JOURNAL OF ADVANCED
BOTANY AND ZOOLOGY
Journal homepage: http://scienceq.org/Journals/JABZ.php
Research Article Open Access

Microbiological Evaluation and Plasmid Profile of Fresh African Mud Catfish (*Clarius gariepinus*) in some Towns in Ekiti State. Nigeria

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Received: April 02, 2017, Accepted: May 03, 2017, Published: May 03, 2017.

ABSTRACT:

Fish farming is one of the fast growing farms in Nigeria. In Nigeria, catfish accounts for about 80% of aquaculture production and are highly nutritious food commodity with wide consumer acceptance. The work is aimed at evaluating the microbiological resistant pattern and plasmids profile of bacteria isolated from catfish samples. The skin surface were swabbed and the gills of the fresh catfish samples were aseptically removed and mascerated for isolation of bacteria. The total plate count ranges from (1.3-2.8)x10^4 CFU/ml. The total coliform count ranges from (0.8-2.4)x10^3 CFU/ml and the total *Escherichia coli* count ranges from (0.3-1.5)x10^3CFU/ml. Ten bacteria genera were identified from the 30 isolates. *Pseudomonas* spp 6(20%), *Staphylococcus aureus* 5(16.7%), *Proteus* spp. 4(13.3%), *Escherichia coli* 4(13.3%). *Klebsiella* spp. 3(10%), *Shigella* spp. 2(6.7%), *Serretia* spp. 2(6.7%), *Enterobacter* spp. 2(6.7%), *Streptococcus* spp. 1(3.3%) and *Aeromonas* spp. 1(3.3%). Eight different antibiotics were used against 30 bacteria. Nine (30%) Nitrofurantoin were resistant, 26(86.67%) to Augumentin, 2(6.67%) to Ofloxacin, 20(66.67%) to cefuroxime, 16(53.33%) to Gentamycin, 23(76.67%) to cefotaxine, 21(70%) to ceftazidine and 8(26.67%) to Ciprofloxacin. Multiple resistance was observed in *Enterobacter* spp.(BS4) with molecular size >2.3130kb_p, *Klebsiella* spp.(CS2) >2.3130kb_p, *Proteus* spp.(CS3) >2.3130kb_p, *Aeromonas* spp.(BG3) >2.3130kb_p, *Pseudomonas* spp.(AG3) with no plasmid, *Pseudomonas* spp.(AG4) <2.3130kb_p, *Pseudomonas* spp.(CG4) 2.3130kb_p, *Shigella* spp.(BG1)<2.3130kb_p, *S.auerus*(CS4)<2.3130kb_p and *S.auerus*(CG2) <2.3130kb_p. Therefore, this study recommends that good hygienic conditions and use of clean water during breeding and processing of catfishes should be strictly adhered to and also, indiscriminate use of antibiotics in feeds and treatment of ponds should be avoided.

Keyword: *Fish farming, Catfish, microbiological resistance, food, aquaculture production*

INTRODUCTION

Catfishes (*Clarias* species) are a diverse group of ray-finned fish. Named for their prominent barbels, which resemble a cat's whiskers, catfish range in size and behavior from the heaviest and longest, the Mekong giant catfish from Southeast Asia and the second longest, the Wels catfish of Eurasia, to detritivores and even to a tiny parasitic species commonly called the candiru, *Vandellia cirrhosa*. There are armour-plated types and also naked types, neither having scales. Despite their name, not all catfish have prominent barbels; members of the Siluriformes order are defined by features of the skull and swim-bladder. Catfish are of considerable commercial importance; many of the larger species are farmed or fished for food. Many of the smaller species, particularly the genus *Corydoras*, are important in the aquarium hobby. Catfish are nocturnal. Catfish have widely been caught and farmed for food for hundreds of years in Africa, Asia, Europe, and North America. Quality and flavor varies, some considering catfish as being excellent food, while others dismiss them as watery and lacking in flavor (1).

