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ORIGINAL ARTICLE

The Antibiotic Resistant Patterns of Bacterial Flora of Fish from Different Aquatic Environments from Ibadan, South-west Nigeria

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ABSTRACT

The physico-chemical properties, bacteriological qualities and antibiotic resistance pattern of isolated bacteria from different aquatic environments and fish obtained randomly at different locations in Ibadan, South West Nigeria were studied. Two hundred and ten samples of fish and water were collected randomly and analysed for bacteriological quality. Antibiotic sensitivity of strains was done using the disc diffusion method. The Antibiotic sensitivity tests conducted on the bacteria isolates revealed multiple drug resistance of four to eight antibiotics among the 32 strains of bacteria belonging to the genera *Escherichia, Staphylococcus, Salmonella* and *Streptococcus.* The relatively high level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment The study established the incidence of contributive resistant bacteria for bacteria isolated from different aquatic environments, captured and cultured fish which have a lot of implication on the safety of the public.

Key words: Antibiotic Sensitivity Tests, Antibiogram, Bacteriology

Introduction

The increase in human population and reports of large number of under-nourished or starving people especially in the developing countries have made the need for food production a major world wide issue of concern [19]. As a result of high prices in conventional sources of animal protein such as livestock and poultry, [35] fish production could be the latest revolution in food production [33].

Fish are generally regarded as safe nutritious and beneficial however aquaculture products have sometimes been associated with certain food safety issues [45]. Food poisoning microorganisms in fish and shellfish are naturally present in water environment referred to as indigenous and those associated with pollution of aquatic environments [12,42]. In Nigeria, pollution of the surface and underground water by oil and solid waste is widespread thereby rendering them unsuitable for man's use [3,25,34,8].

In addition, since many industries lack effluent treatment plants, the untreated wastes are either deposited on the ground or discharged into nearby natural water bodies [15,34]. Due to deposition of human, animal excreta and other environmental wastes into natural water and during the time of rainy season, the faecal matters of various sources are washed from contaminated land and ultimately carried into different water bodies. Consequently, many water resources have been rendered unwholesome and hazardous to human and other living systems [8]. The toxic substances discharged into water bodies are not only accumulated through the food chain [34] but also either limit the number of species or produce dense populations of

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microorganisms [35]. There is interrelationship between poor water quality, fish food and human health [9].

In different studies, Ekundayo [18] on Lagos Lagoon, Ajiwe et al [4] on Ele river and Ibe and Ozer [24] on Otamiri river isolated different bacterial species with potential for causing high proportion of deaths and ill health, in population dependent on the water bodies for water related resources. Beyond the pond and river environment natural occurring substances such as dissolved oxygen, carbondioxide, ammonia, hydrogen sulphate, nitrate hydrogen ions, temperature as well as the salinity of water are of great importance [11] in bacteriology quality of water environment and fish living in it.

This study sets out to evaluate the physico-chemical properties, bacteriological quality of different aquatic environments and the antibiotic resistance pattern of the bacterial isolates from serosal surface and stomach of captured and cultured African catfish (*Clarias gariepinus*) and Tilapia species (*Oreochromis niloticus*).

Materials and methods

Study location:

Three study sites in Ibadan, Southwest Nigeria were used for this study. A commercial fish pond, (A), A fishery institute fish ponds (B), and a River (C). The area and depth of the ponds were determined.

In Situ Measurement of Physico-Chemical Parameters of Water Samples:

At the site of the river, and cultured ponds, Dissolved Oxygen (DO), Water temperature (ºC), Ammonia (NH_3), pH and Total Dissolved Solids (TDS) were measured using a portable Hach meter (Hach Company, Loveland, USA), and Aquacheck® (USA) Water quality test stripes. Mean readings were taken for Morning (8.00 a.m) and afternoon (4.00 p.m) for 3 consecutive times at periodic interval of seven days. The probe of HACH meter was dipped inside the tested water, and stabilized readings of DO, pH, TºC, and Total Dissolved (TDS) displayed on the meter monitor immediately were recorded. Aquacheck® strips were manually dipped inside the tested water. For pH reading, the strip was removed immediately; for Ammonia, the strip was vigorously moved up and down inside the tested water for 30 seconds. Colour change for pH and NH_3 were read against the standards colours in 15 seconds and 30 seconds respectively.

Collection and processing of Fish and Water Samples:

Live African catfish (*Clarias gariepinus)* and tilapia species (*Oreochromis niloticus)* were randomly collected from the study sites. Wild African catfish and wild tilapia fish were collected from Eleyele River, while cultured African catfish and cultured tilapia fish were collected from ponds of the commercial farm and the fisheries institute respectively. A total of 210 tissue samples (skin and stomach) harvested from 48 fishes (24 *Clarias gariepinus*- 12 wild and 12 cultured), with average weight (grams) of 814 ± 82.95 and 1146.67 ± 36.98 for wild and cultured respectively (24 *Oreochromis niloticus* – 12 wild, and 12 cultured) with average weight (grams) of 99.83 ± 21.76 and 65.63 ± 9.6 for wild and cultured respectively were analyzed in this study. Eighteen (18) samples of feral (natural) and cultured pond water were randomly collected from different locations and analyzed. Water samples were drawn in sterile 500ml bottle from three different points of the river and the pond on each occasion. The water samples were brought to the laboratory in cooler box containing ice- packs at temperature below 4ºC. Both fish and water samples were transported directly to the Food and Meat Hygiene Laboratory of Department of Veterinary Public Health and Preventive Medicine, University of Ibadan within 2hrs of sampling. Fish were caught by a local fishing gear and by cast net. Sampling was drawn between 8.00 and 10.00 am in each occasion at periodic intervals of seven days for three consecutive times.

