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Bacterial Flora of Nile Tilapia of Pond Fish and Their Relationship with Predisposing Factors

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Abstract

A cross sectional study was conducted in pond water Nile tilapia (*Oreochromis niloticus*) fish in Sebeta National Fisheries and Aquatic Life Research Center for bacterial isolation and identification. About 133 different types of samples: 100 tissue sample of Nile tilapia (25 samples for each gill, Kidney, skin and intestine), 13 pond water samples, 10 sediments of ponds and 10 fish feed samples were included in the study from four ponds to observe bacterial load of the fish and bacterial infection in relation with pond water, pond sediments and fish feeds. Out 20 bacterial species isolated 14 belongs to Gram negative while 6 isolates belongs to Gram positive organisms. Among the tissue samples of skin, gill, intestine and kidney examined, *Aeromonas salmonicida (atypical) and Aeromonas hydrophila* were present in majority of the fish samples. The bacterial load in intestine is comparably higher than the bacterial load from other organ like skin and gills. Additionally some organisms like *A. hydrophila, Flavobacter spp., Vibrio spp, E.coli*, which are facultative pathogens or agents of food pathogens, were also isolated from fish sample. In conclusion identification of bacteria from pond water, pond sediments and fish feed samples shows that they are important sources of bacterial infection for fish. The present finding of bacterial species in different tissues of fish suggests endow with appropriate control methods for prevention of bacterial causing diseases in fish.

Keywords: Bacteria, *Oreochromis niloticus*, Pond, Sebeta

1. Introduction

The economic importance of fish is greater today than ever before and is steadily growing (FAO, 2004). Fish is a vital source of food for people and contributes about 60% of the world's supply of protein (Canonico *et al.*, 2005). 60% of the developing countries derive 30% of their annual protein from fish (FAO, 1997; Abisoye *et al*., 2011). Ethiopia being a country with stunted growth of children, fish undoutfuly will have a huge contribution in meeting protein demands.

Tilapia is a native African fish from Egypt, East and Central Africa, and as far west as Gambia, which showed the fish held in ornamental ponds (FAO, 2012). The fish *Oreochromis niloticus* belongs to the family Cichlidae, about 16 tilapiine species have been used for aquaculture production out of which ten species are commercially farmed (FAO, 2004). Since 1984, global tilapia production is dominated by three species: the Nile tilapia *O.niloticus* (L.), the Mozambique tilapia *Oreochromis mossambicus* and the blue tilapia *Oreochromis aureus* (Bailey, 1994; Rana, 1997).

Today Nile tilapia, by far, is the most important groups of farm raised food fish around the world, especially in the tropical and semi-tropical areas,

representing more than 80% of total tilapia production (Trewavas, 1991; Fitzsimmons, 2001). The demand for tilapia is much acceding supply and that's why prices of tilapia remain high. Another important advantage is the reproduction and production ability of the species (Bailey, 1994). In spite of the fact that in Ethiopia despite favorable physical conditions aquaculture is almost none existing but fishing still depends on inland water bodies (Nuru, 2012) recognizing the importance of fisheries, the Ministry of Agriculture and Rural Development (MoARD) and the Sebeta Fish Culture Station now called the National Fisheries and other Aquatic Life Research Center (NFALRC) of the Ethiopian Institute of Agricultural Research (EIAR) have been made a huge effort for the development of fisheries in reservoirs such as Koka, Fincha, Melka Wakena and several others could be mentioned as success stories (Abegaz,2010).

The health status of aquatic organisms is uniquely related to their immediate environments, which can contain very high concentrations of microorganisms. Many of these microorganisms are saprophytic, some are pathogenic and both types are capable of infecting fish when conditions become favorable for multiplication (Rodricks, 1991).

In Ethiopia to the author's knowledge this is the first study investigating the bacterial diversity in the environment and fish and because of this the literature available is scarce if not none existent. In other countries studies of this kind have been conducted elsewhere and different results have been recorded.
Given the country has a huge potential in pond fish which will complement the existing water bodies fish resource, there is an urgent need to understand fish pathogens and their interaction with the fish habitat under different agro-ecological zones of Ethiopia. This study was therefore conducted to isolate and identify bacterial flora from apparently healthy fish and pathogenic bacteria from diseased cultured fish; to isolate and identify bacteria from fish habitat and feed and assess the degree of similarity between the bacterial community of fish (*O.niloticus*) and that of the test pond's water and sediment and feed and to describe the physico-chemical characteristics of the pond.