African mud catfish (*Clarius gariepinus*) are highly nutritious food commodity with wide consumer acceptance. In Nigeria, catfish accounts for about 80% of aquaculture production. However, catfish like any other fish species, could result in significant economic loss due to its perishable nature, if adequate preservation techniques are not adopted (2). Various food preservation techniques have been utilized to improve the microbial safety and extend the shelf life of fish in general including freezing, chemical preservation, salting, and smoking (3). Catfish is high in Vitamin D. Farm-raised catfish contains low levels of omega-3-fatty-acids and a much higher proportion of omega-6-fatty-acids. Fish is one of the sources of proteins, vitamins and minerals, and it has essential nutrients required for supplementing both infants and adults diet (4). In Nigeria, fish is eaten fresh and smoked and form a much cherished delicacy that cut across socio-economic, age, religions and educational barriers (5). There are various reasons for the merits of eating fish. One such reason is that fish is less tough and more digestible when compared with beef, mutton, chicken and bush meat. This is possible because of the greater ratio of muscles protein to connective tissues in fish in relation to other animals thus making fish acceptable by infant and adults. Because of its greater digestibility, fish is usually recommended to patients with digestive disorder such as ulcers (6). Fish product has a nutrient profile superior to all terrestrial meats of beef, pork and chicken etc., being an excellent source of high quality animal protein and highly digestible energy. It is a good source of sulphur and essential amino acids such as lysine, leucine, valine and arginine. It is therefore suitable for supplementary diets of high carbohydrate contents (7). Attention has been focused recently on the relationship between fish consumption and reduced incidence of cardiovascular disease. The benefit has been attributed to the nature of the fats in fish. Unlike other fats in other food, it is the only type of fat that supplies omega-3 poly unsaturated fatty acids (PUFA) (8). PUFAs are essential in lowering blood cholesterol level and high blood pressure. It is able to migrate to alleviate

platelet of (cholesterol) aggregation and various arteriosclerosis conditions in adult population. It helps in prevention of asthma, arthritis, psoriasis, and sonic type of cancer (9). It reduces the risk of sudden death from heart attack and reduced rheumatoid arthritis. Omega-3 fatty acids also lower the risk age related muscular degeneration and vision impairment, decrease the risk of bowel cancer, and reduce insulin resistance in skeletal muscle. Fish is abundant to some extent and occur free in nature. This may account for its relatively low cost compare with other meats. Fish is available in most market as fresh, smoked, dried, canned, chilled or frozen and as such the problem of scarcity is removed. The place of aquatic product in the food basket of the nation cannot be over-emphasized. Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual. They are often bacterial species facultative pathogenic for both fish and man and may be isolated from fish without apparent symptoms of disease. The infection source may be fish kept either for food or as a hobby (10). The work is aimed at evaluating the microbiological resistant pattern of isolates and plasmids profile of resistant bacteria isolated from catfish samples purchased in different towns in Ekiti State, Nigeria.

MATERIALS AND METHODS

Collection of samples and study area

Live catfish (*Clarias garipenus*) were purchased in five (5) different locations which include Ido, Ipoti, Ado, Ilawe and Ikere, all in Ekiti State South-Western part of Nigeria. The samples were collected in sterile containers before laboratory examination was carried out.

Samples processing

The skin of the fresh samples were swabbed with swab sticks and each was soaked in separate well labeled MacCartney bottles containing 10mls of peptone water. Five grams (5g) of the fresh gills were aseptically removed and mascerated into separate well labelled MacCartney bottles containing 50mls of peptone water. The stock solutions were then diluted serially in five (5) fold dilutions, 0.5ml of the fifth fold dilutions were then inoculated into Nutrient Agar, EMB Agar and MacConkey Agar using pour plate method.

Characterization and Identification of Isolated Organisms

The characterization of the organism was based on two criteria:

1. Cultural and Morphological characteristics of the colonies
2. Biochemical characteristics

All the isolates were cultured on the prepared medium in duplicates and incubated aerobically at 37°C. The colonies were observed on the agar medium plates while the cell morphology was observed microscopically after staining. Various biochemical tests were carried out on the bacterial isolates for possible identification which includes: Catalase, Coagulase, Oxidase, Spore, Utilization of Citrate, Sugar Fermentation, Nitrate and Indole all according to Leboffe and Pierce (11).