Bacteriological Examination of the Fish Samples:

Samples were processed for bacteriological analyses within 2-4 hrs of sampling following aseptic techniques. Standard length (cm) and weight (g) of the fish samples were measured for each specimen. The bacteriological media, namely Nutrient Agar, (NA) (MicroMaster, Thane, India) and MacConkey Agar (MCA) (MicroMaster, Thane, India) were prepared according to manufacturer's instruction. Bacterial isolates from each specimen were obtained aseptically from the skin (1 cm squared area) and stomach (1 g portion). Each of the samples was homogenized and shaken in 10ml distilled water. The stock solution was serially diluted. One-tenth milliliter of each desired dilution (10^{-10}) was spread onto Nutrient Agar (NA) and MacConkey Agar (MCA) and incubated for 18-24 hours at 37ºC. Surface Plating technique was used by first pouring the media after cooling to 47-55ºC and then allowed to solidify before the diluted samples were aseptically spread. The colony forming counts per gram of sample was determined using standard methods [22,7]. The results were converted to logarithm in base ten.

Each distinct colony on NA and MAC was

further sub-cultured on fresh NA and MAC for evaluation of purity and colonial morphology. The isolates were then identified using gram staining, physiological, biochemical reactions and fermentation of sugars according to standard Taxonomic Schemes [13].

Bacteriological Examination of the Water Samples:

Each water sample collected from river and cultured ponds were analyzed, and total bacterial count (TBC) was determined. Water was serially diluted and then used to inoculate Nutrient Agar and MacConkey Agar (surface spread technique). The plates were then incubated at 37^{o}C for 18-24 hrs. The distinct colony was counted per ml of water sample. The distinct colony was further sub-cultured on freshly prepared NA and MAC for colonial purification. The isolates were equally identified using gram staining method, physiological, biochemical reactions and fermentation of sugar.

Antibiotic Sensitivity Tests:

Strains of each bacterial isolate were tested for their sensitivity and resistance to antibiotics by means of disc diffusion method. They were investigated using Gram positive discs (Abtek Biological Ltd) containing the following: Cotrimoxazole (Cot), 25µg; Gentamicin (Gen) 10µg; Nalidixic acid (Nal), 30µg; Ofloxacin (Ofl) 30µg; chloramphenicol (Chl), 10µg; Augmentin (Aug), 30µg; Amoxycillin (Amx) 25µg; and Tetracycline (Tet), 10 µg, and Poly disc Gram negative, Multi susceptibility Disc (Poly-Tes Med. Laboratories, Enugu, Nigeria) containing the following: Nitrofurantoin (Nit), 100 µg; Ciprofloxacin (Cip), 5 µg; Tetracycline (Tet), 50 µg, Norfloxacin (Nfl), 10 µg; Amoxycillin (Amx), 30 µg; Ofloxacin (Ofl), 5 µg; Chloramphenicol (Chl), 10 µg; Cefuroxime (Cfx), 30 µg; Ampicillin (Amp), 10 µg and Gentamicin (Gen), 10 µg.

The commercial antibiotic discs were placed on nutrient agar plates previously seeded with an 18-24 hr culture of the test organisms using cotton swab. The plates were incubated at 37^{o}C for 48hr, after which zones of inhibition were examined and interpreted accordingly. The resistance pattern of each isolate was constructed from the antibiogram. Earlier, the potencies of all the antibiotics used in the study were confirmed using susceptible *Escherichia coli* strains obtained from Department of Pharmacology, University of Ibadan, Ibadan, Nigeria.

Statistical Analysis:

The data collected from the fish and water samples, were subjected to statistical analysis, using t- test, analysis of variance (ANOVA) and significant means separated using Duncan's Multiple Range Test(DMRT) as outlined by Steel and Torrie. Values of $P<0.05$ were considered significant.

Results and discussion

The physico-chemical attributes of water samples of the different aquatic environments are as presented in Table 2. The temperature of the water ranged from 30.5-31.5^{o}C with higher values obtained in the noon. While pH values in the range of 7.33-7.50 were obtained, there was no indication of the presence of NH_3 in the water samples. The values of dissolved oxygen ranged from 0.15-5.42 mg/L, with the least values obtained for the cultured tilapia water.

The mean total bacteria and enterobacteria counts of the water samples obtained from the ponds and the river are as shown in Table 3. The highest bacteria and enterobacteria counts were obtained from feral water, while the least bacteria and enterobacteria counts were obtained from cultured catfish water (CCW) and cultured tilapia water (CTW) respectively. In each case, the enterobacteria count was lower than the total bacteria count. While there was no significant difference in the bacteria counts of the samples, the enterobacteria count of cultured tilapia water (CTW) was significantly lower than the enterobacteria counts of feral and cultured catfish water (CCW).