2. Materials and Methods

2.1 Description of the study area

The study was conducted at National Fisheries and Aquatic Life Research Centre (NFALRC) which is located about 24 km south-west of the capital city, Addis Ababa. The center is Located 855' 0.012"N, 3837' 0.120"E, at an altitude of 2200m above sea level, the mean annual temperature of the area is about 20°C with relative humidity of 60% and annual rainfall ranges from 860mm-1200mm bimodal rainy season (Zenebe, 2012). In NFALRC, there are 38 research ponds of which 12 are concrete walled ponds and the remaining 20 are earthen ponds. Each concrete pond is constructed from concrete with 100 m^2 area. Five of the earthen ponds have 200 m^2 and two of them have each 900 m^2.The remaining 13 earthen ponds were 100m^2 each. For the purpose of these study four earthen ponds was selected having 100m^2 an average depth of 1.5m with stocking density of three fishes per meter square (Getnet, 2014).

2.2. Study population

The center has got five different exotic and indigenous fish namely Nile tilapia (*O.niloticus*), Tilapia (*Tilapia zilli*), African catfish (*Clarias gariepinus*), Common carp (*Cyprinus carpio*) and Gold fish (*Carassius auratus*). Each species of fishes were stocked with separate ponds. Nile tilapia are known to feed on phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus (FAO, 2012).

2.3 Study Design

A cross sectional study was done for isolation and identification of bacteria from apparently healthy fish and environment. For the pathogenic bacteria, study follow up was conducted daily by the farm attendant and researcher every two weeks for occurrence of any clinical disease and this was done by longitudinal study. The study was conducted during November 2013 to March 2014, where fish, feed and environmental samples were taken four times from four ponds. So a total of 20 live fishes, five dead fish, 12 water samples, ten sediment samples and ten feed samples were sampled from selected four earthen ponds. Data on physicochemical and other factors was also collected.

2.4 Sampling strategy

The number of fish to be sampled per pond was based on an epidemiological sample size determination as described in Fisheries and Oceans Canada (1984); which takes into account population size, assumed prevalence and confidence level where the minimum sample per pond was to consider particularly for bacterial flora study. Therefore, a maximum of six to seven fish per pond was sampled once from each pond. Fish were caught using seine nets.

2.5 Physico-chemical parameters

Data on water quality was collected prior to sample collection and this included temperature, pH, dissolved oxygen and conductivity by thermometer, pH meter, oxygen meter and conductivity meter (Brian, 2006; Environmental Review, 2008). Bacterial water quality was assessed by standard coli form unit following standard procedures (Annex 5).

2.6 Sample collections

2.6.1 Fish samples

Apparently healthy fish were transported in buckets that have been partly filled with water and oxygen and transported to National Animal Health Diagnostic and Investigation Center (NAHDIC) bacteriology laboratory. Fish were killed on the bench and opened and aseptically skin, intestine, kidney and gill samples were collected and inoculated the same day to avoid overgrowth by dominant bacteria. From sick and recently dead fish the surface was first sterilized and an incision was made from behind the gills and to the mid-line and cut along the mid-line towards the anus. The flap of flesh lifted to expose the internal organs. Selected organs were removed aseptically and they were chopped and inoculated onto the plates (Buller, 2004).

2.6.2 Water, sediment and feed samples

Pond water samples were collected in sterile universal bottles (30ml) 15-20 cm below the water surface. Pond sediment samples were scooped out from water and put in sterile universal bottles and transported to NAHDIC bacteriology laboratory. Similarly fifty g of feed samples were taken with sterile universal bottles from feed. (Al-Harbi, 2003).

2.6.3 Morphometric measurements

Total length (cm) and total weight (gm) of each fish was measured before the postmortem examination to know the body condition of the fish (Bwanika *et al.*, 2004). The sex of the fish was also recorded, each sample was dissected ventrally with the aid of a small scissors inserted through the vent, and also semi-circular cut was made laterally on the side of fish for better observation. The gonads which are two parallel tubules located on the dorsal wall of the abdominal cavity were then examined with the naked eye. The males have gonads with smooth exterior, while the females have gonads with a rough exterior. (Holden and Raitt, 1974*;* Olurin ,2006).