Antibiotic Susceptibility Testing

The Kirby-Bauer diffusion method was used to determine the antibiotic susceptibility profiles of the bacterial isolates. The antimicrobial agents tested were: Ceftazidime (30µg), Cefuroxime (30µg), Gentamycin (10µg), Cefotaxine (30µg), Ofloxacin (5µg), Augmentin (30µg), Nitrofurantion (5µg), Ciprofloxacin (5µg). The medium used was Muller-Hinton (MH) Agar. Pure culture of organisms were enriched and activated in nutrient broth and incubated at 37°C to a turbidity of 0.5Mac Farland standards. The Muller-Hinton (MH) Agar was inoculated by streaking using sterile cotton swab of each of the cultures. The antibiotic discs were applied using sterile forceps and sufficiently separated from each other for about 2.5cm in order to prevent over lapping of the zones of inhibition. The agar plates were left on the bench for 30minutes to allow for diffusion of the antibiotics and the plates were incubated invertedly at 37°C for 24hours. Results were recorded by measuring the zones of inhibition and comparing with Clinical and Laboratory Standards Institute (CLSI) interpretative performance standard for antimicrobial disc susceptibility testing (12).

PLASMID EXTRACTION METHODS

A volume of 1.5ml of overnight culture was spinned for 1 minute in a micro-centrifuge to pellet cells. The supernatant was gently decanted, leaving 50-100µl together with cell pellet and vortex at high speed to re-suspend cells completely. 300µl of Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5% were added and mixed by inverting tubes 3-5times until the mixture became sticky. 150µl of 3.0M Sodium acetate was added with a pH of 5.2 to mix completely. It was spinned for 5minutes in micro-centrifuge to pellet cell debris and chromosomal DNA. Supernatant was transferred into a fresh tube and mixed well with 900µl of ice-cold absolute ethanol. It was spinned for 10minutes to pellet plasmid DNA. (White pellet was observed). Supernatant was discarded and the pellet was rinsed twice with 1ml of 70% ethanol and dryed. For further use, re-suspend pellet in 20-40µl of Tris 25mM, EDTA 10mM buffer or distilled water. (TENS Composition: Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5%) (13).

AGAROSE GEL ELECTROPHORESIS

Agarose Gel Electrophoresis is a separation method that can be used to separate DNA based on their molecular weight. The concentration of Agarose used is dependent on the size of DNA to be separated but basically they can be used as follows: Plasmid DNA – 0.8% Agarose. You are provided with the following: 1X TBE buffer (or 1X TAE Buffer) (Tris Boric acid (or Acetic acid) EDTA, Agarose powder, (λ-DNA HIND III digested), 0.5-10µl micropipette and tips, Ethidium bromide (1mg/ml), Loading dye.

Procedure**:**

0.8g of agarose powder (for plasmid DNA) was weighed out and 150mls of 1X TBE buffer was added. It was dissolved by boiling using a magnetic stirrer or microwave oven. It was allowed to cool to about 60°C, and then 10µl of ethidium bromide was added and mixed by swirling gently. It was poured into an electrophoresis tank with the comb in place and a gel thickness of about 4-5mm was obtained and bubbles were avoided. It was allowed to solidify (about 20minutes) and the comb was removed. Then, the tray was placed in the electrophoresis tank. 1X TBE buffer was poured into the tank and the surface of the gel was covered by the buffer. 15µl of sample was mixed with 2µl of the loading dye. Samples were carefully loaded into the wells created by the combs. (Marker was loaded on lane 1 followed by the controls). The electrodes were connected to the power pack in such a way that the negative terminal was at the end where the sample has been loaded. Electrophoresis ran at 60-100V until the loading dye has migrated about three-quarter of the gel. Electrodes were turned-off and disconnected. The gel was observed under Ultra-Violet trans-illuminator (13).

