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Comparative Study on Bacterial Load in Intestine, Gills and Skin of Cultured African Catfish (*Clarias gariepinus)* from Different Locations in Rivers State, Nigeria

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Abstract: *Comparative study on bacterial load in intestine, gills and skin of cultured African catfish (Clarias gariepinus) from different locations (Agip; Aluu and Woji) all in Rivers State were evaluated. The result obtained indicated that the specie Streptococcus accounts for the highest bacterial occurrence (42.9%) and Pseudomonas spp. and E.coli. had the lowest (7.14%). In all the three locations under consideration the highest bacteria (0.0129±0.0040x10^5 CFU/g) was found in the intestine of the fish sampled from Aluu environment, while the lowest (0.0046±0.0019 x10^5 CFU/g) was recorded in the skin of fish sampled from Agip estate. The results of this study revealed a higher degree of bacteriological contamination in different organs of C. gariepinus fish in all the locations. The presence of these pathogenic organisms in these samples of fish could pose a serious threat and hazard to the consumers. Hence, C. gariepinus fish should be processed properly before consumption.*

Keywords: *Micro-biology, Catfish, Aquaculture, Environment, Rivers State*

1. Introduction

Fish and seafood constitute an important food component for a large section of world population (Wafaa *et al.,* 2011). The Food and Agricultural Organization (FAO) (1994) cited by Emikpe *et al.* (2011) asserted that fish contributes about 60% of the world supply of protein and that 60% of the developing countries derives more than 30% of their animal protein from fish. The annual domestic fish production in Nigeria is estimated at 600,000 metric tons per annum while the annual consumption is estimated at 2.66 million metric tons per annum. The deficit or gap is taking care of by importation of fish from outside the country spending ₦100 Billion annually in fish importation (FDF, 2010). Currently the country spends an estimate of ₦125 billion on 1.9 million tonnes of fish per annum. The total demand for fish in the country is 2.7 million tonnes ad which 800,000 tonnes are currently produced locally. The deficit of 1.9 million tonnes is met by imports (FDF, 2014). The need to increase our domestic production and an attempt to reduce the amount of money spent on fish importation can therefore not be over emphasized. Many Nigerians have recognized that Nigeria has market for fish and dived into fish production through aquaculture.

In trying to do this, the African Catfish (*Clarias gariepinus*) is a choice culture fish because it a hardy fish, a delicacy of consumers, and commands good price. The African catfish (C. *gariepinus*) has been reported to be a very important freshwater fish in Nigeria and also the aquaculture industry in Nigeria mainly involves African catfish production (FDF, 2007). It has enjoyed wide acceptability in most parts of are implicated.

Fish acts as an important food vehicle for some zoonotic pathogens such as *Salmonella* and vibrios and the contamination of fish with pathogens is a major public health concern. However, consumption of fin fish and shell fish may also cause disease due to infection or intoxication. Some of these diseases have been specifically associated with pathogen which are resistant to antibiotics (Adebayo – Tayo *et al*., 2012a; Edun *et al*., 2015) and this poses a great risk to human health. Although only a few infectious agents in fish are able to infect humans some exceptions exist that may result in fatalities.

Fish and shell fish not only transmit disease to man but are themselves subject to many diseases and are capable of transmitting many of the established food borne microbial infections and intoxications.

Fish take a larger number of bacteria into their gut from water sediment and food (Adeleye *et al.,* 2010). It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria, particularly the coliform group. Faecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish (Adebayo-Tayo *et al.*, 2012b).

The consumption of fresh African catfish (C. *gariepinus*) is on the increase in both rural and urban centers in Nigeria (FDF, 2007; Emikpe *et al*., 2011). However, there is dearth of information on the microbial load in African catfish (C. *gariepinus*) sampled from ponds. Thus, this study is designed to provide information on bacteria organisms that are found in the gills, intestine and on the skin of *Clarias gariepinus* from ponds of different cultured environment in Rivers State.

2. MATERIALS AND METHODS

Collection of Samples

Twelve live *C. gariepinus* ranging from 30.0cm to 43.2cm in length and 290.2g to 468g in weight were collected from fish ponds of private owned fish farms in Omuike in Aluu, Agip and Woji areas of Rivers state. The fishes were caught using a drag net and were taken immediately to the laboratory for analysis.