2.7 Bacterial count from pond water

Bacterial count of the pond water was conducted as previously described (Al-Harbi and Uddin 2007). Briefly appropriate serial dilutions were made from 10^{-1} to 10^{-6} with sterile physiological saline (0.85% w/v). Aliquots of 1ml of the serial dilutions were inoculated on to petri dish containing tryptone soya agar plates (TSA) in duplicates and incubated at 28^{o}C for 48 hours. Colony forming units (CFU) including pin point sizes were counted with colony counter.

2.8 Bacterial isolation and identification from samples

Bacterial isolation and identification was conducted following procedures for isolation of bacteria from fresh water as described by Buller (2004). Briefly all specimens were inoculated on to Blood agar and incubated at 25^{o}C for 2-5days. If separate isolated colonies where found another sub culture was done on nutrient agar. Some cultures were streaked onto nutrient broth and after overnight incubation; they were sub cultured on blood agar plates. Some bacterial grown good in blood agar plates are shown no to poor growth on nutrient agar, so they are cultured on TSA plates (those of *A. salmonicida* species). From selected young colonies of both nutrient and TSA agar representative pure colonies were taken for preliminary identification of bacteria like Gram's reaction, catalase, oxidase, oxidation-fermentation (O-F), motility, growth on MacConkey agar. After conducting primary identification of bacteria at genus level, those old cultures are revived on brain heart infusion broth, and then followed by selected biochemical tests that included Indole, Urea hydrolysis, Aesculin hydrolysis, Methyl red (MR),

Voges-Proskauer (VP), Lysine decarboxylation (LDC), Triple Sugar Iron (TSI), carbohydrate fermentation like Salicine, Lactose, Arabinose, Sorbitol, Mannitol, Sucrose and Trehalose.

2.9 Statistical analysis.

Data were recorded, checked and coded on Microsoft Excel spreadsheet (Microsoft Corporation) and SPSS statistics version 20 was used for descriptive analysis.

3. Results

3.1 Physicochemical parameters

The physicochemical parameters (mean ±standard deviation) in the studied ponds at different locations of a pond are presented in Table 1. During sampling period water temperature ranged from 17.50± 0.36^{0}C to 21.63± 0.35^{0}C. Dissolved oxygen is vital for survival of pond fish and it varied from 5.43±0.54 to 7.59±0.12 mg/L. The pond water pH was from slightly acidic to slightly alkaline and ranged from 5.84±0.25 to 8.56±0.12, and the total conductivity ranged from 108.63± 0.52 to 145.71±3.31 mg/l.

Table 1:-Temperature, pH, dissolved oxygen and conductivity in water from each of the four ponds.

Pond	Temperature(^{0}C)	DO(mg/L)	pH	Conductivity (mg/L)
1	17.86 ± 0.45	7.59 ± 0.29	8.56± 0.12	123.20 ± 1.40
2	17.50 ± 0.36	5.43 ± 0.54	6.10± 0.28	145.71 ± 3.31
3	21.63 ± 0.35	5.86 ± 0.20	5.84± 0.25	108.63 ± 0.52
4	19.85 ± 0.41	5.80 ± 0.40	8.43± 0.14	123.12 ± 0.55

DO: dissolved oxygen.

3.2 Morphometric measurements

Total length and total weight of each fish to the nearest cm. and gm. was measured to know the body condition of the fish. Sex of each fish was also recorded. (Table 2)

Table 2: Morphometric parameter of the fish samples

Pond	Weight of fish (g)		Height of fish (cm)	
1	22.12	±2.13	196.90	± 51.52
2	25.70	± 3.78	309.60	± 185.27
3	20.63	± 2.05	151.08	± 37.52
4	22.18	± 1.67	188.73	± 34. 37

3.3 Bacterial count of pond water

Among the pond water examined, there was variation in the bacterial count from inlet, centre and outlet among the same pond and different pond (Table 3).