RESULTS AND DISCUSSION

From table 1, the total plate count (TPC) ranges from (1.3-2.8) x 10^4 CFU/ml, total coliforms count ranges from (0.8-2.4) x 10^3 CFU/ml and the total *Escherichia coli* count ranges from (0.3-1.5) x 10^3CFU/ml. This is in accordance with the work of Adedeji et

al. (14) where the microbial loads on the skin and stomach of the *Clarias gariepinus* and *Oreochromis niloticus* were higher. The total bacterial count on fish rarely indicate the quality of the fish but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of affecting health conditions of consumers of such contaminated fish (15). It is generally accepted that fish with microbial load of >10 CFU/ml is likely to be at the stage being unacceptable from the microbiological point of view and unfit for human consumption. The survival of these bacteria is dependent on the conditions prevailing in the aquatic environment and fishes are often simply their hosts (16, 17, 18). The study clearly showed variation in bacterial load in fishes of different sources. High microbial counts may be due to lack of proper maintenance and hygienic practice on the side of the fish pond and improper hygiene and handling procedures adopted by the fish farmers during cropping. This is in agreement with the findings of Abolagba and Melle (19) who reported that lack of proper processing and proper hygiene handling of fish products would result in a very high microbial load. The contaminations observed may result from rupturing fish intestine during poor processing or inadequate washing as intestinal microflora of human or animal origin are the causative agent for food spoilage (20, 21). The higher density of total aerobic bacteria found in the skin and gill might be due to quick proliferation after catching and during transportation and storage. Preservation in low quality ice, handling with contaminated hands could also be responsible for higher density of aerobic bacteria. Fish are very much susceptible to contamination with different bacteria because of their perishable protein content (22).

Ten bacterial genera were identified from the 30 isolates as was shown in table 2. *Pseudomonas* spp. has the most occurrence bacteria with 6 (20%), followed by *Staphylococcus aureus* with 5 (16.7%). Other bacteria identified are *Proteus* spp. and *Escherichia coli* with 4 (13.3%) respectively, *Klebsiella* spp. with 3 (10%), *Shigella* spp., *Serretia* spp. and *Enterobacter* spp. with 2 (6.7%) respectively. The bacteria with the least occurrence are *Streptococcus* spp. and *Aeromonas* spp. with 1 (3.3%) respectively. The isolated bacteria were in accordance with the work of Girogio et al. (23). Isolation of *E. coli* is in agreements with the work of Enayatollah et al. (24), which may be as a result of faceal contamination of fish samples. Higher microbial counts in the skin samples comparative to the gill and flesh may be attributable to handling during harvest and processing and high *Escherichia coli* in all the samples may be due to its ubiquitous nature as it could be found in almost all environment including human skin, water and air during processing.

The prevalence of Gram-negative bacteria in the aquatic environment has been reported by Ermeton et al. (25), especially those of the coliform group. More than 50% of the diseases caused by contaminated water are associated with bacteria from the intestinal micro-biota, such as *Enterobacteriaceae* and the coliform group. The contamination of these environments reflects the poor quality, hygiene and sanitization of the water. Also, 4% of all deaths and 5.7% of all infectious diseases in the world are associated with contaminated water (26). The presence of *Staphylococcus aureus* a normal flora of skin and mucous membrane of humans can be attributed to human contact during handling and processing (27). *Staphylococcus aureus* bacteria were isolated from the catfish samples, which are in relation to the work of Falegan et al. (28) where *Staphylococcus aureus* was isolated from ready to eat fish samples which were collected from five different Local Government of Ekiti, Ekiti State of Nigeria. This may be as a result of lack of hygienic and sanitary measures of processors/seller, poor hygiene/sanitary practices relating to fish products, workhouse, packaging and storage as well as the use of inadequate and inefficient traditional processing facilities. *Staphylococcus aureus* produces a variety of extra cellular enzymes and toxins that have been found to be responsible for food poisoning and can rapidly develop resistance to many antimicrobial agents and pose therapeutic problems (29). *Pseudomonas* spp., *E. coli, Serretia* spp., *Shigella* spp, *Enterobacter* spp. and *Klebsiella* spp. isolated in this study are considered to be opportunistic pathogens, capable of producing infections in immunologically weakened fish or as secondary invaders in fish populations suffering from others diseases (30). Therefore *Pseudomonas* spp. is the major pathogen associated with fresh fish spoilage during refrigeration. The presence of *Klebsiella spp., Escherichia coli* and *Enterobacter* spp. in the fresh fish samples is an indication that the water used for processing was faecally contaminated. The ponds and rivers that harbor the fish may be the source of contaminants due to indiscriminate deposition of human, animal excreta and other environmental wastes into natural water, land and during the rainy season especially, as the faecal matter from various sources are washed from contaminated land into different water bodies. Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. Run-off from roads, parking lots and yards can carry animal wastes into natural water course and ponds. Birds can also be a significant source of bacteria. Swans, Geese and other water fowls can all elevate bacteria counts in water bodies and ponds (31).