Preparation of Media

Nutrient Agar was used for the isolation of bacteria from the fish samples and it was obtained commercially in powdered form. The media was prepared according to the manufacturers guide; 28.0g was dissolved in 1L of distilled water and sterilized by autoclaving at 121°c for 15 minute. The media was allowed to cool and then poured into sterile disposable petri dishes and allowed to solidify.

Preparation of Samples/ Microbiological Analysis

The fishes were first killed and their length and weight were measured. The fish skin was which was placed on a clean foil and weighed to get 1g of skin; 1g of intestine and 10g of gills. The fish tissues where then put into 9ml of distilled water to give 1:10 dilution and shaken thoroughly. 1ml of the pond water samples was also pippeted into 9ml of distilled water to also give 1:10 dilution. The stock solution was serially diluted up to 10^{-5} as described by Willey *et al.* (2008). Plating (spread plate method) was done by inoculating 0.1ml of the dilution on nutrient agar in duplicate plates using 10^{-4} and 10^{-5} and spreading with a sterile glass spreader, the plates were then incubated for 18-24 hours at ambient temperature. The plates were examined after incubation and the number of colony forming units (CFU) that developed were counted and recorded.

Isolation

Isolation of the colonies was done by sub-culturing representative colonies on a freshly prepared nutrient agar. This was then incubated at 31^{0}C for 24 hours to obtain pure cultures.

Characterization/Identification of Isolates

The characterization of the organisms was based on colonial, morphological and biochemical characteristics of colonies. Macroscopic examination of surface colonies on nutrient agar medium was used to determine the colour, edge, elevation, surface, shape and arrangement of microorganisms. Morphological characteristics were studied on the oil immersed slide under the microscope after gram staining.

Gram's Stain

The gram staining technique was used to differentiate the gram positive from gram negative isolates based on the gram staining technique described by Christian Gram in 1884 (Willey *et al*., 2008). The principle of the test is based on the cell wall properties of the two bacterial classes. A smear of the isolate was made and fixed on a grease free slide and passed over a flame. Firstly crystal violet was poured on the smear which was rinsed off after one minute; lugos iodine (which is the mordant) was then poured on the smear and rinsed off after one minute. Few drops of ethanol was then used to decolorize the smear which was rinsed off after 5 seconds. Lastly, fuchsin (which is the counter stain) was poured on the smear and was rinsed off after one minute. The slide was air dried afterwards, immersion oil was then dropped on the slide (a drop) which was placed under the microscope and viewed using a magnification of x100.

Biochemical Test

The Biochemical tests that were carried out on the bacteria isolates were Catalyst, Coagulase Motility, Oxidaze, Sugar fermentation, Methyl-Red and Voges-Proskauer test.

Catalase Test

This test detects aerobic bacteria based on the present of enzyme catalase that convert hydrogen peroxide to water and oxygen. It is used to differentiate *Streptococcus* catalase negative from *Staphylococcus* catalase positive and *Bacillus* catalase positive from *Clostridium* negative (Willey *et al*., 2008). A loop full of the organism test was smeared on a clean grease-free slide, drop of 3% hydrogen peroxide was added to it. Presence of effervescence to indicate hibernation of oxygen is a positive test while no effervesence is a negative test.

Coagulase Test

This test detects the enzyme coagulase that causes plasma to clot. It is an important test used in the differentiation of *Staphylococcus aureus* from *S. epidemidis* (Willey *et al*., 2008). The slide coagulase method, as described by Ogbulie *et al.,* (2001) was used. A drop of plasma was dropped on a slide and a loopfull of the isolate was added to the plasma on the slide and the mixture rocked for 10 seconds to observe for clumps that depicts a positive test.

Motility Test

This test as described by Cheeseborough, (1985) is said to separate motile from non-motile bacteria based on the knowledge that motile bacteria "swarm" in semi solid agar to give a diffuse spreading growth that is easily detected by the naked eye. 7.25g of nutrient agar was dissolved in 500ml of distilled water. The mixture was boiled and stirred for proper dissolution and 10ml was dispensed into test tubes and autoclaved at 121°C for 15 minutes. The medium was allowed to become semi-solid in the test tubes and overnight culture was stabbed into the medium and incubated at 37°C for 24 hours. A positive test is indicated by a swarming movement away that is maybe at the top or bottom from the stab line while a negative test would remain in the stab line.

Oxidase Test

This test depicts the presence of oxidase enzymes in the isolates that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye (Tetramethy – p – phenylenediamine) to reduce the dye to deep purple (Cheeseborough, 1985). The wet filter paper method was used. A strip of filter paper was soaked with oxidase reagent and placed in a petri dish and a speck of culture smeared on it using glass rod. Deep intense purple colour depicts a positive test while no colour change is negative.