Table 3: Bacterial count of different pond water from different locations

Pond	Inlet (CFU)		Center (CFU)		Outlet (CFU)	
1	3.70	$\pm 2.82\times10^5$	1.05	$\pm 2.12\times10^5$	2.60	$\pm 7.07\times10^5$
2	1.70	$\pm 16.97\times10^5$	2.35	$\pm 10.6\times10^5$	3.30	$\pm 12.72\times10^5$
3	5.75	$\pm 13.43\times10^5$	8.65	$\pm 6.36\times10^5$	8.40	$\pm 24.04\times10^5$
4	7.85	$\pm 24.74\times10^5$	3.85	$\pm 14.84\times10^5$	3.90	$\pm 2.82\times10^5$

3.4 Bacterial isolation

About 132 (100 tissue samples of 25 tilapia fish, 12 water samples, 10 sediment of ponds and 10 feed samples) different types of samples processed in the laboratory for bacterial isolation. A total of 14 Gram negative and 6 Gram positive bacteria were isolated from apparently health fish and pond habitat. Bacterial isolates from the pond water, sediment, feed, organ of fish were identified to species level as much possible.

Bacteriological examination of the various organs in fish revealed variation in bacterial presence among different organs of fish. Eight microflora common to all the fishes contains six Gram negative bacteria: *A.hydrophilia, A.cavai, A.salmonicida, Klebsiella pneumonie, Plesiomonas shigelloides and vibrio spp.* and two Gram positive bacteria; *Staphylococcus epidermidis* and *Corynebacterium phocae.*

Gram negative rod shaped bacteria dominated all populations. From those bacteria *A. salmonicida, A.hydrophilia, P. shigelloides, Vibrio spp. and staphylococcus epidermidis* were most frequently isolated bacteria and common to all four ponds. Other bacterial species which were also isolated but not present in all the ponds were *Flavobacterium spp. Pseudomonas angulliseptica, Pseudomonas aeroginosa and Corynebacterium pseudodiphtheriticum.*

3.5 Comparison of bacterial isolates from different samples

When the bacterial isolates from different samples (Fish, water, sediment and feed) were compared *Aeromonas hydrophilia* (24.8%), *Aeromonas salmonicida* (24.8%), *Coryenebacterium spp (14.3%), Vibrio spp (12.8%) and Aeromonas cavie* (10.5%) were found in majority of the samples. (Table 4)

Among the Gram negative bacteria *Aeromonas salmonicida* (17.86 %) is present in most of the bacterial isolates while *Pseudomonas aeruginosa* (0.51%) was presents the least (Figure 1).