Eight different antibiotics were used against 30 bacteria isolates. 9 (30%) isolates were resistant to Nitrofurantoin, 26 (86.67%) isolates were resistant to Augumentin, 2 (6.67%) isolates were resistant to Ofloxacin, 20 (66.67%) isolates were resistant to cefuroxime, 16 (53.33%) isolates were resistant to Gentamycin, 23 (76.67%) isolates were resistant to cefotaxine, 21(70%) isolates were resistant to ceftazidine and 8 (26.67%) isolates were resistant to Ciprofloxacin. This study contradicts the work of Adedeji et al. (14) which shows high sensitivity pattern of bacteria isolated from fresh fish samples to Augmentin, Tetracyclin, Nitrofurantoin, Cotrioflaxacin, Amoxicillin and Chloroamphenicol and Gentamicin. Maximum resistance of the bacteria isolates was found in Amoxicillin. ` Antibiotics are given as a feed supplement in the aquaculture industry and as a result, the surrounding environment is directly exposed to these drugs. Remains of feed containing antibiotics and undigested antibiotics in fish faeces are deposited in the sediment of the fish farms. Since many of these antibiotic substances are broken down slowly, they contribute towards an accumulation of antibiotics in the environment surrounding the farms which can result in local bacteria selecting genes resistant to antibiotics (32).The development of acquired resistance in aquatic bacteria can result in a reservoir of resistant bacteria which spread resistance by means of horizontal gene transfer. Many aquatic bacteria and bacteria pathogenic to humans belong to the same group, such as *Aeromonas, Acinetobacter, Kluyvera, Vibrio* and *Yersinia* and this makes it easier for the bacteria to transfer resistant genes to other bacteria in the group. The presence of identical factors in bacteria found in aquatic environments and in clinical pathogens is clear proof that resistant genes from bacteria in aquaculture have spread to human pathogens (32).

Antibiogram of the antibiotic resistant pattern showing twenty (20) different patterns of resistance with the third group exhibiting five different patterns of resistance to antibiotics. Second, fourth and sixth groups exhibiting three different patterns of resistance

to antibiotics, fifth and seventh groups exhibiting two different patterns of resistance to antibiotics and the first and eighth groups with one pattern of resistance to antibiotics. The resistant pattern of each of the bacteria to different antibiotics was shown in table 3. The antibiotics resistant pattern of *Shigella* spp. shows that four different antibiotics- AUG-CXM-CRX-CAZ were resistant. For *Staphylococcus aureus*, *Enterobacter* spp. and *Pseudomonas* spp., the antibiotics resistant pattern was seven antibiotics- NIT-AUG-CXM-GEN-CRX-CAZ-CPR. For *Klebsiella* spp., the antibiotics resistant pattern was to the eight antibiotics- NIT-AUG-OFL-CXM-GEN-CRX-CAZ-CPR. For *Serratia* spp., the antibiotics resistance pattern was seven antibiotics- NIT-AUG-OFL-CXM-GEN-CRX-CAZ. For *Proteus* spp., the antibiotics resistant pattern was six antibiotics- AUG-CXM-GEN-CRX-CAZ-CPR. For *Escherichia coli*, the antibiotics resistant pattern was four antibiotics- AUG-CXM-CRX-CAZ. For *Aeromonas* spp., the antibiotics resistant pattern was six antibiotics- NIT-AUG-CXM-GEN-CRX-CAZ.

