pathogenic *Vibrio* spp. and *Aeromonas hydrophilla*), (ii) enteric bacteria that are present due to feacal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli* and *Staphylococcus aureus*), and (iii) bacteria introduced during processing, storage and preparation for consumption (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfrigens* and *Salmonella* spp.) [10]. The flora of fish is directly related to the health of the fish and also affects other animals in the ecosystem. The bacterial diseases of fish include fin rot, furunclulosis, hemorrhagic ulcers and septicemic disorders, vibriosis, yersiniosis, enteric septicemia, columnaris, mycobacteriosis, bacterial gill disease and bacterial kidney disease [11, 12].

Lake Chivero was constructed in 1952 by the impoundment of the Manyame River to supply water to Harare city. City of Harare as well as its satellite townships lies within its own catchment area discharging sewage and industrial effluent into the lake's main tributaries; the Manyame, Marimba and Mukuvisi rivers thus increasing the organic content of the lake. Eutrophication was reported in the lake as early as the 1960's [13]. In Zimbabwe, fish are distributed countrywide in lakes and rivers such as lakes Kariba and Chivero. Lake Chivero is of particular interest because it is located downstream of Harare city effluent outfall and has occasionally received untreated or insufficiently treated sewage and industrial waste [14] and is hypereutrophic [15]. The composition of dissolved nutrients such as dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) are very high, reported at 1,840 μgL^{-1}DOP [15]. Eutrophication can occur naturally (rainfall runoff) or can be due to anthropogenic activities (domestic and industrial waste). It drastically increases ammonia concentration leading to bioaccumulation and biomagnification of pathogenic organisms that are otherwise less concentrated in freshwater environments [16, 17] and this increases the prevalence of fish pathogenic and opportunistic bacteria which are usually low in clean aquatic systems.

Lake Chivero is a habitat of several cichlid fish species such as native (*Tilapia rendalli*, *T. sparrmanii* and *Pseudocrenilabrus philander*) and introduced (*Oreochromis* spp. and *Serranochromis robustus*) [18]. Several studies addressing water quality and fish parasitology [19] have been done quite extensively in the Manyame system including Lake Chivero. Microcystin levels in the lake were recorded at 19.9 μgL^{-1} exceeding the recommended level of 1.0 μgL^{-1} [20]. Blue green algae has been correlated to increase fish populations [21], however, massive algal blooms of up to 42.2 μgL^{-1} resulted in the fish kills from February 2003 to November 2004 [22]. Human pathogenic bacteria such as *E. coli*, *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp. and faecal *Streptococcus* were isolated from the lake [23]. Parasites including *Ascaris lumbricoides* eggs, *Entamoeba histolytica*, *Isospora beli*, *Taenia* spp. and *Schistosoma mansoni* were reported in the same study. The evidence of the diversity of these microorganisms in the Lake can significantly alter the microflora of fish.

The bacterial flora of fish in such a eutrophic environment was of particular interest in this study. This study was designed to investigate the bacterial flora (whether resident or transient flora) of *Oreochromis niloticus* in Lake Chivero. *Oreochromis niloticus* was introduced in the lake in the 1980's [24] and is highly invasive due to its wide range of trophic and ecological adaptations. *Oreochromis niloticus* is currently the most dominant fish species in the lake and can easily transcend other fish species. It dominates aquaculture worldwide (up to 72 % production annually), is essential for economic development and protein security [25] and is most preferred as food locally [26]. Bacteria such as *Streptococcus* spp. can lead to high fish mortalities if they infect internal organs such as the kidney [9, 27]. Furthermore, the fish from such a contaminated environment can be a source of infection to the public who so incessantly eat fish from the lake. The quantitative and qualitative estimation of the bacterial flora of *O. niloticus* was of importance as it ascertained the possible health implications associated with the bacterial diversity of *O. niloticus* on the fish as well as humans. The objectives of this study were to isolate and identify bacteria infecting the different organs of *O. niloticus* in Lake Chivero, to determine the prevalence of the bacterial isolates and compare their diversity and abundance on different organs. We hypothesise that the mean abundances of the bacterial isolates on different fish organs (skin, gills, muscle, intestine, liver and kidney) are the same.

MATERIALS AND METHODS

Study site

The study area, Lake Chivero is situated 32 km from Harare capital city; coordinates (-17.872781, 30.797493) (Figure 1). The lake has a shore length of approximately 48 km and a surface area of 2632 ha. The maximum depth is 27 m and the maximum width is 8 km with a capacity of 2.5 x 10^8 m^3 of water [28].