Several bacteria were isolated (table 4) from different parts of the fish and water used in this study. Predominant organisms identified in African catfish and Tilapia fish studied were not different. Eleven different organisms were isolated, with nine different types from skin samples, five different types from stomach and six different types from sampled water.

High levels of resistance to antibiotics (60-80%) were obtained among the *E. coli* isolates as shown in Table 5. Only gentamicin was active against all the bacteria isolates. Cefuroxime, Chloramphenicol, Amoxillin, Tetracycline and Ciprofloxacin were not active against any of the isolates. The total resistance of the bacteria to the antibiotics range from 12.5% for Norfloxacin to 100% for Ampicillin, Cefuroxime, Chloramphenicol, Amoxillin, Tetracycline and Ciprofloxacin. The cumulative effect of the antibiotics as obtained in this study is Gentamicin > Norfloxacin > Nitrofurantoin > Ofloxacin > Ampicillin = Cefuroxime = Chloramphenicol = Amoxillin = Tetracycline = Ciprofloxacin.

High levels of resistance were obtained among the *Salmonella* isolates (50-70%). Gentamicin, Chloramphenicol and Amoxillin had 100% activity against all the isolates, while Norfloxacin, Tetracycline and Ciprofloxacin were not active against any of the isolates obtained from different sources. The total resistance of the bacteria to the

antibiotics range from 37.5% for Ampicillin to 100% for Norfloxacin, Tetracycline and Ciprofloxacin as shown in Table 10. The cumulative effect of the antibiotics as obtained in this study is Gentamicin = Chloramphenicol = Amoxillin > Ampicillin > Cefuroxime = Ofloxacin > Nitrofurantoin > Norfloxacin = Tetracycline = Ciprofloxacin.

High levels of resistance were obtained among the Staphylococcal isolates (50-87.5%). Only gentamicin was active against all the isolates. The total resistance of the bacteria to the antibiotics ranged from 37.5% for Ofloxacin to 100% for Nalidixic acid, Cotrimoxazole and Amoxillin as shown in Table 6. The cumulative effect of the antibiotics as obtained in this study is Gentamicin>Ofloxacin=Tetracycline> Augmentin> Nitrofurantoin > Nalidixic acid = Cotrimoxazole=Amoxillin.

High levels of resistance were obtained among the Streptococcal isolates (75-87.5%) as shown in Table 7. None of the Antibiotics tested had 100% activity against all the isolates. The total resistance of the bacteria to the antibiotics range from 37.5% for Ofloxacin and Nitrofurantoin to 100% for other antibiotic. The antibiotic resistant patterns of some bacterial isolates obtained from the fish samples are shown in Table 8. Strains of *E. coli, Staphylococcus, Salmonella* and *Streptococcus* evaluated in this regard showed multiple drug resistance ranging from four to eight antibiotic.

Discussion:

The average physico-chemical parameters of the aquatic environments which were recorded during the study were found suitable for the culture of fresh water African catfish and Tilapia [11] in tropical environment. A significantly lower level of dissolved oxygen was recorded in cultured Tilapia water. Though Tilapia can survive low dissolved oxygen, this could unduely stress the fish, leading to low survival rate [11]. A concentration of at least 5mg/L [29,6] is recommended as ideal for fresh water fish aquaculture.

Feral water showed a significantly higher total dissolved solids (>68.30%) above recommended level of less than 40% [6]. This could be as a result of mass pollution of the environments, hence higher bacteria load observed in the feral water studied. All the water quality variables studies were suitable for bacterial proliferation particularly temperature ranging from 30.50 – 31.50^{o}C [39]. A low level of organic substances in the water, low temperature, good oxygenation and the appropriate pH assures that the bacteriological status of the water is good and that the abundance of heterotrophic bacteria remaining low. The presence of bacteria in the aquatic environment can be used to evaluate its sanitary and bacteriological state as well as the state of fish in it. The survival of these bacteria is dependent on the prevailing condition in the aquatic environment and fish are often the hosts [43,16]. Poor water quality has been linked with low fish product quality and potential human health risks [11]. The results obtained from different aquatic environments, meet the requirements set by the World Health Organisation (1989) for water used in the fish cultivation which permits 10^3 fecal bacterial from the coliform group per 100cm^3.

The Antibiotic sensitivity tests conducted on the bacteria isolates to ascertain their level of resistance to commonly used antibiotics revealed multiple drug resistance of four to eight antibiotics among the 32 strains of bacteria belonging to the genera *Escherichia, Staphylococcus, Salmonella* and *Streptococcus.* The relatively high level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment [44,1,40,32]. Antibiotics prescriptions in hospitals are give without clear evidence of infection or adequate medical indication. Broad spectrum antibiotics are sometimes given in place of narrow spectrum drugs as a substitute for culture and sensitivity testing with consequential risk being super infections and the selection of drug resistant mutants [36]. In developing countries, drugs are available to the public and thus people may practice self administration of antibiotics and further increase the prevalence of drug-resistant strains. The long standing practice of using low doses of antibiotics for a long period of time for growth promotion and arbitrary use of antibiotics in animal husbandry is a strong contributor to the development of the antibiotic resistant bacteria in the environment. The consumption of antibiotic is enormous, and it has been estimated that the antibiotic market consumption world wide lies between 100,000-200,000 tons [47].