Sugar Fermentation Test

This test shows the ability of microorganisms to ferment certain sugars. Three sugars were used; two disaccharides (lactose and maltose) and one monosaccharide (glucose) (Cowan, 1974). 3g of peptone powder was dissolved in 180ml of distilled water in appropriately labeled conical flasks. 0.1g of phenol red was dissolved in 50ml of distilled water and 2ml of the resulting indicator solution dispensed into each conical flask and shaken thoroughly. The solution was dispensed in 5ml amounts into test tubes with inverted Durham's tubes and autoclaved for 15minutes. The test tubes were then inoculated with loop ful of test organisms and incubated for 24 hours. The test was observed for acid production leading to colour change (red to yellow) as well as gas production that causes the displacement of the liquid in the inverted Durham's tubes which indicates a positive test.

Methyl-Red Test

This test was employed to check the ability of microorganisms to produce sufficient acid during glucose fermentation and conditions such that the pH of an old culture is sustained below a value of 4.5 (Cheeseborough, 1985). Buffered glucose broth was prepared by dissolving 5g each of peptone and dipotassium hydrogen phosphate (K_2HPO_4), dispensing 5ml amount into test and autoclave at 121°C for 15 minutes. 10% glucose was prepared by dissolving 5g of glucose in 50ml of distilled water and sterilized by boiling for 6 minutes. 0.25ml of the resulting glucose solution was added to each tube and inoculated with the organism. The set up was incubated at 37°C for 48 hours after

which 4 to 5 drops of methyl red reagent was added to the solution, shaken and read at once. Positive reaction is indicated by a bright red colour while a yellow colour shows a negative test.

Voges-Proskauer Test

The principle of this test is the fact that bacteria ferment carbohydrates with the production of Acetyl methyl carbinol ($C_4H_8O_2$) or its reduction product 2, 3-Butylene glycol ($C_4H_{10}O_2$) (Carton, 1993). Buffered glucose broth was inoculated with the test organisms and incubated at 37°C for 48 hours. Three 3ml of alpha-naphthol and 1ml of 40% potassium hydroxide (KOH) were added and the mixture properly shaken. A colour change to pink depicts a positive test while no colour change depicts a negative test.

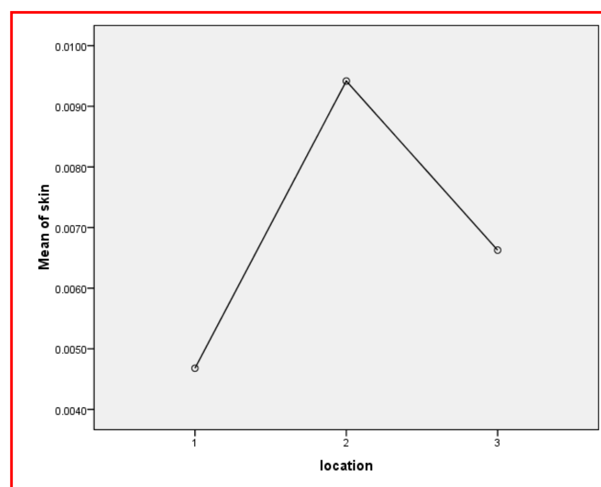
3. Results and Discussion

All fish samples examined in this study looked physically healthy based on their appearance but they were all infected with the "ever –ubiquitous" bacteria. The bacteria load of all skin, intestine and gills of all the fish sampled from the different locations showed that the fishes are contaminated. Table 1 shows the microbial load found in different parts of *C. gariepinus* from the ponds of the different locations. There was no significant difference ($p<0.05$) in the bacteria load in the skin, gills and intestines from Agip, Aluu and Woji. However, fish from Aluu had the highest bacteria count in the skin (0.0094±0.0033 x 10^5cfu/g). This was followed by fish from Woji (0.0066±0.0102 x 10^5cfu/g) and then Agip (0.0046±0.0019 x 10^5cfu/g). For gills, fish from Agip had the highest bacteria count (0.0255±0.0128 x 10^5cfu/g). This was followed by fish from Woji (0.0109±0.0030) and then Aluu (0.0073±0.0030). For intestine, fish from Aluu had the highest bacteria count (0.0129±0.0040). This was followed by Woji (0.0094±0.0030) and then Agip (0.0079±0.0026).

