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Bacteriological and Elemental Quality of *Clarias gariepinus* (cat fish) Samples from River Lavun, Bida Niger state, Nigeria

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: River Lavun, located near Bida is a source of domestic water for the populace. Fish from this river is consumed by general populace. Polluted river water contaminates its fish, it is therefore, necessary to evaluate bacteriological and elemental quality of fish from this river which have been reported earlier to be polluted.
Objective: To evaluate the bacteriological and elemental quality of fish from River Lavun, with a view of assessing its potential health hazard.
Methodology: Three live cat fish (*Clarias gariepinus*) were obtained from River Lavun monthly for six months for analyses. Using standard methods, microbial load was determined, while susceptibility profiles of the identified isolates was obtained using disc diffusion technique. Elemental contents of the fish were determined using atomic absorption spectrophotometer.
Results: Heterotrophic plate counts ranged from 1.5±0.3 to 5.4±0.6×10^5 (cfu/g) while faecal coliform counts ranged between 3.8±1.2 and 6.8±2.4×10^2 (cfu/g). The Enterobacteriaceae constituted 69.8% of the isolates comprising, mostly *Klebsiella* spp. (20.8%), *Enterobacter* spp. (17.0%), *Escherichia coli* (13.2%), *Salmonella* spp. (9.4%) and *Serratia* spp. (5.7%). *Staphylococcus* spp. constituted as much as 30.2%. A high proportion of these organisms were resistant to erythromycin, tetracycline, ampicillin, amoxicillin-clavulanate and cefuroxime. However, *Staphylococcus* spp. were generally susceptible to the test antibiotics. A high number of isolates (67.9%) were multi-drug resistance. Three elements: Iron, Zinc and Nickel were above permissible limit in fish prescribed by FAO/WHO.
Conclusion: Fish from this river is polluted with some pathogenic bacteria and contains some elements that could be hazardous on consumption.
Keywords: Bacteriological, contamination, resistance, elemental.

INTRODUCTION

Fish from rivers are now receiving increasing attention as potential source of animal protein and essential nutrients for human diets (Fawole, 2007). Fish meat is known for its high nutritional quality, relative low fat content, saturated fat, cholesterol and high levels of poly unsaturated fatty acids, proteins and minerals such as calcium, phosphorous, sodium, potassium and magnesium (Salihu *et al*., 2012), In Nigeria, fish is the preferred source of the much desired animal protein compared to poultry, beef, mutton, pork and veal. It is comparatively cheaper

and highly acceptable, with little or no religious bias, which gives it an advantage over pork or beef (Feldhusen, 2000).

In the past, it was thought that fish harvested from open waters (marine and fresh) were generally safe, principally because of the practice of quick chilling of fish and fisheries products soon after harvesting. This notion, according to Reilly *et al*. (1997) was borne out of the lack or paucity of epidemiological evidence of fish-borne diseases. Recent evidence from fisheries reports and studies in the areas of water pollution, fish handling and preservation, water management/fish feeding practices in aquaculture and some cultural practices of fish preparation and raw fish consumption have suggested otherwise (Reilly *et al*., 1997; Atiribom *et al*., 2007; Obasohan *et al*., 2010; Olayiwola and Adedokun, 2015).

The expansion of fish production facilities in the effort to meet animal protein supply through increased fish production has placed increased requirements of quality and product safety on producers, marketers and regulators. This assertion was emphasized by Ihuahi and Omojowo (2005), who opined that the issue of quality and safety of fish and fisheries products have become a serious concern to consumers and regulators in both producing and importing countries.

Pathogenic microbes cause many diseases in both wild and cultured fish. They may vary from a primary pathogen to that of an opportunist invader of a host rendered moribund by some disease process (Inglis *et al*., 1994). Fish may harbour pathogens on or inside its body after exposure to contaminated water or food. Most commonly reported pathogens in fish include: *Samonella, Shigella, Leptospira, E. coli, Vibrio, Mycobacterium* spp., viruses and hookworm larvae. Salihu *et al*. (2012) reported the isolation of bacterial pathogens from fish caught from River Sokoto. Some of these bacterial isolates were *E. coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris* and *Salmonella typhimurium.*

Noga (2000) observed that the prevalence of infectious diseases depends on the interaction between the fish and the pathogens of the aquatic environment, hence the bacteria flora of the fish depicts the level of bacteria in the water environment (Torimiro *et al*., 2014). Salihu *et al*. (2012) also revealed the presence of Enterobacteriaceae and some Gram-positive bacteria in fish from Sokoto River. These contaminated fish if consumed could cause serious health problems to human population.