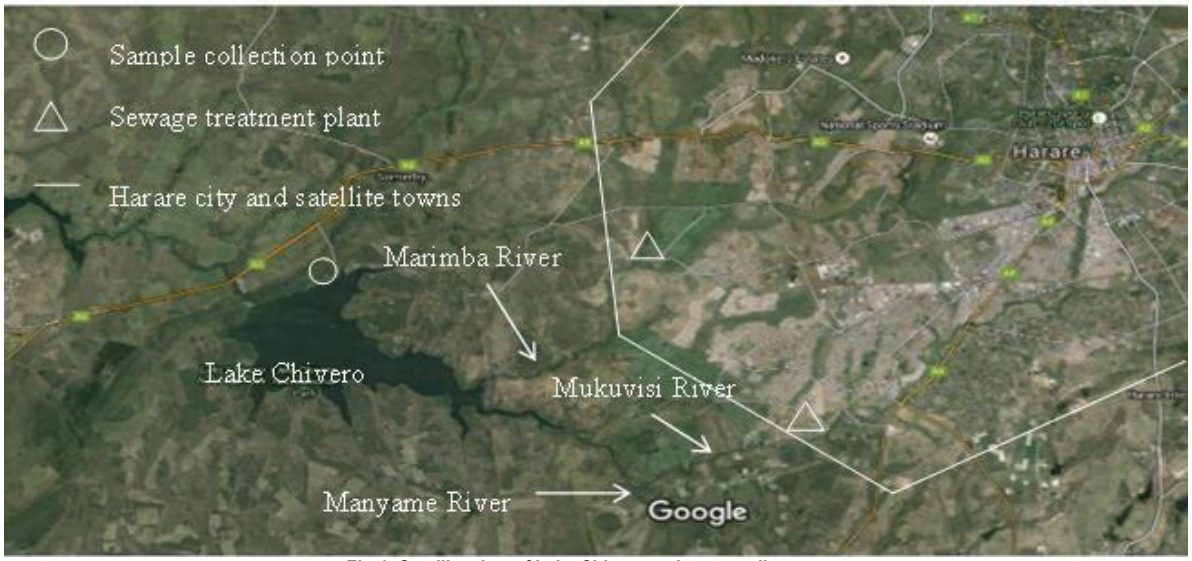

Fig-1: Satellite view of Lake Chivero and surrounding areas

Sample collection and preparation

A total of thirty (30) fish samples (*O. niloticus*) were collected in February 2016 at the fisheries at Kamba Caravan Park station situated at Lake Chivero (Figure 1). The fish were caught by the fisheries gill nets set over night on various locations around the lake. Immediately upon arrival of boats on the shore and before handling by vendors, fish samples were randomly selected, placed in sterile zip-lock bags separately, stored on ice and transported to the Central Veterinary Laboratory (Bacteriology section) at Division of Veterinary Services in Harare. Upon arrival at the laboratory, the samples were processed immediately for identification of bacteria.

For each fish sample portions of the skin (including scales and underlying epidermis tissue), gills, muscle, intestines, liver and kidney were aseptically cut and placed in separate universal bottles. The dissection of all samples was sequential; starting by the dissection of the skin followed by the muscle, gills, intestine, liver and finally kidney. One (1) gram portions of the skin (approximately 8 cm^2), muscle, gills, intestines and liver, and the entire kidney were removed and mixed in phosphate buffered saline (PBS) in a ratio of 1 part sample to 9 parts PBS to make a 10^{-1} dilution. The diluted specimens were then homogenized using the IKA® T25 Basic Ultra TURRAX® homogenizer (IKA, China). Serial dilutions were then performed in exponents of ten where needed.

Culture preparation and enumeration of colony forming units

The homogenate (100 µl) was then pipetted onto sheep blood agar (MAST, United Kingdom) prepared plates for the general culture of all bacteria and onto Listeria selective agar (MAST, United Kingdom) prepared plates for the selective culture of *Listeria* spp. and then spread evenly on the surface of the media. One (1) millilitre of the homogenate was pipetted onto the centre of chromogenic Compact Dry *Escherichia coli*Coliform ready to use plates (Nissui Pharmaceuticals, Japan) for the selective culture of *E. coli* and other coliforms. All plates were incubated inverted at 37 ± 2^0C for 24 ± 2 hours. All samples showing positive growth for *E. coli* were then cultured on chromogenic CHROMagar O157 (Life Technologies, France) prepared plates for the culture of *E. coli* O157:H7 by pipetting 50 µl of sample onto the surface of the media, spreading and followed by incubation at 37 ± 2^0C for 24 ± 2 hours.