Different level of resistance of the bacterial isolated to individual antibiotics and various degree of sensitivity were obtained among the isolates. This could be so because strains of the same micro-organisms isolated from different sources may present diverse level of resistance as a means of surviving [26]. Multiple drug resistance is an extremely serious public health problem [36] and it has been found to be associated with the outbreak of major epidemics throughout the world [30,14]. Thus, the multiple drug resistance shown by these bacteria is worrisome because of the public health implications. Handling and consumption of fish materials of this kind can expose the handler to certain risks either from the water or from the fish themselves.

Staphylococci are gram-positive facultative anaerobic bacteria. They are widespread among mammalians where they belong to the healthy microflora of skin and mucosa. However,

staphylococci are also common human and animal pathogens. The coagulase-positive species *Staphylococcus aureus* are the species with the broadest pathogenic potential. In contrast to *S. aureus*, members of the heterogeneous group of coagulase-negative staphylococci (CNS) are regarded as less pathogenic bacteria. CNS indeed represents a substantial part of the saprophytic microflora in humans and they rarely cause disease in immunocompetent outpatients. In recent decades, however, coagulase-negative staphylococci have emerged as nosocomial pathogens in immunocompromised individuals. Specifically, *Staphylococcus epidermidis* is a common cause of line-associated septicemia and other polymer-related infections. Nosocomial isolates of both *S. aureus* and CNS are characterized by increasing resistance towards antibiotics which is a great challenge for the management of hospital-acquired infections [49].

Gündogan *et al*. [20] reported high prevalence of resistance of strains of *E. coli* and *Serratia marcescens* isolated from samples of raw minced calf meat, chicken carcasses and meatballs (ready-to-eat meat) to a number of antibiotics, including nalidixic acid, tetracycline, ampicillin, kanamycin, chloramphenicol and erythromycin. *S. marcescens* is recognized as an opportunistic pathogen and strains of it are now resistant to commonly used antibiotics [21]. However, up to 1950 the species was thought to be a harmless saprophytic organism [5]. In the same vein, *Proteus* species are highly resistant to antibiotics; therefore infections caused by *Proteus* species are difficult to cure [48]. Their plasmids are responsible for spreading antibiotics resistance genes in a microbial population. A large number of *Proteus* species has varied multi-drug resistant markers that are encoded on transferable plasmids.

Table 1: The Descriptive analysis of the study sites

Sites	*Latitude	*Longitude	Area (m^2)	Water depth (m)
A	7.3878 N	3.8964 E	120,270	1.1
B	7.3878 N	3.8964 E	20,045	1.0
C	7.3878 N	3.8964 E	NA	NA

A, commercial fish pond; B, pond of fishery institute; C, Eleyele river, NA- Not available *Source: http// www.Wikipedia.com/june 2010.

Table 2: Physico-chemical properties of the different aquatic environments

Systems	Temp (°C)		DO (mg/L)		Ph		NH_3 (ppm)		TDS (%)	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
CCW	30.5± 0.27[a]	31.33± 0.33 [a]	5.40± 0.03 [a]	5.42± 0.07 [a]	7.50± 0.03 [a]	7.33± 0.09 [a]	0.00	0.00	8.83± 0.08 [b]	8.83± 0.18 [b]
CTW	30.6± 0.04[a]	31.40± 0.25 [a]	0.15± 0.01 [b]	0.15± 0.01[b]	7.50± 0.03 [a]	7.50± 0.03 [a]	0.00	0.00	23.47± 11.69 [b]	23.47± 11.69 [b]
FW	30.67±0.07[a]	31.5± 0.20 [a]	4.91 ± 0.92 [a]	4.61±0.0 [a]	7.33±0.09	7.50±0.03 [a]	0.00	0.00	68.3±012.56 [a]	64.50±10.95 [a]

CCW, Cultured Catfish Water (A) CTW, Cultured Tilapia Water (B) FW, Feral (Natural) Water (C);;; *Means in the same column with the same letters are not significantly different using Duncan Multiple Range Test at P<0.05.

Table 3: Mean total bacteria load and Enterobacteria count (x 10^2) of different aquatic environments studied.

SAMPLES		Total Bacteria count	Enterobacteria count
		Mean± SEM n=9	Mean± SEM n=9
A	Cultured Catfish Water (CCW)	224.27± 41.79a (4.35)*	71.00± 27.50b (3.85)*
B	Cultured Tilapia Water (CTW)	196.33± 42.31a (4.29)*	111.33± 18.44a (4.05)*
C	Feral Water (FW)	252.67± 24.53[a] (4.40)*	138± 22.69a (4.14)*

*Means in the same column with the same letters are not significantly different according to Duncan Multiple Range Test at P<0.05; *, values in parenthesis are $\log_{10}$ CFU/ml.

Table 4: Bacterial isolates obtained from different parts of fish and water

Samples	Bacterial isolates
Water	*Staphylococcus aureus, Escherichia coli, Pseudomonas* sp, *Klebsiella* sp, *Streptococcus* sp, and *Proteus* sp.