Elemental toxicants could enter fish directly through the digestive tract due to consumption of contaminated water and food or non-dietary routes across permeable membranes such as gills (Burger *et al*., 2002). Goldstein (1990) and Malik (2004) have revealed that fish acts as a bio-indicator of heavy metal levels in aquatic environment and when their concentrations exceed the required levels, they became toxic and cause several health problems. Therefore introduction of heavy metals into food chain threatens human health.

In Nigeria, Odoemelan (2005) reported an accumulation of heavy metals such as Ni, Cu, Mn, Pb, Zn, Fe, Hg, Cr, V and Cd in fish from Oguta Lake. Different fish samples from Kaduna River in Nigeria have also been found to contain toxic elemental contaminants such as Hg, Cd, V, Zn and Fe which were identified in appreciable amount in all the fish samples studied (Nwaedozie, 1998). Since fish have been recognized as good bio-accumulators of organic and inorganic pollutants (King and Jonathan, 2003), these contaminants cause unhealthy effects to the fish and this may be transferred to man through contaminated fish.

In an earlier report, River Lavun was found to be contaminated with various bacteria with worrying levels of minerals and metals. It has become imperative to determine if the fish from this river is safe for consumption.

MATERIALS AND METHODS

Collection of Fish Samples

Three samples of live *Clarias gariepinus* (Cat fish), caught from River Lavun were obtained from fisher men monthly, for six months. The fish samples were transported in a disinfected container containing the river water, to the laboratory and they were cleaned with sterile distilled water.

Preparation of Fish Samples for Microbiological Analysis

One gram (1.0 g) each of the fish muscle (skin and flesh) samples were weighed aseptically, and macerated in 9.0 mls of 0.1 % peptone water and serially diluted three fold and labelled appropriately

(Salihu *et al*., 2012). This was used for the microbiological analysis.

A 1.0 ml from the 10^{-3} dilution was used for heterotrophic bacterial count while 1.0 ml from the 10^{-2} dilution was used for faecal coliform count.

Heterotrophic (Standard) Plate Count

Heterotrophic plate count was carried out using the pour-plate method as described by America Public Health Association, APHA (1998). A 1.0 ml from the 10^{-3} mixture was aseptically transferred into labelled sterile Petri-dishes. Aliquots of 15 ml sterile molten Plate Count Agar was then poured into the plates and properly mixed to ensure effective even distribution of the water samples in the agar media. The plates were allowed to set (solidify) and thereafter, placed in incubators at 37°C. The number of colony forming units were counted after an incubation period of 48 hours. The values were multiplied by the dilution factor to calculate the actual microbial levels.

Determination of Faecal Coliform Count

One millilitre (1.0 ml) from 10^{-2} mixture was aseptically transferred to the centre of a prepared Eosine Methylene Blue, EMB agar. Using a sterile rod, the mixture dropped was spread evenly on the media surface. The plates were incubated at 44.5°C for 24 hours. Lactose fermenting colonies formed were counted as faecal coliform in cfu/ml and the value multiplied by the dilution factor to get the actual level of the bacterial in each of the inoculums.

Preliminary Identification of the Isolates

One milliliter (1 ml) of stock culture were mixed with 9.0 ml of peptone water as pre-enrichment and incubated at 37°C for 24 hours. The 24 hours culture was then streaked on several selective media: MacConkey Agar, Salmonella-Shigella Agar, E.M.B Agar and Mannitol Salt Agar. They were incubated at 37°C for 24 hours except E.M.B Agar which was incubated at 44.5°C for 24 hours. The colonies of bacteria were Gram stained.

The isolates were further identified biochemically using Catalase, Coagulase and Oxidase test.

Further identification up to specie level was done using Microbact GNB 12E (Oxoid) test strip for isolates that are oxidase-negative, nitrate-positive and glucose-fermenting Gram negative bacilli (GNB), and Microbact Staphylocacal 12S was used to identify isolates (both coagulase- positive and coagulase-negative) that are Gram-positive cocci, non-motile, non-spore forming, and catalase-positive.