The colony forming units based on the different colony morphological characteristics (colour, elevation, shape, structure, surface and type of haemolysis) observed on the different media were counted. Estimates of colony forming units per gram (cfu/g) were obtained using the formula:

$$\text{cfu/g} = \frac{(\text{cfu counted}) \text{ x } (\text{reciprocal of dilution factor})}{(\text{volume of homogenate plated, ml})}$$

The morphologically different colony forming units were then picked using a sterile straight wire and sub-cultured onto sterile blood agar plates by streaking to isolate pure cultures of bacteria. The plates were incubated at 37 ± 2^0C for 18 to 24 hours and the isolates obtained were used for the identification of bacteria.

Bacterial identification

Bacterial identification was carried out in two steps; primary identification and then secondary identification. Primary identification included macroscopic examination of the colonies, the Gram stain for cellular morphology identification, and primary biochemical tests (catalase and oxidase tests). The Bergey's Manual of Determinative Bacteriology [29] was then followed to identify bacteria to the species level where possible.

Secondary identification was carried out using media that incorporates biochemical tests, namely MacConkey agar (MAST, United Kingdom), triple sugar iron agar (TSI), Simmon's citrate, urease, indole, sugar fermentation tests and analytical profiling index (API 20 NE) kits. These tests were carried out where appropriate following the Bergey's Manual of Determinative Bacteriology identification flow chart.

Data summarization

The different isolates of bacteria identified were grouped based on the primary biochemical test results. Two other groups; total *E.coli* and total coliforms, were used to assess the levels of faecal and environmental contamination of the different organs respectively. The prevalence of the different bacterial isolates identified in the different organs was then expressed as percentages. The mean abundance of the bacterial isolates on all the samples per organ was calculated as well as the standard error of the mean and counts of isolates were presented as the mean count plus/minus (±) standard error of the sample mean.

Statistical Analysis

The distribution of the data for all organs and isolates was tested for normality using the Shapiro-Wilk test and was observed to be not normally distributed at 0.05 level of significance. As a result, a non-parametric analysis of variance (ANOVA), Kruskal-Wallis test for completely randomized designs was used to test the distribution of each isolate in the different organs separately at 0.05 level of significance. Where there was significance difference in the omnibus ANOVA, multiple comparison tests were conducted using the Conover-Inman test with a Benjamin-Hochberg (B-H) p-value adjustment [30, 31]. The B-H p-value adjustment method was used to control the false discovery rate (the expected proportion of significant results which are in fact false positive results). All statistical analysis and graphical presentations were carried out using R statistical software [32].

RESULTS

Isolation and identification of bacteria

A total of twelve (12) bacteria were isolated and seven (7) were identified to genus level, four (4) were identified to species level while one was identified to family level. There were five (5) notable groups to which these bacteria belonged based on the primary biochemical tests conducted. These were (i) Gram positive cocci-shaped catalase positive, (ii) Gram positive rod-shaped catalase positive, (iii) Gram negative cocci-shaped oxidase negative, (iv) Gram negative rod-shaped oxidase negative and (v) Gram negative rod-shaped oxidase positive bacteria (Table 1). The Gram negative rod-shaped oxidase negative (Enterobacteriaceae family) group was the most represented with six (6) members namely *E.coli* including *E. coli* O157:H7 serotype, *Citrobacter* spp, *Enterobacter* spp, *Klebsiella* spp, *Shigella* spp. and *Edwardsiella tarda.*

Table-1: Different groups of bacteria with representative members identified in *O. niloticus* in Lake Chivero (February, 2016).

Group	Primary biochemical tests	Bacteria identified
(i)	Gram positive, cocci-shaped, catalase positive	*Staphylococcus* spp.
(ii)	Gram positive, rod-shaped, catalase positive	*Bacillus subtilis*, *Listeria ivanovii* and unidentified isolate.
(iii)	Gram negative, cocci-shaped, oxidase negative	*Acinetobacter* spp.
(iv)	Gram negative, rod-shaped, oxidase negative	*E.coli* including *E. coli* O157:H7, *Citrobacter* spp, *Enterobacter* spp, *Klebsiella* spp, *Shigella* spp. and *Edwardsiella tarda.*
(v)	Gram negative, rod-shaped, oxidase positive	*Aeromonas* spp.