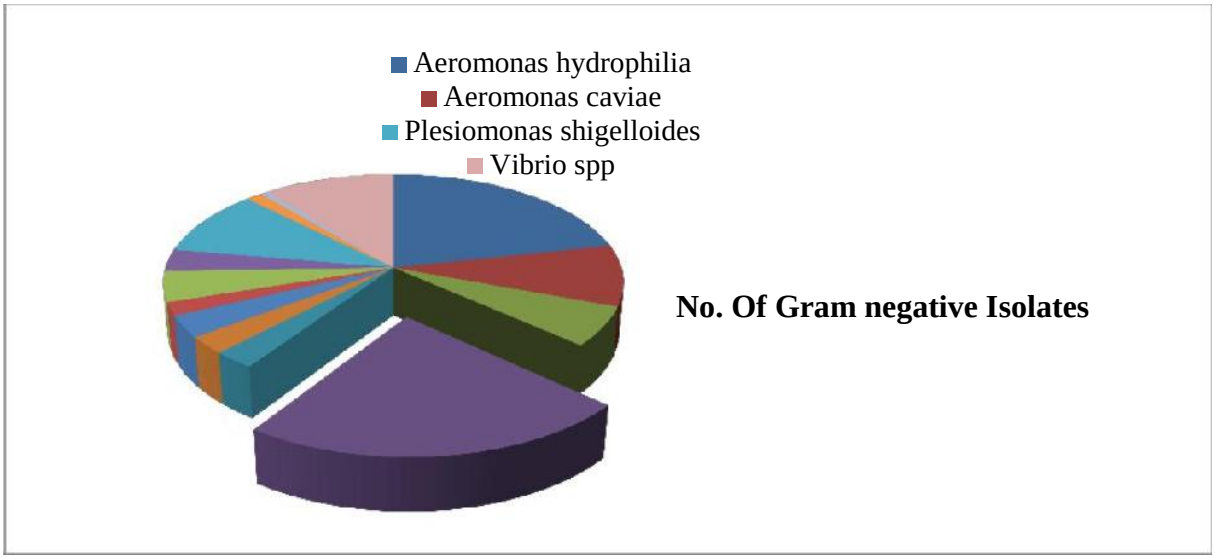

Figure1:-Total of Gram Negative bacterial isolates from different samples

Table 4: Total of bacterial isolation of different samples

Bacteria	Organ of fish			Water			Sediments			Feed			Total		
	No	**+ ve**	**%**	**No**	**+ ve**	**%**	**No**	**+ ve**	**%**	**No**	**+ ve**	**%**	**No**	**+ ve**	**%**
A. hydrophila	100	27	27	13	6	46.2	10	0	0	10	0	0	133	33	24.8
A. cavie	100	11	11	13	3	23	10	0	0	10	0	0	133	14	10.5
A. salmonosida	100	32	32	13	1	7.7	10	0	0	10	0	0	133	33	24.8
A. sobria	100	9	9	13	0	0	10	0	0	10	0	0	133	9	6.8
C. frundi	100	3	3	13	1	7.7	10	0	0	10	0	0	133	4	3
E. tarda	100	4	4	13	0	0	10	0	0	10	0	0	133	4	3
E. coli	100	2	2	13	1	7.7	10	0	0	10	2	20	133	5	3.8
Flavobacterium spp	100	3	3	13	0	0	10	0	0	10	0	0	133	3	2.3
K. pneumonia	100	6	6	13	1	7.7	10	0	0	10	0	0	133	7	5.3
Pastuerella Spp	100	2	2	13	2	15	10	2	20	10	0	0	133	6	4.5
P. shigelodes	100	12	12	13	3	23	10	0	0	10	0	0	133	15	11.3
Pseudomonas Spp	100	3	3	13	0	0	10	0	0	10	0	0	133	3	2.3
Vibrio spp	100	13	13	13	2	15	10	2	20	10	0	0	133	17	12.8
Bacillus spp	100	4	4	13	0	0	10	0	0	10	0	0	133	4	4
Coryenebacterium spp.	100	15	15	13	0	0	10	2	20	10	2	20	133	19	14.3
Staph. Epidermis	100	7	7	13	1	7.7	10	1	10	10	2	20	133	11	8.3
Staph warnari	100	3	3	13	3	23	10	0	0	10	3	30	133	9	6.8

Among Gram positive bacteria isolated from different samples *Staph. Epidermis* (5%) and *Corynebacterium phocae* (4%) are present in most of the samples. (Figure 2)