Table 5: The antibiogram of some strains of *E. coli* obtained from the samples

Isolates	Nit	Gen	Amp	Cfx	Chl	Ofx	Amx	Nfx	Tet	Cip	***%
*EC	-	+	-	-	-	-	-	+	-	-	70
*EC	+	+	-	-	-	-	-	+	-	-	70
**EC	+	+	-	-	-	-	-	+	-	-	80
**EC	+	+	-	-	-	-	-	-	-	-	80
#EC	-	+	-	-	-	+	-	+	-	-	70
#EC	+	+	-	-	-	+	-	+	-	-	60
##EC	+	+	-	-	-	+	-	+	-	-	60
##EC	+	+	-	-	-	-	-	+	-	-	70
Ø Total	25	0	100	100	100	50	100	12.5	100	100	Nit,

Nitrofuratoin 100µg; Gen, Gentamicin 10µg; Amp, Ampicillin 10µg; Cfx, Cefuroxime 30µg; Chl, Chloramphenicol 10µg; Ofl, Ofloxacin 5µg; Amx, Amoxyclin 30µg; Nfl, Norfloxacin 10µg; Tet, Tetracycline 50µg; Cip, Ciprofloxacin 5µg; EC, *E. coli*; Ø, % resistance of the isolates to each antibiotics; *** cumulative % resistance of each bacterial strain to all the antibiotics; #, wild tilapia isolate; ##, cultured tilapia isolate; *, wild catfish isolate; **, cultured catfish isolate; +, susceptible to antibiotics; -, resistant to antibiotics.

Table 6: The antibiogram of some strains of *Salmonella sp* obtained from the samples

Isolates	Nit	Gen	Amp	Cfx	Chl	Ofl	Amx	Nfx	Tet	Cip	***%
*SA	+	+	+	-	+	-	+	-	-	-	50
*SA	-	+	+	+	+	-	+	-	-	-	50
**SA	-	+	+	-	+	-	+	-	-	-	60
**SA	-	+	+	-	+	+	+	-	-	-	50
#SA	-	+	-	-	+	-	+	-	-	-	70
#SA	-	+	-	+	+	-	+	-	-	-	60
##SA	-	+	+	-	+	-	+	-	-	-	60
##SA	-	+	-	-	+	+	+	-	-	-	60
Ø Total	87.5	0	37.5	75	0	75	0	100	100	100	Nit,

Nitrofuratoin 100µg; Gen, Gentamicin 10µg; Amp, Ampicillin 10µg; Cfx, Cefuroxime 30µg; Chl, Chloramphenicol 10µg; Ofl, Ofloxacin 5µg; Amx, Amoxyclin 30µg; Nfl, Norfloxacin 10µg; Tet, Tetracycline 50µg; Cip, Ciprofloxacin 5µg; SA, *Salmonella* sp; Ø, % resistance of the isolates to each antibiotics; *** cumulative % resistance of each bacterial strain to all the antibiotics; #, wild tilapia isolate; ##, cultured tilapia isolate; *, wild catfish isolate; **, cultured catfish isolate; +, susceptible to antibiotics; -, resistant to antibiotics.

Table 7: The antibiogram of some strains of *Staphylococcus aureus* obtained from the samples

Isolates	Nal	Gen	Ofl	Tet	Aug	Nit	Cot	Amx	**%
*ST	-	+	-	-	-	-	-	-	87.5
*ST	-	+	-	+	-	-	-	-	75.0
**ST	-	+	+	-	+	-	-	-	62.5
**ST	-	+	+	+	-	-	-	-	62.5
#ST	-	+	+	+	-	-	-	-	62.5
#ST	-	+	+	+	-	+	-	-	50
##ST	-	+	-	-	+	-	-	-	75
##ST	-	+	+	+	-	-	-	-	62.5
Ø Total	100	0	37.5	37.5	75	87.5	100	100	

Nal, Nalidixic acid 30µg; Gen, Gentamicin 10µg; Ofl, Ofloxacin 30µg; Tet, Tetracycline 10µg; Aug, Augmentin 30µg; Nit, Nitrofurantoin 30µg; Cot, Cotrimoxazole 25µg; Amx, Amoxycillin 25µg; Ø total, % resistance of the isolate to each antibiotics; ***, Cumulative % resistance of each bacterium to all the antibiotics; +, positive/ susceptible; -, negative/resistant; *ST, Wild catfish isolate, ** ST, Cultured catfish isolates; # ST, Wild Tilapia isolate; ##ST, cultured Tilapia isolate.