Table 1. *Bacteria count found in different parts of C. gariepinus*

Location	N	Skin (10^5 CFU/G)	Gills (10^5 CFU/G)	Intestine (10^5 CFU/G)
Agip	12	0.0046±0.0019	0.0255±0.0128	0.0079±0.0026
Aluu	12	0.0094±0.0033	0.0073±0.0030	0.0129±0.0040
Woji	12	0.0066±0.0102	0.0109±0.0030	0.0094±0.0030

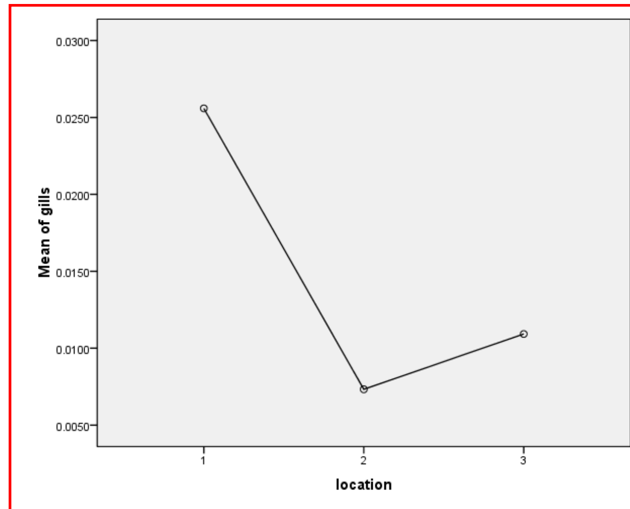
The gills had the highest bacteria count (0.0255±0.0128 x 10^5cfu/g) while the skin had the least bacteria count (0.0046±0.0019 x 10^5cfu/g) for fish from Agip. The intestine had the highest bacteria count (0.0129±0.0040 x 10^5cfu/g) the gills had the least had the least bacteria count (0.0073±0.0030 x 10^5cfu/g) for fish from Aluu. The gills had the highest bacteria count (0.0109±0.0030 x 10^5cfu/g) while the skin had the least bacteria count (0.0066±0.0102 x 10^5) for fish tissues from Woji. These contamination was less in contrast with the work done on wild and cultured *C. gariepinus* in Ibadan by (Emikpe *et al*., 2011) and also with the work done on *C. gariepinus* in Abeokuta by (Oladosu *et al*., 2011). This is higher than the set standard for the acceptable level of microbiological safety of foods which is 1-100cfu/g (Ayinla *et al*., 1994).



Key: Location 1 Agip; Location 2 Aluu ; Location 3 Woji

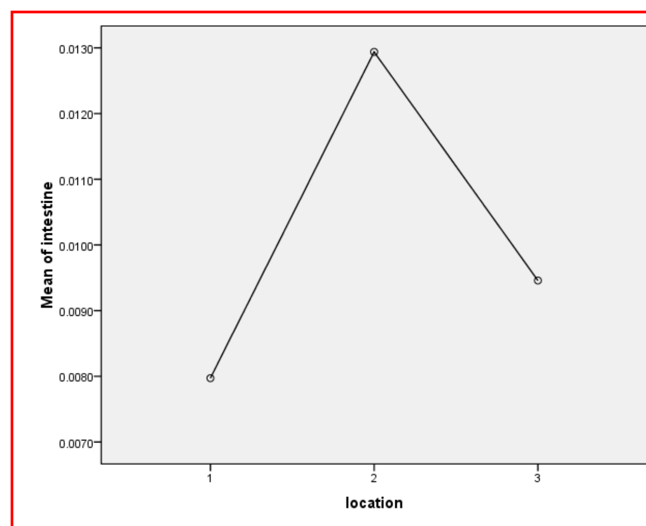
Figure 1. *Incidence of bacteria load on the skin of C. gariepinus from the different locations*

Most bacteria species were identified, present in *Clarias gariepinus (Table 2), which include both pathogenic and normal flora. These bacteria species found in the tissues of C. gariepinus in* this study were similar to the ones isolated in cultured *C. gariepinus* by Emikpe *et al*. (2011) and Oladosu *et al*. (2011). The occurrence of these bacteria species in different organs of fish (Figures1 to 3), could be an indication of presence of certain predisposing as handling of the fish, feeding of the fish, changing water as at when necessary and also cleaning and disinfecting the ponds. Some normal floral of humans such as *Staphylococcus sp*. *Streptococcus* sp were found predominant in the fish tissues from the different location. This could be based on the obtainable practices in the fish farm of the various locations such as handling could have introduced these bacteria species.