Prevalence of bacteria

The prevalence of bacterial isolated in the different organs is shown in Table 2 below. *Staphylococcus* spp. were most prevalent in primarily all organs ranging from 53% on liver to 100% on the skin while the *E. coli* O157:H7 serotype was not isolated from the intestines, kidney and liver.

Table-2: Prevalence (% infected, N=30) of the bacterial isolates on different organs of *O. niloticus* in Lake Chivero (February, 2016)

Isolate	Skin	Gills	Muscle	Intestine	Liver	Kidney
Acinetobacter spp.	60.0	33.3	66.7	20.0	60.0	60.0
Aeromonas spp.	6.7	33.3	13.3	20.0	33.3	26.7
B. subtilis	26.7	20.0	6.7	66.7	46.7	53.3
Citrobacter spp.	20.0	20.0	13.3	26.7	0.0	13.3
E. tarda	6.7	26.7	40.0	33.3	20.0	26.7
Enterobacter spp.	13.3	13.3	20.0	26.7	20.0	6.7
E. coli	93.3	86.7	73.3	10.0	3.3	0.0
E. coli O157:H7	13.3	16.7	6.7	0.0	0.0	0.0
L. ivanovii	46.7	33.3	33.3	40.0	0.0	0.0
Klebsiella spp.	13.3	0.0	20.0	33.3	46.7	40.0
Shigella spp.	66.7	46.7	26.7	0.0	0.0	0.0
Staphylococcus spp.	100.0	86.7	80.0	60.0	53.3	66.7
Unidentified	100.0	80.0	86.7	6.7	0.0	26.7

Enumeration of bacteria

The dominance in terms of grand means of the five groups indicated in Table 1 was as follows: group (iv) most dominant (4.706×10^4 cfu/g), to group (iii), to group (i), to group (v) and to group (ii) least dominant (5.183×10^3 cfu/g). The Enterobacteriaceae family (group iv) was subdivided further into the coliform group (Figure 2), namely *E.coli*, *Citrobacter* spp, *Enterobacter* spp, *Klebsiella* spp, and the non-coliform group namely *Shigella* spp. and *Edwardsiella tarda.* Coliforms were present on all organs, with highest total coliform counts on the skin ($2.778 \times 10^4 \pm 3.848 \times 10^3$ cfu/g) and lowest on the liver ($1.398 \times 10^2 \pm 2.384 \times 10^1$ cfu/g). *Escherichia coli* was present on all organs except the kidney, with highest counts on gills ($7.57 \times 10^2 \pm 4.042 \times 10^2$ cfu/g) and lowest on liver (4.333 ± 6.022 cfu/g).

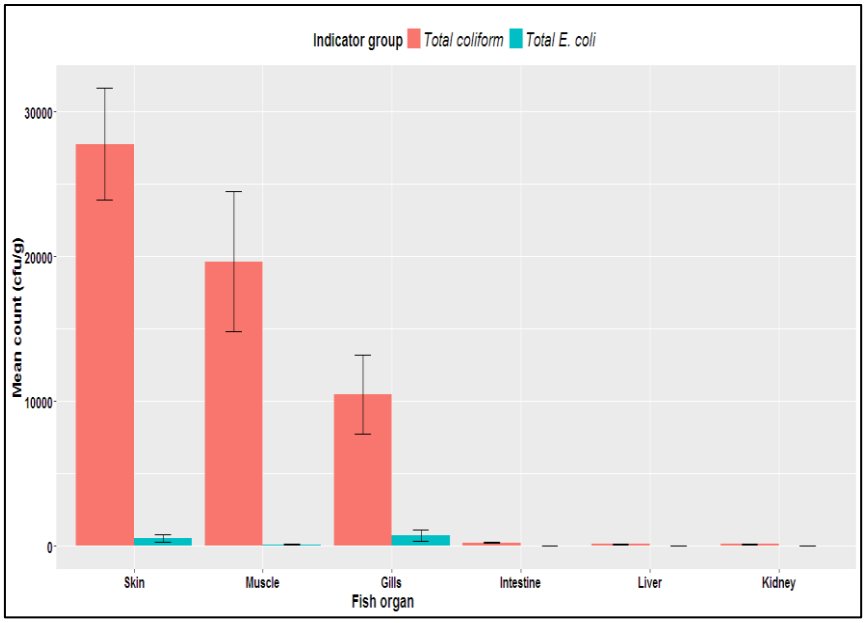

Fig-2: Mean *E.coli* and total coliform counts on different *O. niloticus* organs in Lake Chivero (February, 2016). Error bars represent the standard error of the sample mean

Staphylococcus spp. was isolated on all the organs and ranged from $8.03 \times 10^2 \pm 2.403 \times 10^2$ cfu/g on the liver to $1.46 \times 10^5 \pm 4.014 \times 10^4$ cfu/g on the skin, while *E. coli* O157:H7 serotype was least abundant ranging from zero cfu/g on intestines, liver and kidney to $2.4 \times 10^2 \pm 1.571 \times 10^2$ cfu/g on the skin (Figure 3).