Table 8: The antibiogram of some strains of *Streptococcus sp* obtained from the samples

Isolates	Nal	Gen	Ofl	Tet	Aug	Nit	Cot	Amx	***%
*SP	-	-	-	-	-	+	-	-	87.5
*SP	-	-	+	-	-	-	-	-	87.5
**SP	-	-	-	-	-	+	-	-	87.5
**SP	-	-	+	-	-	-	-	-	87.5
#SP	-	-	-	-	-	+	-	-	87.5
#SP	-	-	+	-	-	-	-	-	87.5
##SP	-	-	+	-	-	+	-	-	75
##SP	-	-	+	-	-	+	-	-	75
Ø Total	100	100	37.5	100	100	37.5	100	100	

Nal, Nalidixic acid 30µg; Gen, Gentamicin 10µg; Ofl, Ofloxacin 30µg; Tet, Tetracycline 10µg; Aug, Augmentin 30µg; Nit, Nitrofurantoin 30µg; Cot, Cotrimoxazole 25µg; Amx, Amoxycillin 25µg; Ø total, % resistance of the isolate to each antibiotics; ***, Cumulative % resistance of each bacterium to all the antibiotics; +, positive/ susceptible; -, negative/resistant; *SP, Wild catfish isolate, ** SP, Cultured catfish isolates; # SP, Wild Tilapia isolate; ##SP, cultured Tilapia isolate.

Table 9: The resistant pattern of some of the bacterial isolates

No of antibiotics	Resistance pattern	Bacterial isolates
4	Nal Aug Cot Amx	#ST
	Cfx Ofl Nfx Tet Cip	*SA
	Nit Ofl Nfx Tet Cip	*SA
	Nit Cfx Nfx Tet Cip	**SA
5	Nal Aug Nit Cot Amx	*ST
	Nal Aug Nit Cot Amx	#ST
	Nal Aug Nit Cot Amx	#ST
	Amp Cfx Chl Amx Tet Cip	#EC
	Amp Cfx Chl Amx Tet Cip	##EC
	Nit Cfx Ofl Nfx Tet Cip	**SA
6	Nit Amp Cfx Ofl Tet Cip	#SA
	Nit Amp Ofl Nfx Tet Cip	#SA
	Nit Amp Cfx Nfx Tet Cip	##SA
	Nal Tet Aug Nit Cot Amx	*ST
	Nal Ofl Tet Nit Cot Amx	**ST
	Nal Ofl Aug Nit Cot Amx	**ST
	Nal Ofl Tet Nit Cot Amx	##ST
	Nal Gen Tet Aug Cot Amx	##SP, ##SP
7	Nit Amp Cfx Chl Amx Tet Cip	*EC
	Amp Cfx Chl Ofx Amx Tet Cip	*EC
	Amp Cfx Chl Ofx Amx Tet Cip	**EC

Table 9: Continue

	Nit Amp Cfx Chl Amx Tet Cip	♯EC
	Amp Cfx Chl Ofx Amx Tet Cip	♯♯EC
	Nit Amp Cfx Ofl Nfx Tet Cip	♯♯SA
	Nal Gen Ofl Tet Aug Cot Amx	*SP
	Nal Gen Tet Aug Nit Cot Amx	*SP
	Nal Gen Ofl Tet Aug Cot Amx	**SP, ♯SP
	Nal Gen Tet Aug Nit Cot Amx	**SP, ♯SP
8	Amp Cfx Chl Ofx Amx Nfx tet Cip	**EC

EC, *E. coli*; ST, *S. aureus*; SP, *Streptococcus*; SA, *Salmonella*, *isolate from wild catfish; **, isolate from cultured catfish; ♯, isolate from wild tilapia; ♯♯, isolate from cultured tilapia.

In Nigeria, several studies have reported high levels of multiple-drug resistance in bacterial isolates such as *E. coli, S. aureus, S. marcescens, P. vulgaris, S. pyogenes, B. cereus, Klebsiella* sp, *Micrococcus* sp, *P. aeruginosa, Enterobacter* sp, *S. equi, S. epidermidis* and *B. subtilis* from diverse environmental samples [26,2,27,28]. These studies among other things showed steady rise in the resistance of various isolates to different antibiotics. Since, there is continuous interaction among different components of the environment; it is may not be a surprise that a high level of antibacterial resistance is obtained in the present study. A number of practices that contribute to the emergence, development and spread of resistance in bacteria are rampant in the country.

In conclusion, the study has established the incidence of contributive resistant bacteria for bacteria isolated from different aquatic environments, captured and cultured fish which have a lot of implication on the safety of the public. The safety of the public in this regard through the following recommendations:

Sanitation within the metropolis should be improved through the provision of public toilets, and enactment of effective policy for the collection and disposal (management) of municipal solid waste. All these would drastically reduce the pollution of running water and rivers with human and domestic wastes.

The sanitary conditions under which fishes are reared or cultured in ponds should be improved by following standard or good practices; such as use of good quality water, use of feeds with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public among other things. The farmers should embrace standard operating practices as applicable to fish farming. The workforce should be educated on the maintenance of good hygienic practices, and should be provided with necessary working and safety equipment.

The long-age practice of using antibiotics as growth promoter in agriculture should be discouraged. This is widely practiced in poultry and fish rearing in Nigeria. Drug residues can be carried to the fish through the use of poultry droppings as feed for the fish or through the use of antibiotics as growth promoter in a fish population. These practices can enhance the incidence, selection, proliferation and dissemination of resistant bacteria in the environment. Another implication is that, there may be high concentration of drug residues accumulating in the muscles of the fishes, thereby reducing their overall quality.