Antibiotic Susceptibility Testing

The susceptibility of the different species of isolates was determined according to European Committee on Antimicrobial Susceptibility Testing, EUCAST (2014). A total of 10 standard antibiotics impregnated discs were used as test antibiotics, namely Chloramphenicol (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg), Tetracycline (30 µg), Ampicillin (10 µg), Trimethoprim/Sulphamethoxazole (25 µg), Amoxicillin-clavulanate (30 µg), Cefuroxime sodium (30 µg) and Nitrofurantoin (300 µg).

The result was interpreted using the interpretation criteria published by EUCAST (2014). The isolates were reported as sensitive (S), intermediate (I) and resistant (R) to the various antibiotics depending on the sizes of the zones of inhibition.

Multiple antibiotic resistant (MAR) index was determined as described by Krumperman (1983).

Preparation of Fish Samples and Determination of Elemental Composition

One gram (1.0 g) each of fish muscle was weighed and dissolved in a 10 ml mixture of $HNO_3 - H_2O_2$ (1:1) and digested for 2 hours at 160°C. The digest was cooled, filtered and transferred to 100 ml volumetric flask and filled up to the level with de-ionized water (Olaifa *et al.*, 2004). This was used for the elemental analysis.

Digested fish samples were analysed using flame Atomic Absorption Spectrophotometer (model AA240FS, Varian), and the readings were recorded. The dilution factor of the sample was used to determine the final concentration of the various elements in the fish samples.

RESULTS

Bacteriological Analysis of Fish

Heterotrophic Plate and Faecal Coliform Counts of fish caught from River Lavun are shown in Fig. 1. In April (beginning of rainy season), Heterotrophic plate count (HPC) was $1.7\pm0.4\times10^5$ CFU/g while Faecal coliform count (FCC) was far lower having value of $6.8\pm2.4\times10^2$ CFU/g. These values however decreased

slightly in May and June and increased again in July. The highest counts of HPC of $5.4\pm0.6\times10^5$ were obtained in September (peak of rainy season) while FCC was highest in April. There was significant difference between the HPC and FCC of fish at p=0.011.