Diversity of bacterial mean abundance

The diversity of bacteria isolated in this study varied between different organs as shown in Figure 3 below. The sum total of bacterial isolates ranged from 8.837×10^3 cfu/g on the kidney to 3.609×10^5 cfu/g on the skin and there was a significance difference ($p = 2.2 \times 10^{-16}$) of the mean abundances of isolates on all the organs. The skin and muscle had the greatest diversity of bacteria as they were infected with all the twelve isolates, while the kidney had the least diversity, not infected with *E. coli*, *L. ivanovii* and *Shigella* spp.

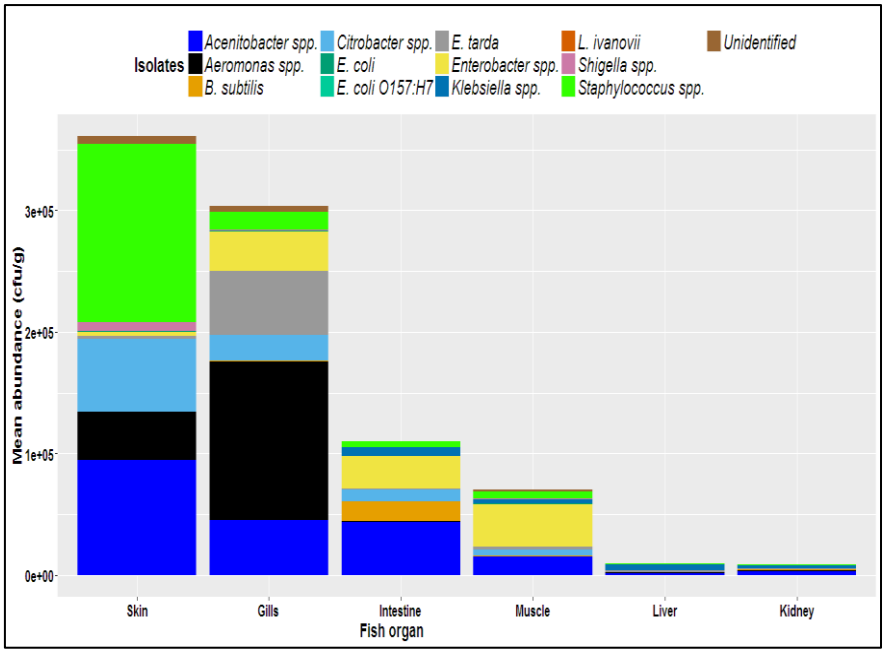

Fig-3: The bacterial diversity and abundance on the organs of *O. niloticus* in Lake Chivero (February, 2016)

A more specific analysis of the diversity and abundance of the bacterial isolates individually between the different organs is shown in Table 3 below. There was no significance difference ($p > 0.05$) in the mean abundance of *Aeromonas* spp, *Citrobacter* spp, *E. tarda* and *Enterobacter* spp. between the different organs. However, the mean abundances of these bacteria varied greatly. There were very high counts of *Aeromonas* spp. and *E. tarda* on the gills at $1.311 \times 10^5 \pm 4.39 \times 10^4$ cfu/g and $5.298 \times 10^4 \pm 2.8 \times 10^4$ cfu/g respectively, while low counts of these bacteria were recorded on the liver ($7.033 \times 10^2 \pm 3.611 \times 10^2$ cfu/g) and kidney ($3.3 \times 10^2 \pm 1.238 \times 10^2$ cfu/g) (Figure 3). *Enterobacter* spp. were very high on gills, intestine and muscle and lowest in the liver with counts of $3.23 \times 10^4 \pm 1.587 \times 10^4$ cfu/g, $2.725 \times 10^4 \pm 1.567 \times 10^4$ cfu/g, $3.477 \times 10^4 \pm 1.775 \times 10^4$ cfu/g and $1.167 \times 10^2 \pm 4.868 \times 10^1$ cfu/g respectively. In addition, highest counts of *Citrobacter* spp. were recorded on the skin ($6.0 \times 10^4 \pm 3.078 \times 10^4$ cfu/g) and not present on the liver.