Plasmid profiles have been reported to be useful in tracing the epidemiology of antibiotic resistance (33). However, in this study, resistance to various antimicrobial agents was not associated with presence of plasmids. This was because no particular molecular size plasmid could be associated with any particular antimicrobial resistance. Multiple resistance was observed in *Enterobacter* spp.(BS4) with molecular size >2.3130kb$_p$, *Klebsiella* spp.(CS2) >2.3130kb$_p$, *Proteus* spp.(CS3) >2.3130kb$_p$, *Aeromonas* spp.(BG3) >2.3130kb$_p$, *Pseudomonas* spp.(AG3) with no plasmid, *Pseudomonas* spp.(AG4) <2.3130kb$_p$, *Pseudomonas* spp.(CG4) 2.3130kb$_p$, *Shigella* spp.(BG1) <2.3130kb$_p$, *Staphylococcus aureus* (CS4) 2.3130kb$_p$ and *Staphylococcus aureus* (CG2) <2.3130kb$_p$. Resistance of the bacteria isolates to various antimicrobial agents may be located either on chromosomes, plasmids or transposons. For example, methicillin resistance gene (mec4916) has a chromosomal locus, and is probably maintained on a mobile element (34). The result of the plasmid analysis of the isolates indicated that majority of the bacterial isolates possessed extra chromosomal DNA coded on plasmid which is responsible for the resistance of the isolates to the antibiotics they were subjected to. The possession of the plasmid by the isolates may be through mutation or through the chemicals present in the water used for the ponds.

Table 1: The Microbial load of the catfish samples collected from five different towns in Ekiti State.

Code	Towns	Ponds	Samples	TPC 10^4CFU/ml	TCC 10^3CFU/ml	TECC 10^3CFU/ml
A	Ido-Ekiti	1	Gill	2.4	1.3	0.8
			Skin	1.8	0.9	0.4
		2	Gill	2.2	1.6	0.9
			Skin	1.7	1.0	0.3
		3	Gill	2.5	1.1	0.8
			Skin	1.8	0.8	0.3
B	Ipoti-Ekiti	1	Gill	2.6	1.8	1.1
			Skin	2.0	1.4	0.7
		2	Gill	2.4	1.1	0.7
			Skin	2.1	1.0	0.5
		3	Gill	1.9	1.2	1.0
			Skin	1.4	1.0	0.7
C	Ado-Ekiti	1	Gill	2.8	2.4	1.5
			Skin	2.4	1.8	1.5
		2	Gill	2.3	1.6	1.2
			Skin	2.0	1.1	0.8
		3	Gill	2.0	1.2	1.0
			Skin	1.3	0.9	0.8
D	Ilawe-Ekiti	1	Gill	2.2	1.5	1.0
			Skin	2.0	1.1	0.5
		2	Gill	2.1	1.0	0.6
			Skin	2.0	1.0	0.5
		3	Gill	1.7	1.1	1.1
			Skin	1.2	1.0	0.7
E	Ikere-Ekiti	1	Gill	2.3	2.1	1.4
			Skin	2.0	1.7	1.4
		2	Gill	2.0	1.2	1.1
			Skin	2.1	1.1	0.6
		3	Gill	1.9	1.0	0.8
			Skin	1.3	0.9	0.8

Key: TPC- Total plate counts, CFU- Colony forming unit, TCC- Total coliform count, TECC- Total *Escherichia coli* count.

Table 2: Frequency of occurrence and the percentage distribution of bacteria isolated from catfish samples.