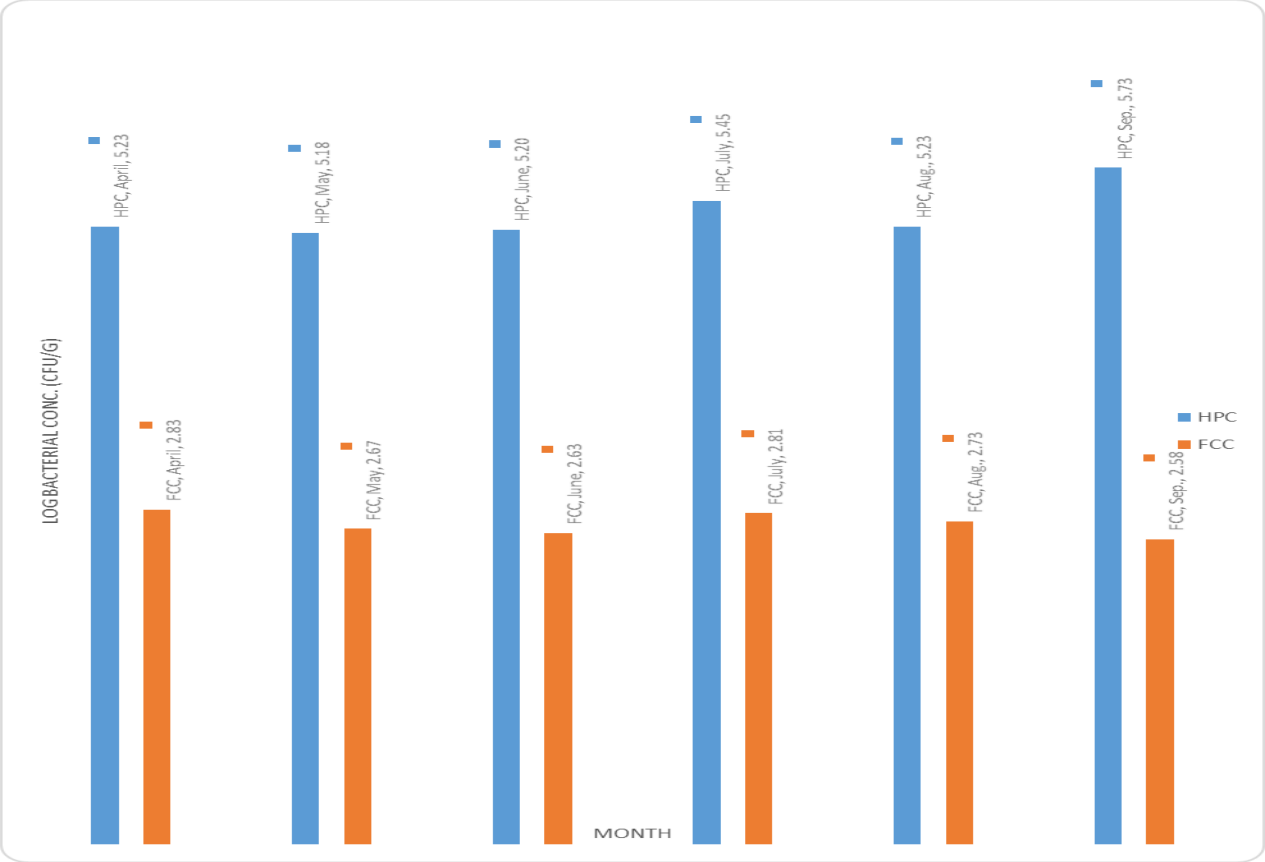

Figure 1: The mean values of HPC and FCC of fish from April to September, 2014.

Key: HPC = Heterotrophic Plate Count
FCC = Faecal Coliform Count

Fifty three (53) isolates belonging to 20 different species were identified. Organisms identified belong mostly to Enterobacteriaceae family (69.8%). Among the Enterobacteriaceae members isolated, *Klebsiella* spp. were the highest with 29.7% occurrence followed by *Enterobacter* spp. (24.3%); *E.coli* (18.9%) and *Salmonella* spp. (13.5%). The other isolates were *Staphylococcus* spp. (30.2%). Of the *Staphylococcus* isolates, eight different species were isolated as shown in Table 1.

Antibiotic Susceptibility Profiles of the Isolates

The results of the antibiotic susceptibility tests of the isolates are presented in Table 2. It shows that majority of the organisms were susceptible to the inhibitory activities of gentamicin, nitrofurantoin, ciprofroxacin and chloramphenicol but resistant to the tetracyclines, erythromycin and ampicillin especially with *Klebsiella* spp., *E. coli* and *Salmonella* spp. Except with Cefuroxime, the staphylococci spp. were generally susceptible to all the antibiotics tested.

Multiple Antibiotic Resistant (MAR) indices presented on Table 3 shows that most of the isolates are multi-drug resistant being resistant to two or more classes of the antibiotics tested. The highest MAR index of 0.7 were observed with *E. coli* isolate.

All the *Serratia* and *Klebsiella* isolates exhibited multiple drug resistance. Majority of the Enterobacter isolates (88.9%), 85.7% of E. coli and 80.0% of *Salmonella* spp. were found to be multi-drug resistant. In contrast, only a few of *Staphylococci* isolates (12.5%) exhibited multiple drug resistance.

Elemental Analysis of Fish Samples

Concentration of the elements in fish is presented in Table 4. Silver (Ag), Cadmium (Cd) and Lead (Pb) were below detectable limit. However, there were traces of Cobalt (Co), Manganese (Mn) and Copper (Cu) while Calcium (Ca), Iron (Fe), Zinc (Zn), Nickel (Ni), Potassium (K) and Sodium (Na) were found in high concentration. In most cases, concentration of elements in the sample fish decreases from April to August. The Fe and Zn and that of Ni at August sampling were well above the FAO/WHO acceptable limit.