There is increased need for the regulatory agencies such as National Agency for food and Drug Administration and Control (NAFDAC) to set standard guidelines for the fish industry in Nigeria. This is necessary in view of the rapid expansion that the industry has witnessed in recent years, and its attendant consequences on the public health of the nation. The enforcement of such guidelines will also assist the farmers to meet international set standards which can enhance their capabilities to export fish stocks to other countries.

References

1. Abbar, F and H.K. Kaddar, 1991. Bacteriological studies on Iraq Milk Products, journal of Applied Bacteriology, 71: 497-500.
2. Adewoye, S.O. and A. Lateef, 2004. Assessment of the Microbiological Quality of Clarias gariepinus Exposed to an Industrial Effluent in Nigeria. The Environmentalist, 24: 249-254.
3. Ajayi, S.O. and O. Osibanjo, 1981. 'Pollution Studies on Nigerian Rivers. II: Water Quality of Some Nigerian Rivers,' *Environmental Pollution*. (Ser. B) 2: 87-95.
4. Ajiwe, V.I.E., B.O. Nnabuike, C.C. Onochie and V.O. Ajibola, 2000. Surface water pollution by effluents from some industries in Nnewi area Nigeria, Journal of Applied Sciences, 3: 1265-1280.
5. Anía, B.J., 2008. *Serratia*: overview. http://emedicine.medscape.com/article/228495-overview. Retrieved 24-April 09.
6. ANZECC, 2000. Australia and New Zealand Environment and conservation council.
7. APHA, 1995. 'Standard Methods for the examination of Water and Wastewater. 19th ed. American Public Health Association.
8. Bakare, A.A., A. Lateef, O.S. Amuda and R.O. Afolabi, 2003. The Aquatic Toxicity and Characterization of Chemical and Microbiological Constituents of Water Samples

from Oba River, Odo-Oba.. Nigeria: Asian Journal of Microbiology, Biotechnology and Environmental Science, 5: 11-17.
9. Birley, M. and K. Lock, 1988. Health and peri-urban natural Resource production. Environment and Urbanization, 10(1): 89-106.
10. Boon, J.H. and F.A. Huisman, 1996. 'Viral, Bacterial and Fungal Diseases of Siluroidei, Living Resources., 9: 153-164.
11. Boyd, C.E., 1998. Water quality in warm water fish ponds. Auburn University Press, England. pp: 36.
12. Brett, J.R., Environmental factors and growth, In: Fish Physiology (Hora, W.S., Randal, D.J. and Brett, J.R. eds) academic Press. New York. pp: 260.
13. Buchanan, R.M. and N.E. Gibbons, 1974. *Bergey's Manual of Determinative Bacteriology* (8th Ed.). Baltimore, MD: The Williams and Wilkins Company.
14. Canton, R., T.M. Coque and F. Baquero, 2003. Multi-resistant gram-negative bacilli: from epidemics to endemics. Nosocomial and hospital-related infections. *Current Opinion in Infectious Diseases*., 16: 315-325.
15. Chukwura, E.I. and G.C. Okpowasili, 1997. 'Impact of Brewery Wastewater on Recipient Aquatic Environments,' in N. Okafor, G. Okereke, E. Miambi, and S. Odunfa (eds), Biotechnology for Development in Africa: Proceedings of an International conference organized by Foundation for African Development through International Biotechnology (FADIB) held at Enugu, Nigeria, 9-13 Feb; 1997. Ochumba Press Ltd. Enugu, ISBN 978 2791 19-9. pp: 225-233.
16. Del Rio-Rodriquez, R.E., V. Inglis and S.D. Millar, 1997. Survival of Esherichia coli in the intestine of fish Aquaculture Research, 28: 257-264.
17. Doyle, M.P. and M.C. Ericson 2006. Closing the door on the feacal coliform assay, Microbe, 1: 162-163.
18. Ekundayo, J.A., 1977. Environmental consequences of pollution of the Lagos lagoon. Bulletin of Science Association of Nigeria, 3: 290-299.
19. FAO, 2000. FAO Yearbook. Fisheries Statistics; aquaculture production 1998, vol 86/2, FAO Fish ser. No. 56, FAO State. Ser. No. 154, Rome pp: 169.
20. Gündogan, N., A. Devren and S. Citak, 2006. Incidence, protease activity and antibiotic resistance of *Escherichia coli* and *Serratia marcescens* isolated from meat, chicken and meatball samples. *Archiv für Lebensmittelhygiene,* 57: 113-117.
21. Hejazi, A. and F.R. Falkiner, 1997. *Serratia marcescens*. *Journal of Medical Microbiology*, 46: 903-912.
22. Horsley, R.W., 1977. A review of bacterial Foora of teleosts and elasmobranches, including methods for its analysis. Journal of Fish Biology, 10: 529-533.
23. Huisman, E.A. and C.J.J. Richter. 1987. Reproduction, Growth, Health Control and Aquacultural potential of African Catfish, Clarias gariepuns (Burchell,1822). Aquaculture, 63: 1-14.
24. Ibe, K.M. and T.E. Ozoh, 2000. Assessment of bacteriological and physicochemical qualities of Otamiri river, Southeastern Nigeria, Journal Science Engineering and Technology, 7: 2341-2441.
25. Kukulu, S.E. and O. Osibanjo, 1992. 'Pollution Studies on Nigeria Rivers. Trace metal levels of surface waters in the Niger Delta Area,' *International Journal of Environmental Studies*, 4: 287-292.
26. Lateef, A., 2004. The Microbiology of a Pharmacentical Effuent and its Public Health Implications, 'World Journal of Microbiology and Biotechnology, 22: 167-171.
27. Lateef, A., J.K. Oloke and Gueguim E.B. Kana, 2005. The prevalence of bacterial resistance in clinical, food, water and some environmental samples in Southwest Nigeria. *Environmental Monitoring and Assessment,* 100: 59-69.
28. Lateef, A., J.K. Oloke, Gueguim E.B. Kana and E. Pacheco, 2006. The Microbiological Quality of Ice Used to Cool Drinks and Foods in Ogbomoso Metropolis, Southwest, Nigeria. Internet Journal of Food Safety, 8: 39-43.
29. Lawson T.B., 1995. Fundamentals of Aquacultural Engineering, New York: Chapman and Hall.
30. Levy, S.B., 2001. Antibiotic resistance: consequences of inaction. *Clinical Infectious Diseases,* 33: S124-S129.
31. Ligia, M., 2003. Bacterial microflora in the gastrointestinal tract of Nile tilapia, Oreochromis nitoticus, cultured in a semi-intensive system.
32. Malik, A. and M. Ahmad, 1994. Incidence of Drug and Metal Resistance in E. Coli strains from Sewage Water and soil, Chemical and Environmental Research, 3: 3-11.
33. Noga, E.J., 2000. Fish disease diagnosis and treatment (Iowa: Iowa State University Press) pp: 367.
34. Odiete, W.O., 1999. Environmental Physiology of Animals and Pollution, Diversified Resources Ltd. Lagos, 1st ed. pp 187-219, ISBN 978-028-957-7.
35. Okafor, N., 1985, *Aquatic and waste Microbiology*, 1st Ed., Fourth Dimension publishing Company Ltd. Enugu, pp. 187-219. ISBN 9781561270.