S/N	Bacteria	Frequency of occurrence	Percentage distribution
1	*Shigella* spp	2	6.7
2	*Pseudomonas* spp	6	20
3	*S. aureus*	5	16.7
4	*Streptococcus* spp	1	3.3
5	*Klebsiella* spp	3	10
6	*Serretia* spp	2	6.7
7	*Proteus* spp	4	13.3
8	*E. coli*	4	13.3
9	*Aeromonas* spp	1	3.3
10	*Enterococcus* spp	2	6.7
		30	100

Table 3. The resistance pattern of each of the bacteria to different antibiotics.

Bacteria (n)	Antibiotics								
	NIT	AUG	OFL	CXM	GEN	CRX	CAZ	CPR	Resistance pattern
Shigella spp. (2)	-	1	-	2	-	2	2	-	AUG-CXM-CRX-CAZ
S. aureus (5)	1	5	-	2	2	4	3	1	NIT-AUG-CXM-GEN-CRX-CAZ-CPR
Pseudomonas spp. (6)	3	1	-	5	5	4	4	3	NIT-AUG-CXM-GEN-CRX-CAZ-CPR
Streptococcus spp. (1)	1	1	-	1	-	1	1	-	NIT-AUG-CXM-CRX-CAZ
Klebsiella spp. (3)	1	3	1	2	2	2	2	1	NIT-AUG-OFL-CXM-GEN-CRX-CAZ-CPR
Serretia spp. (2)	1	2	1	1	2	2	1	-	NIT-AUG-OFL-CXM-GEN-CRX-CAZ
Proteus spp. (4)	-	4	-	4	3	4	3	2	AUG-CXM-GEN-CRX-CAZ-CPR
E. coli (4)	-	4	-	1	-	2	3	-	AUG-CXM-CRX-CAZ
Aeromonas spp. (1)	1	1	-	1	1	1	1	-	NIT-AUG-CXM-GEN-CRX-CAZ
Enterobacter spp. (2)	1	2	-	1	1	1	1	1	NIT-AUG-CXM-GEN-CRX-CAZ-CPR

KEY: CAZ- CEFTAZIDIME (30µg), CRX-CEFUROXIME (30µg), GEN- GENTAMYCIN (10µg), NIT-NITROFURANTOIN (5µg), CPR-CIPROFLOXACIN (5µg), AUG-AUGMENTIN (30µg), OFL-OFLOXACIN (5µg) and CXM-CEFOTAXINE (30µg).

Table 4: Multiple resistant isolates and plasmid molecular sizes (kb_p).

S/N	Multiple resistant isolates	Plasmid molecular sizes (kb_p)
1	*Enterobacter* species (BS4)	>2.3130
2	*Klebsiella* species (CS2)	>2.3130
3	*Proteus* species (CS3)	>2.3130
4	*Aeromonas* species (BG3)	>2.3130
5	*Pseudomonas* species (AG3)	-
6	*Pseudomonas* species (AG4)	<2.3130
7	*Pseudomonas* species (CG4)	2.3130
8	*Shigella* species (BG1)	<2.3130
9	*Staphylococcus auerus* (CS4)	2.3130
10	*Staphylococcus auerus* (CG2)	<2.3130

KEY: BS4-B SKIN 4, CS2-C SKIN 2, CS3-C SKIN 3, BG3-B GILLS 3, AG3-A GILLS 3, AG4- A GILLS 4, CG4-C GILLS 4, BG1-B GILLS 1, CS4-C SKIN 4 AND CG2-C GILLS 2

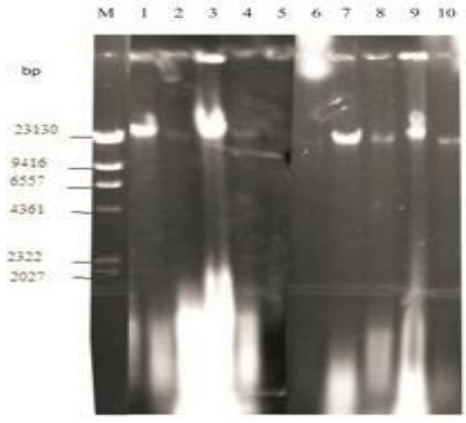

Fig 1: Plasmid profile of the multiple antibiotic resistant isolates from the catfish samples

KEY: M- MARKER MOBILITY, BP-BASE PAIR, 1-*ENTEROBACTER* SPP, 2-*KLEBSIELLA* SPP, 3-*PROTEUS* SPP, 4-*AEROMONAS* SPP, 5-*PSEUDOMONAS* SPP, 6-*PSEUDOMONAS* SPP, 7-*PSEUDOMONAS* SPP, 8-*SHIGELLA* SPP, 9-*STAPHYLOCCOCUS AUERUS*, 10-*STAPHYLOCCOCUS AUERUS*.