Staphylococcus spp. had the greatest diversity and significance difference between the organs in which the skin had the greatest count followed by the gills and finally the kidney and liver (Table 3). The skin was in most cases the highly infected organ and was dominated by *Acinetobacter* spp., *Aeromonas* spp., *Citrobacter* spp., *Staphylococcus* spp. and the unidentified isolate. It was noted that internal organs namely, intestines, kidney and liver were least infected with bacteria except for the highest counts of *B. subtilis* on the intestines and of *Klebsiella* spp. on intestines, liver and kidney (Figure 3). The infection of the muscle with bacterial isolates was intermediate in most cases in relation to other organs (Table 3).

Table-3: Kruskal-Wallis test (p-value) for the isolates mean abundance variation between different organs of *O. niloticus* in Lake Chivero (February, 2016) and Conover-Inman multiple comparison tests with B-H p-value adjustment

Bacterial isolate	Kruskal-Wallis test	Conover-Inman test (Organs with significance difference of isolate)
Acinetobacter spp.	5.44×10^{-4}	S > G > I > M > K > L
Aeromonas spp.	5.508×10^{-2}	-
B. subtilis	2.34×10^{-5}	I > M > G > K > S > L
Citrobacter spp.	8.733×10^{-2}	-
E. tarda	7.493×10^{-2}	-
Enterobacter spp.	3.486×10^{-1}	-
E. coli	2.20×10^{-16}	G > S > M > I > L > K
E. coli O157: H7	1.32×10^{-2}	S > G > M > I, K, L
L. ivanovii	2.95×10^{-6}	S > I > G, M > K, L
Klebsiella spp.	5.09×10^{-4}	I > L > M > K > S > G
Shigella spp.	1.84×10^{-13}	S > M > G > I, K, L
Staphylococcus spp.	2.20×10^{-16}	S > G > M > I > K > L
Unidentified	2.20×10^{-16}	S > G > M > K > I > L

G = gills, I = intestine, K = kidney, L = liver, M = muscle and S = skin.

DISCUSSION

In this study it was noted that the most prevalent bacteria were not the most abundant except for the *Staphylococcus* spp. On the skin *Staphylococcus* spp. was most prevalent and also most abundant ($1.46 \times 10^5 \pm 4.014 \times 10^4$ cfu/g). On the gills, *E. coli* was most prevalent but *Aeromonas* spp. were most abundant ($1.311 \times 10^5 \pm 4.39 \times 10^4$ cfu/g) while the intestines were dominated by *Acinetobacter* spp. ($4.379 \times 10^4 \pm 2.749 \times 10^4$ cfu/g) although *B. subtilis* was most prevalent. The kidney was also dominated by *Acinetobacter* spp. ($3.057 \times 10^3 \pm 9.904 \times 10^2$ cfu/g) and *Staphylococcus* spp. were most prevalent, while in the liver *Acinetobacter* spp. were most prevalent and *Klebsiella* spp. were most abundant ($5.06 \times 10^3 \pm 2.358 \times 10^3$ cfu/g).

The most represented group of bacteria in this study was the Enterobacteriaceae family owing to its wide distribution in the environment including the soil, water and plants as well as in humans and animals alimentary canals [33]. Six (6) members of this family were associated with *O. niloticus* in Lake Chivero of which *Enterobacter* spp. were predominant (1.644×10^4 cfu/g) and *E. coli* O157:H7 was least dominant (5.444×10^1

cfu/g). The bacteria were isolated from all organs of the fish tested in this study and this was consistent with study by Hassan *et al*. [34], in which *Citrobacter* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp. and *Salmonella* spp. were identified on the gills, intestine, liver and kidney. Although the Enterobacteriaceae family was most represented group, *Acinetobacter* spp. and *Staphylococcus* spp. were most abundant and similar results were reported by Alyl *et al*. [35].

Members of the Enterobacteriaceae family, namely *Citrobacter* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp. and *Salmonella* spp., are generally harmless to fish and can be considered part of the bacterial flora of fish especially in eutrophic environments. However, the bacteria were reported to cause increased mortalities of *O. niloticus* of up to 43.3 % caused by *Enterobacter cloacae* though no clinical signs of infection were observed on the fish organs [36]. In this study the skin showed signs of infection namely reddening on the ventral surfaces of the fish (Figure 4).