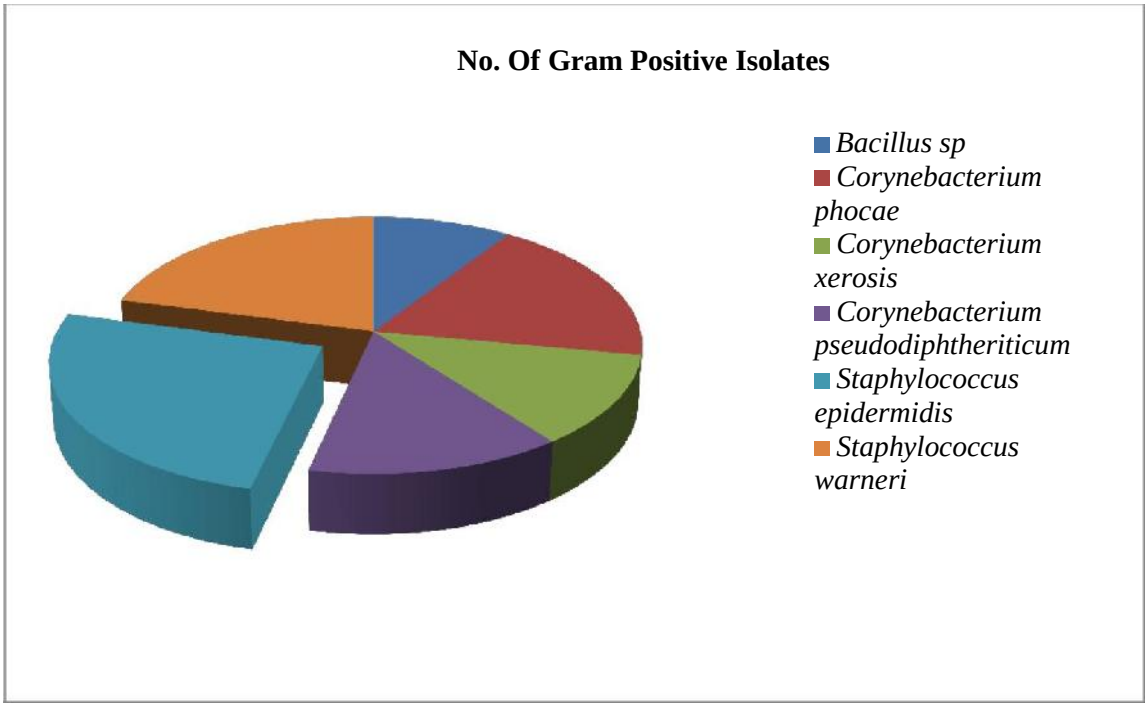

Figure 2: Total of Gram Positive bacterial isolates from different samples

Among the total 196 isolates, 158 (80.61%) isolates are from fish while 25 (12.76%), 8 (4.08%) and 5 (2.55%) belongs to water, feed and sediment samples. (Figure 3)

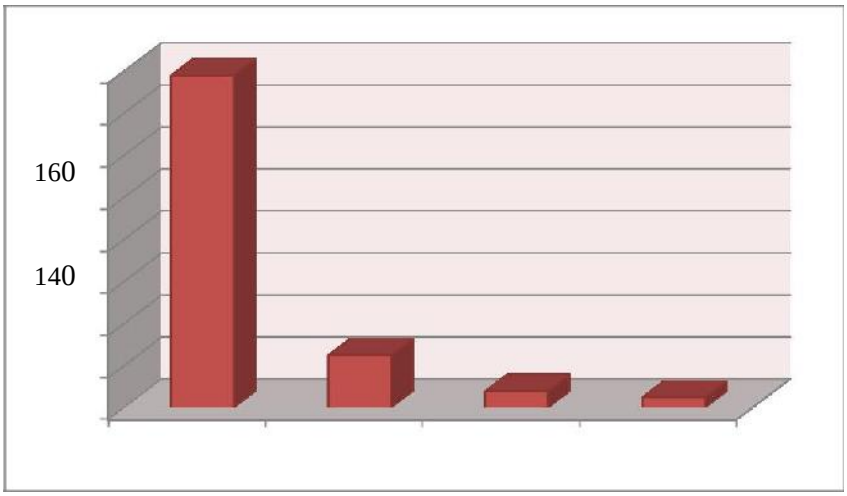

Figure 3: Total no. of samples

Among the 158 isolates from fish samples, *Aeromonas salmonicida* (17.34%) and *Aeromonas hydrophilia* (13.27%) are dominant among Gram negative bacteria while *Staph. Epidermis* (3.5%) and *Corynebacterium phocae* (3.5%) are dominant among Gram positive bacterias. *Vibrio spp* (6.6%) and *Plesiomonas spp* (6.12%) are also present in some fish samples (Table 5 & 6).

Table 5: Total no. of Gram negative isolates from fish samples

Gram Negative bacteria	No. Of isolates	% of Isolates
Aeromonas hydrophilia	26	13.26
Aeromonas caviae	11	5.61
Aeromonas sobria	9	4.59
Aeromonas salmonicida	34	17.34
Citrobacter frundii	3	1.53
Edwardsiella tarda	4	2.04
Escherichia coli	2	1.02
Flavobacterium spp.	3	1.53
Klebsiella pneumonia	6	3.06
Pasteurella Spp.	2	1.02
Plesiomonas shigelloides	12	6.12
Pseudomonas anguilliseptica	2	1.02
Pseudomonas aeruginosa	1	0.51
Vibrio spp.	13	6.63
Total	128	65.30

Table 6: Total no. of Gram positive isolates from fish samples

Gram Positive Bacteria	No. Of isolates	% of Isolates
Bacillus spp.	4	2.04
Corynebacterium phocae	7	3.57
Corynebacterium xerosis	3	1.53
Corynebacterium pseudodiphtheriticum	6	3.06
Staphylococcus epidermidis	7	3.57
Staphylococcus warneri	3	1.53
Total	30	15.30