Location 1 - Agip ; Location 2- Aluu; Location 3- Woji

Figure 2. *Incidence of bacteria load in the gills of C. gariepinus from the different locatio*



Location 1 - Agip ; Location 2- Aluu; Location 3- Woji

Figure 3. *Incidence of bacteria load in intestines of C. gariepinus from the different location*

Table 2. *Diversity and incidence/occurrence of Bacteria in C. gariepinus*

S/No	Bacterial Species	Frequency of Occurrence	% Occurrence
1.	Pseudomonas sp	2	7.14
2.	Streptococcus sp	9	32.1
3	Bacillus sp	3	10.7
4	*E.coli*	2	7.14
5	Staphylococcus sp.	12	42.9
TOTAL		**28**	**100**

The isolates were characterized using various characteristics as tested by biochemical test, plate colonial morphology. The bacteria identified are shown in Table 3.The bacteria isolates identified in *C. gariepinus* include: *Staphylococcus sp, Streptococcus sp, Pseudomonas sp, Bacillus sp and Escherichia coli.* The coagulase *Staphylococcus* positive species are the species with the broadest pathogenic potential for causing infection of the skin, deeper tissues and organs, pneumonia, enteritis and pseudomembranous enterocolitis and food poisoning. In contrast to the coagulase *Staphylococcus* positive species members of the heterogenous group of coagulase negative *Staphylococci* (CNS) are regarded as less pathogenic bacteria (Efiuwewere and Ajiboye, 1996; Ogbulie *et al*., 2007). The presence of enteric organisms such as *E. coli* is particularly an indicator of fecal contamination in water bodies (indicator Organisms) (Willey *et al*., 2008). Contamination with *E. coli* in fish tissue in Aluu could be explained as a result of runoff. Some strains of *E. coli* are capable of causing food borne disease, ranging from mild enteritis to serious illness leading to death. There's a risk that pathogenic strains of *E. coli* may be present in pond water when animal manure such as bovine used in fertilizing ponds (WHO 1997).

Table 3. Characterization of Bacteria Isolates

S/N	COLONIAL MORPHOLOGY							BIOCHEMICAL TEST										ISOLATED ORGANISM
		Pigmentation	Surface	Edge	Elevation	Shape	Arrangement	Gram Reaction	CAT	COA	MOT	OXI	GLY	LAC	MAL	MR	V.P	
1	AGFS	Yellow	Glossy	Entire	Raised	Round	Cocci in clusters	+	+	+	-	-	+	+	+	+	-	*Staphylococcus sp*
2	AGFIN	Creamy	Wrinkled	Undulated	Slightly raised	Irregular	Rod	+	+	-	+	-	+	+	+	+	-	*Bacillus sp*
3	AGFG	Creamy	Smooth	Undulated	Raised	Irregular	Rods	-	+	-	+	-	+	+	+	+	-	*E.coli*
4	AGFG	Yellow	Glistering	Entire	Covex	Round	Cocci in chains	+	-	-	-	-	+	+	+	+	-	*Streptococcus sp*
5	AGFG	Cream	Glossy	Entire	Raised	Round	Cocci In Clusters	+	+	+	-	-	+	+	+	+	-	*Staphylococcus sp*
6	AGFIN	Yellow	Glistering	Rhizoid	Flat	Irregular	Cocci in chains	+	-	-	-	-	+	+	+	+	-	*Streptococcus sp*
7	AGFIN	Cream	Glistering	Serrated	Convex	Irregular	Cocci in chains	+	-	-	-	-	-	+	+	-	-	*Streptococcus sp*
8	AGFIN	Pink	Glistering	Entire	Slightly raised	Irregular	Cocci in chains	+	+	+	-	-	-	+	+	-	-	*Streptococcus sp*
9	AGFS	Cream	Dull	Entire	Flat	Irregular	Rod	+	+	-	+	-	+	+	+	-	-	Bacillus sp
10	AGFG	Cream	Glistering	Rhizoid	Flat	Irregular	Cocci in chains	+	+	-	+	+	-	+	+	+	-	*Streptococcus sp*
11	AGFG	Yellow	Dull	Serrated	Flat	Round	Cocci in clusters	+	+	+	-	-	+	+	+	+	-	*Streptococcus sp*
12	AGFIN	Cream	Dull	Entire	Raised	Round	Cocci in clusters	+	+	+	-	-	+	+	+	-	-	*Streptococcus sp*
13	AGFS	Cream	Glistering	Entire	Convex	Round	Cocci in clusters	+	+	-	-	-	-	+	+	+	-	*Streptococcus sp*
14	ALFS	Cream	Glossy	Serrated	Flat	Round	Cocci in clusters	+	+	-	-	-	-	+	+	+	-	*Streptococcus sp*
5	ALFIN	Cream	Wrinkled	Entire	Slightly	Irregular	Rod	+	+	-	+		+	+	+	+	-	Bacillus sp