Fig-4: Reddening of skin on the ventral surface of *O. niloticus* in Lake Chivero (February, 2016)

Escherichia coli and other coliforms were present on fish samples tested. The presence of *E.coli* and a count of not less than 10^1 cfu/g coliforms do not conform with the standards stipulated by the CODEX Alimentarius [37] and the Zimbabwe food standards regulations [38]. Although *E. coli* is part of the normal flora of humans and is harmless, certain strains of the bacterium are pathogenic and are responsible for gastrointestinal illnesses. The presence of *E. coli* and other coliforms in high numbers on the skin, gills and muscle organs of the fish (Figure 2) indicated that these organs are the most likely contaminated or infected with bacteria in the environment (water contaminated with sewage and industrial effluent). The presence of *E. coli* and other coliforms is not directly harmful but is a presumptive indication of faecal contamination and that other pathogenic bacteria especially enteric ones may be present [17, 39].

Escherichia coli was highly prevalent (greater than 70 %) and abundant (greater than 10^1 cfu/g) on the skin, muscle and gills of *O. niloticus* deeming the fish unfit for human consumption. Furthermore, human pathogenic *E. coli*, namely *E. coli* O157:H7 (an enterohaemorrhagic serotype) was isolated on the skin, muscle and gills increasing the risk of disease outbreak in consumers. In 2006, *E. coli* O157:H7 caused a diarrheal outbreak that resulted in over 205 illnesses and 3 deaths in the United States of America [40]. Other strains of *E. coli* such as enterotoxigenic, enteroinvasive, vero-cytotoxin producing and enteroaggregative O104:H4 have been the cause of fatal diarrheal illnesses such as the 2011 *E. coli* O104:H4 outbreak that claimed 48 lives with 857 haemolytic-uremic syndrome reported cases in Germany [41]. Other human pathogenic bacteria were also present, namely *L. ivanovii*, *Shigella* spp and *Staphylococcus* spp., and *E. tarda* is an opportunistic pathogen causing gastrointestinal illnesses [33]. Gastrointestinal

illnesses (nausea, vomiting, diarrhoea and abdominal pain) resulting from consuming contaminated food products are alarming causes of concern globally. Though the symptoms are usually mild and only lasting a few days and a small proportion is reported, over a million cases are reported annually resulting in thousands of deaths [42].

The external organs of *O. niloticus* had the highest load of bacteria (Figure 3) and this can be explained by the fact that these organs are constantly in contact with the environment which results in increased contamination and infection with bacteria. The skin was infected with all bacteria isolated and had the highest bacterial load. The underlying muscle tissue was also infected with all bacterial isolates though the bacterial count was lower than that of the gills and the skin. This suggests that bacteria present in the environment will infect the skin and end up in the muscle. The reduced counts of bacteria on the muscle are similar to the study by Mandal *et al*. [43]. One of the causes of the reduction of bacterial load in the internal organs mainly muscle, intestine, liver and kidney of *O. niloticus* is the up-regulation of genes encoding the expression of viperin, an anti-viral and anti-bacterial protein [44]. This was indicated by the reduction in prevalence and abundance of bacterial isolates from the skin to the kidney (Table 2, Figure 3). In this study, fish pathogenic bacteria were identified. *Edwardsiella tarda* is the major pathogen of *O. niloticus* causing edwardsiella septicaemia [27, 45]. It causes swelling of the abdomen due to ascites and haemorrhages on the body wall [46]. *Aeromonas* spp. are responsible for haemorrhagic septicaemia including reddening of the body wall and haemorrhages in the viscera and peritoneum [47, 48]. Some of the fish sampled in this study showed these symptoms (Figure 4). Some of the bacteria isolated in the present study have been listed as emerging opportunistic pathogens of fish [49] causing mortalities of up to 20% in trout and carp, and are well known for transmitting antibiotic resistance genes [50, 51].

The presence of large numbers of bacteria in freshly caught fish in the lake is a cause of concern. The concentration of bacteria increases to high levels that pose danger to consumers when improper handling and storage is practised [10]. At high concentrations, bacteria produce toxins that can lead to consumer illness. The major microorganisms involved are *Staphylococcus aureus* and *Clostrodium botulinum* [52]. *Staphylococcus* spp. were the most dominant and prevalent microorganism on the skin and muscle in this study and could result in diseases such as neonatal meningitis and mastitis in humans [51]. These bacteria give evidence that the lake is heavily polluted by sewage effluent among other pollutants. A few years ago the Harare City Council banned the consumption of Lake Chivero fish due to an outbreak of typhoid fever in Harare and other areas [53].