3.6 Bacterial isolates from different organ of the fish

Among the bacterial isolates,*A.hydrophila*, *A.Salmonosida*, Coryenebacterium Spp and Plesiomonas Spp are dominant bacterial species in all the organs of the fish. While Vibrio spp (20%) are predominant in gills compared to other organisms. Similarly *A. hydrophila* (32%) is in intestines, *A. salmonosida* (16%) in kidney. (Table 7)

Table 7: Bacterial isolate from different organ from fish samples

No	Bacteria	Skin		Gill		Intestine		Kidney	
		+ ve	**%**	**+ ve**	**%**	**+ ve**	**%**	**+ ve**	**%**
1	*A. hydrophila*	10	40	7	28	8	32	2	8
2	*A. cavie*	4	16	4	16	2	8	1	4
3	*A. salmonosida*	10	40	12	48	6	24	4	16
4	*A. sobria*	0	0	0	0	6	26	3	12
5	*Citrobacter spp*	1	4	2	8	0	0	0	0
6	*Edwardsella tarda*	0	0	2	8	2	8	0	0
7	*E.coli*	0	0	1	4	0	0	1	4
8	*Flavobacter spp.*	0	0	2	8	1	4	0	0
9	*K. pneumonia*	3	12	0	0	2	8	1	4
10	*Pastuerella Spp*	0	0	2	8	0	0	0	0
11	*Plesiomona spp*	5	20	3	12	4	16	0	0
12	*Pseudomonas Spp*	1	4	2	8	0	0	0	0
13	*Vibrio spp*	2	8	5	20	5	20	1	4
14	*Bacillus spp*	0	0	1	4	2	8	1	4
15	*Coryenebacterium Spp*	7	28	4	16	1	4	3	12
16	*Staph epidermis*	3	12	2	8	0	0	2	8
17	*Staph warneri*	1	4	2	8	0	0	0	0

4. Discussion

The physicochemical parameters were within a range suitable for tilapia culture except for the temperature of the pond. But variations observed in the parameters of the four ponds studied could be attributed of the influences of the micro-climatic conditions of fish ponds in the area. Since the water sample was collected at different period, there was variation in the bacterial loads from different ponds. This could be due to the reason the temperature of water was close to optimum for growth of many bacteria.

The isolation and identification of bacteria from fish and its environment is essential to establish strategies to prevent and combat disease. In this study the presence of high bacterial load in the fish and its environments were observed. Analysis of the relationship between the microbial content of the pond water and the fish organs revealed the isolation of similar types of bacteria in both environments. *Aeromonas hydrophilia, Aeromonas salmonicida, E. coli, Pasteurella spp. and Staphylococcus spp.* were observed in parallel in fish and water. This is an indication that the surrounding water has an influence on the composition of the microflora of the fish. Similar observation were reported by Al-Harbi and Uddin (2007)

The frequencies of bacterial isolates were shown no significant variation between sex groups and among the three different location of the water within one pond. But there was significant difference was observed between ponds. This result is in partial agreement with the reports of (Silva and Widanapathirana, 1984; Ogbondeminu, 1993). This variation probably due to the water quality of the pond was deteriorated and it is important to note that all these variation in results may occur because of differences attributed to the nature of the ponds from which the fish were recovered.

In this study bacterial distribution in different organs and tissue of fishes showed significant variation that the presence of bacterial load in different organs. Bacterial load is more in intestine compared to other organs. The results are in agreement with the previous finding (Sugita *et al*., 1985; Chowdhury *et al.,* 1989; Ogbondeminu *et al.,* 1991; Al-Harbi and Uddin, 2004).