36. Prescott, L.M., J.P. Harles and D.A.K. lein, 1999. Microbiology 4th Ed. Mc Graw Hill; New York, pp 678-697.ISBN 0-697-354393
37. Raghavan, R.P., 2003. Incidence of human pathogenic bacteria in shrimps feeds. A study from India. NAGA. Worldfish Centre Quarterly, 26: 22-24.
38. Sagua, V.O., 1986. A review of recent advances in Commercial tilapia Culture. In proc. 5th Nigeria (FISON) Ilorin. pp: 18-26.
39. Shankar, C.M., H. Mahmud, S.R. Mohammad, H.M. Mozaffor, H.M. Zahid and M.D. Sirajul, 2009. Coliform Bacteria in Nile Tilapia, Oreochromis niloticus of shrimp-Gher, Pond and Fish Market: World Journal of Fish and marines Sciences, 1(3): 160-166.
40. Silva, A.A.L. and E. Hoffer, 1993. 'Resistance to Antibiotics and Heavy metals in *E. coli* from Marine Environment,' *Environmental Toxicology and Water Quality*, 8: 1-11.
41. Sowunmi, A.A., M.A. Okunubi and M.O. Efuntoye, 2008. Occurrence of bacteria in gill and buccal cavity of Clarias gariepinus (Burchell, 1822) and Tilapia zilli (Gervais) from Lekki lagoon, Southwest Nigeria.
42. Spaulding, A.V. and T.A. Blasco, World Mangrove Atlas, 1997. International Society for Mangrove Ecosystem, Japan, 94-97.
43. Trust, T.J. and R.A.H. Sparrow, 1974. The bacterial flora in the alimentary tract of freshwater salmonid fishery can. J. Microbiol., 20: 1219-1228.
44. Umoh, V.T., A.A. Adesiyun and N.E. Gomwalk, 1990. Antibiogram of Sterphylococcal Strains isolated from Milk and Milk Products, 'Zentralblatt fun veterina-medizin (B) 37: 701-706.
45. WHO, 2007. Food safety issues associated with products from Aquaculture. Report of a Joint FAO/NACA/WHO study Group, WHO Technical reprint Series: 883 + Geneva.
46. WHO, 1989. Health guidelines for the use of wastewater in agriculture and aquaculture. Report of a WHO scientific group, World Health Organisation, Geneva, pp: 1-74.
47. Wise, R., 2002. Antimicrobial Resistance:- Priorities for Action; Journal of Artimicrobical Chemotherapy, 49: 585-586.
48. Yah, S.C., N.O. Eghafona, S. Oranusi and A.M. Abouo, 2007. Widespread plasmid resistance genes among *Proteus* species in diabetic wounds of patients in the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. African Journal of Biotechnology, 6: 1757-1762.
49. Ziebuhr, W., 2001. *Staphylococcus aureus* and *Staphylococcus epidermidis*: Emerging Pathogens in Nosocomial Infections. *Contributions to* Microbiology, 8: 102-107.
50. Zmyslowka, I., D. Lewandowska, J. Guziur, 2000a. Microbiological study of Ide (Leuciscus idw L) from ponds of different trophy-Arch. Pol. Fish., 8: 259-269.