CONCLUSION AND RECOMMENDATION

The presence of these bacterial isolates in catfish is indicative of public health risk in contacting diseases associated with these organisms. Compliance with standard microbiological measures to prevent contamination by these organisms becomes very necessary and should be ensured. Fish farming has promoted the spread of several bacterial diseases, which in turn has led to the

increased use of antibiotics. Concerns about the consequence of antibiotic use on public health have encouraged the development of strict regulations controlling the use of antibiotics and have led to a few antibiotics being licensed for use in aquaculture. The high proportion of antibiotic resistant bacteria that persist in sediments and farm environments may provide a threat to fish farms because they can serve as sources of antibiotic resistance genes for fish pathogens in the vicinity of the farms. It can be revealed that lack of proper storage facility after capture and insanitary conditions during processing are the major sources of contamination identified in this study. Because resistant bacteria may be transferred to humans and are capable of transferring their resistance elements to opportunistic human pathogens, the implementation of efficient strategies to contain and manage resistance-gene emergence and spread is critical. One strategy for reducing antibiotic use in aquaculture in addition to the potential effects on human health, inefficiencies in antibiotic treatment of fish illnesses lead to significant economic losses, is to implement rearing practices that minimize the level of stress on the fish and that reduce the likelihood that infections requiring antibiotic treatment will occur. Proper hygienic practice must be ensured by the fish farmers in other to avoid much contamination of the fishes during breeding. Future prediction and prevention of antibiotic resistance must also depends on the research investments in the development of microbial source tracking as well as in the ecology, including water ecology of antibiotic-resistant microorganisms. The multiple antibiotic resistant (MAR) showed by the bacteria isolates and the plasmid profile analysis indicated that the gene responsible for the resistance of some of the isolates to the antibiotics is located on the R-plasmid, which is of significant health importance, which should be helpful for health care personnel in proper monitoring of rural waters and suggest possible solutions to problems that may arise from these resistance strain that could invade the communities from drinking or usage of the contaminated water. Therefore, precaution should be taken to prevent water contamination during harvesting as well as post harvest handling of fish. The safety of the public then depends on the improvement of sanitation within the metropolis by provision of public toilets, and enactment of effective policy for the collection and disposal (management) of municipal solid waste as these would drastically reduce the pollution of running water and rivers with human and domestic waste. 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 microbial load of fish can also be improved through regular disinfection of catching gears or working equipment, and brief immersion of caught fishes in disinfecting solution such as brine water to reduce the microbial load on the fish before storing at cold temperature or sold to the public. The public should be enlightened on the inherent danger that may accompany handling fresh fish or consumption of improperly cooked fish. Several alternatives to antibiotics has being recommended including probiotics, phage therapy and essential oils, high level of hygiene, and minimal use and correct dose of antibiotics. . In view of the findings of this research work it is therefore recommended that good hygienic conditions and use of clean water during processing should be strictly adhered to. After harvest, fresh fish should be properly stored at low temperatures so as to inhibit survival of mesophilic bacteria. Some of these measures have been successfully used to control bacterial infections in aquaculture facilities. In addition, fish ponds water must be changed timely to prevent infections and disease outbreak.

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Citation: Akoja, S. O, *et al*. (2017). Microbiological Evaluation and Plasmid Profile of Fresh African Mud Catfish (Clarius gariepinus) in some Towns in Ekiti State. Nigeria, J. of Advanced Botany and Zoology. V5I104. DOI: 10.5281/zenodo.1000075.