The diversity of fish pathogens (*Aeromonas* spp. and *E. tarda*) were found to be more prevalent on internal organs whereas opportunistic pathogens were highly prevalent on most organs (Table 2). This suggests that *O. niloticus* is at risk of disease from these bacteria. Members of the Enterobacteriaceae family and human pathogenic bacteria are more prevalent and significantly abundant on the skin and muscle, organs that are handled and consumed, increasing the risk of disease outbreak in consumers. *Oreochromis niloticus* in Lake Chivero is thus a potential source of cross contamination of other food products especially those consumed uncooked from which many food poisoning cases are reported [10].

Streptococcus spp. has been identified in Lake Chivero water [23]. However, none of the fish sampled in this study were infected with *Streptococcus* spp. This bacterium is one of the etiological agents of bacterial kidney disease together with *Renibacterium salmoninarum* in Nile tilapia [10]. Kidney infection with *Streptococcus iniae* can results in devastating mortalities of fish [54].

Lake Chivero is infested by aquatic parasites [23] which are prevalent on fish [19]. The presence of parasites can result in concurrent infection of fish with parasites and bacteria. A study has shown that concurrent infection of fish with *Gyrodactylus niloticus* and *S. iniae* results in *O. niloticus* mortalities of greater than 40% in contrast to 7% and 0% due to *S. iniae* and *G. niloticus* alone respectively [55]. In the same study the parasite was shown to harbour viable bacteria after several cycles of infection suggesting that the parasite was a potential mechanical vector of the bacterium. This can also be the case in Lake Chivero where both parasites and bacteria are prevalent which could result in increased concurrent infections and mortalities.

In the current study there were some important limitations. Anaerobic bacteria could not be identified due to the specialized equipment required for sample collection, namely sterile air-free containers and anaerobic transport medium [33]. Thus, in this study the bacterial flora described includes aerobic and facultative aerobic bacteria only, although obligate anaerobic bacteria such as *Clostrodium* spp. and *Eubacterium* spp. are also associated with fish [9]. A culture-based technique was used in this study and it has disadvantages which mainly include out-competition of slow growing microorganisms by fast-growing ones and the failure to identify unculturable microorganisms [46]. Several microorganisms are extremely fastidious and require specialized supplemented media for growth in the laboratory [56]. In the current study only one such bacterium, *L. ivanovii*, was cultured on listeria selective media.

CONCLUSIONS

The abundance and diversity of bacteria varied between different organs and this indicates stressful environmental conditions that may negatively impact the populations of *O. niloticus* in the lake. The results indicated *O. niloticus* to be heavily infected with bacteria some of which are known to be pathogenic to fish and others pathogenic to humans. The fish therefore do not satisfy the food safety standards stipulated by the CODEX Alimentarius and are deemed unfit for consumption. Individuals who consume fish from Lake Chivero are therefore at risk of illness especially children, the elderly and immunocompromised individuals.

Consumers that intend to eat the fish should be certain that the fish are thoroughly cooked to reach an internal temperature of 100^0C for a minute [52]. Though disease outbreak is self-limiting, people that consume fish from Lake Chivero are at a greater risk of suffering from infections due to exposure to the different pathogens isolated.

Additional Points

RECOMMENDATIONS

Measures should be put in place to remove water hyacinth plants in Lake Chivero and invest in sewage treatment in order to reduce the nutrient load in the lake which may indirectly increase the environmental stress for the fish and more likely to cause fish to suffer from various bacterial diseases. Certain forms of *Staphylococcus* spp. have high antibiotic resistance, antimicrobial susceptibility tests of bacteria associated with *O. niloticus* in Lake Chivero should thus be carried out to ascertain the danger that may be encountered during treatment of humans [57].

Molecular-based techniques including polymerase chain reaction, 16S rRNA and reverse transcriptase sequencing should be employed to carry out the molecular systematics of bacteria associated with the fish [58]. This will enable identification of all bacterial groups including unculturable ones as well as avoid the challenges that may be encountered when culturing anaerobic bacteria. However, culture-based methods may still give valid results provided several different media that target different genera of bacteria are used [59]. These genera include bacteria such as *Salmonella* spp., *Vibrio* spp., and *Campylobacter* spp. which are often associated with fish [10] and fish pathogenic bacteria such as *Flavobacterium* spp. and *Flexibacter* spp. that require cytophaga agar to be cultured [27].

Data Availability

The data used to support the findings and conclusions of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this publication.

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