Table 1: Distribution of bacteria Isolates from fish samples collected from River Lavun

Organism	Frequency (n)	Percentage (%)
Enterobacter gergoviae	7	13.2
Enterobacter sakazaki	1	1.9
Enterobacter aerogens	1	1.9
Serratia marcescens	1	1.9
Serratia rubidaea	2	3.8
Citrobacter freundii	1	1.9
Klebsiella pneumoniae	9	17
Klebsiella oxytoca	2	3.8
Escherichia coli	7	13.2
Salmonella sp	3	5.7
Salmonella arizonae	2	3.8
Shigella sonnei	1	1.9
Staphylococcus aureus	3	5.7
Staphylococcus xylosus	3	5.7
Staphylococcus saprophyticus	4	7.5
Staphylococcus simulans	2	3.8
Staphylococcus capitis subsp. *Ureoly*	1	1.9
Staphylococcus hominis	1	1.9
Staphylococcus auricularis	1	1.9
Staphylococcus chromogens	1	1.9
Total	**53**	**100**

Table 2: Antibiotic Resistance Profiles of selected Bacteria Isolates from fish in River Lavun.

Antibiotics	Percentage Resistant (%)					
	Entr. spp (n=9)	*Serr.* spp (n=3)	*Kleb.* spp (n=11)	*E. coli* (n=7)	*Salm.* sp (n=5)	*Staph.* spp (n=16)
Ampicillin	77.78	33.33	100.00	71.43	80.00	12.50
Amox. / Clav.	55.56	66.67	18.18	57.14	80.00	6.25
Nitrofurantoin	0.00	0.00	0.00	28.57	0.00	6.25
Gentamicin	0.00	66.66	18.18	28.57	0.00	43.75
Ciprofloxacin	0.00	0.00	9.09	0.00	0.00	6.25
Tetracycline	88.89	100.00	100.00	85.71	80.00	25.00
Erythromycin	0.00	100.00	100.00	85.71	100.00	0.00
SMZ/TMP	0.00	33.33	18.18	28.57	20.00	0.00
Cefuroxime	33.33	66.67	27.27	71.43	80.00	50.00
Chloramp.	11.11	0.00	0.00	28.57	20.00	6.25

Amox. / Clav. : Amoxicillin / Clavulanic acid combination
SMZ/TMP: Sulphamethoxazole trimethoprim combination
Chloramp.: Chloramphenicol
Entr. = Enterobacter *Serr. = Serratia* *Staph*. = Staphylococcus
Kleb. = Klebsiella *Salm. = Salmonella*

Table 3: MARI of bacteria isolates from water and fish samples collected from River Lavun.

MARI	**No. of organisms with MARI value**						
	***Entr.* spp.** (n=9)	***Serr.* spp.** (n=3)	***Kleb.* spp.** (n=11)	***E. coli*** (n=7)	***Salm.* spp.** (n=5)	***Staph.* spp.** (n=16)	**Percentage (%)**
0.0	0	0	0	0	0	4	7.80
0.1	0	0	0	1	0	4	9.80
0.2	1	0	0	0	1	6	15.69
0.3	4	0	5	0	0	2	21.57
0.4	4	2	4	1	3	0	27.45
0.5	0	0	0	0	0	0	0.00
0.6	0	1	2	4	1	0	15.69
0.7	0	0	0	1	0	0	1.96

MARI: Multiple Antibiotic Resistance Index
Entr. = Enterobacter *Citr. = Citrobacter* *Serr. = Serratia*
Kleb. = Klebsiella *Salm. = Salmonella* *Shig. = Shigella*
Staph. = Staphylococcus

Table 4: Concentration of elements in fish collected from River Lavun

Elements	Concentration (mg/100g)			
	April	June	August	FAO/WHO Limit
Ca	226.28±96.00	135.19±56.95	36.86±11.85	-
Ag	0.00	0.00	0.00	-
Co	0.27±0.07	0.01±0.01	0.10±0.09	-
Fe	13.70±4.17	9.61±1.60	9.98±0.66	0.08
Cd	0.00	0.00	0.00	0.25
Mn	0.32±0.31	0.00	1.13±0.86	-
Pb	0.00	0.00	0.00	0.03
Zn	3.66±0.39	4.54±0.76	2.75±0.46	0.1
Ni	0.01±0.01	0.19±0.08	23.00±0.09	8.0
Mg	52.79±10.24	45.87±4.15	35.15±4.36	-
Cu	0.29±0.05	0.11±0.06	0.45±0.07	-
K	553.33±142.95	450.00±17.32	323.33±184.75	-
Na	230.00±20.00	163.33±15.28	130.00±20.00	-

DISCUSSION

Bacterial flora of fish depicts the levels of contamination of the water environment (Torimiro *et al.*, 2014). Fish caught from this river have relatively high heterotrophic and faecal counts during the peak of rainy season which might have resulted from heavy river contamination.