Aeromonas spp., Citrobacter spp., E.coli, Pasteurella spp., Plesiomonas spp., pseudomonas spp., vibrio spp. and Staphylococcus spp. are bacterial species isolated from the gill of the fish during the study period, this may be due to that, the surface and gills of fish are populated by a diverse array of bacteria and the most important health problems are related to gill conditions and thought to be the route of infection for a variety of pathogens. The bacterial flora on the fish surface reflects the aquatic environment in which they are reared (Shewan and Hobbs, 1967, Austin, 1993).
In this study there are also numerous types of bacteria isolated from the kidney of fish. This is in concordant with many authors. The isolation of bacteria species from the kidney of apparently healthy fish have frequently been reported (Apun *et al*., 1999; Eshetu, 2000; Gebeyehu, 2003). However Fitzsimmons (2001) described that the presence of microorganisms in internal fish organs could indicate the breakdown of immunological defense mechanisms. It was also noted that bacterial species were isolated from the kidney, although the internal organs of healthy fish should be sterile (Sutine *et al.*, 2007).

Snieszko(1974) have pointed out that the occurrence of an infectious agent in a fish could not be necessarily an abnormal event or it will not lead to a disease situation. Moreover, that under natural conditions, most infectious agents coexist with their host without causing disease. However stress factor are frequently blamed for the incidence of many disease outbreaks and dissemination of infection. As in this present study, there was an outbreak in the fishes of earthen pond four by *Aeromonas salmonicida.*

In the present study, high bacteria load in skin, gill and intestine may be due to the high metabolic activity of fishes associated with increased feeding rates at higher temperatures. The bacterial flora of pond water sediments reflects the bacterial composition of the gills and intestine. It should be noted that gill disease may be initiated by opportunistic bacteria already resident in gill surface. The present study revealed that potential disease causing bacteria such as *A. salmo nosida*, *A. hydrophila*, *Plesiomonas spp*, *Coryenebacterium spp* (Austin & Austin, 1993) were present in significant numbers.

Isolation of *A. hydrophila, Flavobacter spp., Vibrio spp, E.coli*, which are facultative pathogens or agents of food pathogen, is of importance. Although the presence of these bacteria is not often associated with fish disease or enteric disease in humans, the health implications of the introduction of these organisms into natural water via the fish feces in the aquaculture water should not be ignored. The presence of other bacteria such as *Pleisomonas shigelloides* can pose some risk to consumers. This bacterium have been implicated in a number of outbreaks of sporadic and epidemic gastroenteritis, particularly in tropical and subtropical countries (Pilar Hernandez and De Garcia, 1997).

5. Conclusions and Recommendations

Different bacterial species were identified from Gill, Skin, intestine and kidney of *O.niloticus* fish. During this study the water samples, pond sediments and fish feed were also examined and different bacteria were isolated. About 14 Gram negative and 6 Gram positive bacterial species were isolated from different organs of the fish and its environments. Of these *Aeromonas hydrophilia, Aeromonas salmonicida, Aeromonas cavie, Plesiomonas spp., Vibrio spp Coryenebacterium spp*.and *Staphylococcus spp*. were the major bacterial species isolated, but some bacterias like *Pseudomonas spp., Flavobacterium spp. Bacillus spp, Edwardisella tarda, Citrobacter frundii* and *Pasteurella spp* were less frequently isolated. Types of bacteria isolated in pond water, sediments and feed samples were also isolated in fish. Identification of the bacteria from feed, sediments and pond water shows that they are important sources of bacterial infection for fish. Apart from this some organisms like *A. hydrophila, Flavobacter spp., Vibrio spp, E.coli*, which are facultative pathogens or agents of food pathogens, were also isolated from fish sample.

Therefore, the present study results indicate that the bacterial flora of apparently healthy *O.niloticus* can be influenced by the microflora of the aqueous environment. The commensal bacterial flora included facultative pathogens which under conditions of stress could give rise to fish epizootics or even human diseases (zoonosis). With the information obtained in this study, the knowledge of microflora of the *O.niloticus* culture system will help in the management aspects, by enabling recognition and correction of the abnormal conditions that can be a prelude to the onset of bacterial fish disease or any zoonosis.

The present finding of bacterial species in different tissues of fish and its habitats suggests that appropriate control methods for prevention of bacteria causing diseases in fish should be taken care. Therefore it is important to provide appropriate hygienic condition

for fish feed and water sources were important to prevent bacterial contamination from feed and water sources. The construction of concrete walled ponds is also advisable to prevent sediment (soil) based bacterial infection of fish. It is crucial to regulate the bacterial load in aquaculture system by adopting management practices like regular water exchange and feed regulation in order to safeguard against infectious agents.

Conflicts of Interest: The authors should declare the absence of conflicts of interest.

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