					raised													
16	ALFG	White	Glistering	Entrire	Convex	Round	Cocci in chains	+	-	-	-	-	-	+	+	-	-	*Streptococcus sp*
17	ALFG	Yellow	Glossy	Serrated	Raised	Round	Cocci in clusters	+	+	+	-	-	-	+	+	+	-	*Streptococcus sp*
18	ALFIN	Cream	Dull	Entire	Flat	Irregular	Rods	-	+	-	+	-	+	+		+	-	*E.coli*
19	WFS	Pink	Dull	Entire	Raised	Irregular	Rods	-	+	-	+	+	-	+	+	-	-	*Pseudomonas sp*
20	WFS	Write	Glistering	Entire	Convex	Round	Cocci in chains	+	+	-	-	+	-	+	+	+	-	*Streptococcus sp*
21	WFS	Cream	Glistering	Entire	Convex	Irregular	Rods	-	+	-	+	=	+	+	+	-	-	*Pseudomonas sp*
22	WFG	Cream	Dull	Entire	Flat	Round	Cocci in clusters	+	+	-	-	-	-	+	+	+	-	*Staphylococcus sp*
23	WFG	White	Glistering	Serrated	Convex	Irregular	Cocci in chains	+	+	-	-	+	-	+	+	+	+	*Streptococcus sp*
24	WFG	Cream	Glossy	Serrated	Raised	Round	Cocci in clusters	+	+	+	-	-	+	+	+	-	-	*Staphylococcus sp*
25	WFS	Cream	Glossy	Round	Flat	Round	Cocci in clusters	+	+	+	+	-	-	+	+	+	-	*Staphylococcus sp*
26	WFS	Yellow	Glistering	Round	Convex	Round	Cocci in chains	+	+	-	-	-	+	+	+	+	-	*Streptococcus sp*
27	WFIN	Cream	Dull	Entire	Raised	Irregular	Cocci in cluster	+	+	+	-	-	+	+	+	+	-	*Staphylococcus sp*
28	WFIN	Yellow	Glossy	Entire	Raised	Round	Cocci in clusters	+	+	+	-	-	+	+	+	+	-	*Staphylococcus sp*

Key : AL-Aluu; AG- Agip; W-Woji; F-Fish; Cat- Catalyst; Coa- Coagulase; Mot- Motility; Oxi-Oxidase; Glu-Glucose; Lac-Lactose; Mal- Maltise; M.R-Methylred; V.P-Voges-Proskauer

The high bacteria load in these fishes as a source of proteins to humans, poses a hazard to the consumers health as some of the isolated species as *Bacillus sp, Staphylococcus,* are noted for very severe disease of man while some other species such as *Pseudomonas, E. Coli* may cause diseases in certain fish species such as Tilapia or be a source of zoonosis to humans (Edun *et al*., 2007).

4. CONCLUSION AND RECOMMENDATION

This study have shown that fish samples from the different locations were all contaminated with most bacteria species which include normal flora as well as the pathogenic forms of bacteria. The isolation of these organisms from the tissues of *C. gariepinus* is worrisome because of their potential in causing ill-health to human. It is okay to assume that these organisms might be introduced into the ponds by human healthy carriers through handling. Based on this study which identified the presence of bacteria organisms it is therefore recommended that better aquaculture practices be employed so as to reduce the chances of these bacterial contamination. The sanitary conditions under which fishes are reared in ponds should be improved by following standard practices such as use of good quality water free of contamination, treatment of organic manure before introduction into ponds, the use of feed free of contaminants regular draining of pond after specific period of time etc. the microbial load of fish can also be improved through regular disinfection of working equipment, brief immersion of the fishes in disinfecting solution such as brine water to reduce the microbial load on the fish before storage or before it is sold to the public for consumption. Before consumption, cooking of fish properly should also be done, as heat kills most of the microorganism if not all.

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