The type of microorganism found associated with fish depends on the aquatic habitat of fish and are known to be affected by certain factors like salinity level and bacterial load of the habitat (Diler *et al*., 2000). In fish, the non-indigenous pathogens may not be pathogenic but could cause infection if ingested by man. Novotny *et al.* (2004) reported that food-borne pathogens associated with fish and fish products include *Clostridium botulinum* type E and *Vibrio parahaemolytiens.* Other potentially human pathogenic bacteria associated with fish include *C. perfringes, Staph.* spp., *Salmonella* spp., *Shigella* spp., *V. cholerae* and other vibrios. Outbreaks usually occur due to the ingestion of insufficiently heat-treated fish or products contaminated after/during their processing (US DAFSIS, 2011).

Most bacterial isolates from fish in this study are multiple antibiotics resistant, This high proportion imply that the study area is a potential source of infectious outbreak. The highest MAR index of 0.7 was seen with the *E. coli* isolate, a worrying development as *E. coli* isolate is a well-known pathogen. Effective management of infection that may arise from this organism is thus a problem. Contaminated food such as fish is major sources of enteric pathogens, causing several foodborne disease outbreaks. Consumption of the fish with presence of antibiotic- resistant bacteria is a major public health concern as antibiotic-resistant bacteria could be transferred to humans, contributing to the spread and persistence of antibiotic-resistant bacteria in environments.

Iron (Fe) is one of the most abundant metals in the earth's crust. It's an essential trace element required by all forms of life. The average daily intake of iron has been estimated to be 17mg/day for males and 9-12mg/day for females (FAO/WHO, 2011). Hazard of pathogenic organisms may be increased because of the presence of Fe since most of these organisms need Fe for their growth (Tiwana *et al*., 2005). The Fe values of the fish were found to be above the permissible limit of standards for drinking water by FAO/WHO (2011). These high Fe values observed might be due to contaminated river water. This could lead to toxicity. Though excessive iron is not stored in the body, impaired ability to regulate iron absorption may result in siderosis in liver, pancreas, adrenals, thyroid, pituitary and heart and which could

manifest as cirrhosis, adrenal insufficiency, heart failure or diabetics.

The work of Adeniyi *et al*. (2012) is in line with this study having reported Zn concentration of 38.24mg/kg in fish which exceeded the FAO/WHO (2011) limit of 1mg/kg.

The values of macro elements (Na, K, Mg and Ca) were generally high in the fish samples; and it concur with results of Joanna *et al*. (2009) who analysed fish samples from the Mazurian Great Lakes Poland. The concentrations of K, Na, Ca, and Mg in their study were higher than the values in this work. This present study showed that the most abundant macro element present in the fish samples was potassium. This is in line with the result of Adeniyi *et al*. (2012).

Elemental composition of water diffuses into fish and could accumulate in it. While Tulay *et al*. (2014) reported that levels of trace elements such as Al, B, Ba, Cr, Cu, Fe, Mn, Ni and Zn in various fish species collected from Sakarya region, Turkey, were found to be below limit values provided by Turkish Food Codex (TFC), FAO and WHO, concentrations of heavy metals (Cd and Fe) in fish collected from Densu River, Accra, Ghana were found to be above the maximum limits by WHO as well as FAO, Tiimub and Mercy (2013) however reported that Pb, Hg, and As were below detectable limit in their own work. Nwaedozie (1998) on the other hand, reported the presence of Hg, Cd, V, Zn and Fe in appreciable amount in all the fish samples from River Kaduna.

Variations in the concentration of different nutritional components in fish have been attributed to the ability of fish to absorb and convert essential nutrients from the diet or water bodies where they live (Adewoye *et al*., 2003).

CONCLUSION

Fish from River Lavun is contaminated with disease-causing bacteria and therefore should be treated adequately before consumption.

A high number of the isolates particularly the Enterobacteriaceae are multiple drug resistant with attendant health implication. Commonly used antibiotics such as ampicillin, tetracycline, and erythromycin are relatively ineffective others such as quinolones, chloramphenicol and nitrofurantoin are effective alternatives.

The presence of some heavy metals makes the fish from this River water unsafe for consumption.

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