ANTIBIOTIC RESISTANT PATTERNS OF BACTERIAL ISOLATES OBTAINED FROM CATFISH AND TILAPIA FISH TYPES CONSUMED IN IBADAN

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Abstract
This study was carried out to identify antibiotic resistant bacteria isolated from Catfish (Clarias gariepinus) and Tilapia (Oreochromis sp.) fish types at Eleyele, University of Ibadan fish farm and Asejire all within Ibadan metropolis. Antibiotic susceptibility testing was carried out on Ninety (90) of the previously isolated bacterial isolates using the agar gel diffusion technique. Antibiotics used were Gentamycin, Ofloxacin, Nitrofurantoin, Ampicillin, Augmentin, Amoxicillin, Ceftazidime, Cefuroxime, Ciprofloxacin, Ceftriaxone, Erythromycin, Amikacin and Ticarcillin. Various level of multi drug resistance was observed among the isolates. The bacterial population identified from this study includes; Staphylococcus aureus, Salmonella sp., Shigella sp., Pseudomonas aeruginosa, Klebsiella pneumonia, Klebsiella oxytoca, Klebsiella ozaenae, Acinetobacter baumanii, Proteus vulgaris, Escherichia coli, Enterobacter aerogenes, Citrobacter freundii and Moraxella catarrhalis. Twelve (12) of the isolates were further subjected to plasmid profile analysis in which, plasmids of different sizes were found for 10 of the isolates. It was observed that only one Shigella sp., out of the Five (5) isolates had lost the plasmids and became responsible to the earlier resistant antibiotics while the other four isolates Klebsiella pneumoniae, Moraxella catarrhalis, Staphylococcus aureus maintained their resistance to the antibiotics. The spread of multidrug resistance pathogens has constituted a major impediment to the control of infectious diseases, and since this study has shown that fish samples could be a reservoir of bacteria carrying R-plasmids as well as genes responsible for resistance, therefore, the indiscriminate use of antibiotics should be discouraged and also national regulations on the use of antibacterial should be introduced and enforced.

Keywords: *Antibiotics; Fish; Multidrug resistance; plasmids; isolates*

Received: 15.02.2018 Reviewed: 31.05.2018 Accepted: 06.06.2018

1. INTRODUCTION

Antibiotics are used in human and veterinary medicine to treat and prevent diseases and for other purposes including growth promotion in food animals (Phillips *et al.*, 2004). The use of antibiotics plays a major role in the emerging public health crisis of antibiotic resistance (Torrence, 2001). Despite that the majority of antibiotic use occurs in agricultural settings, relatively little attention has been paid to how antibiotic use in farm animals contributes to the overall problem of antibiotic resistance (Landers *et al.*, 2012).
Antibiotic resistance is a looming public health crisis. International, national and local antibiotic stewardship campaigns have been developed to encourage the judicious use of antibiotics and limit unnecessary exposure to it, with the ultimate goal of preserving their effectiveness for serious and life-threatening infections (Avorn *et al.*, 2001; Belongia *et al.*, 2005).
Also, there is a considerable debate in veterinary medicine regarding use of antibiotics in animals raised for human consumption (food animals). The potential threat to human health resulting from inappropriate antibiotic use in food animals is significant, as pathogenic-resistant organisms propagated in these livestock are set to enter the food supply and could be widespread in food products (Ramchandani *et al.*, 2005; Garofalo *et al.*, 2007).
Commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans (Diarrassouba *et al.*, 2007; Mena *et al.*, 2008). While antibiotic use in food animals may represent a risk to human and animal health, the degree and relative

impact have not been well characterized (Landers *et al.*, 2012).

Extensive use of antibiotics in fish production as growth promoters, prophylaxis and treatment of infections have led to misuse of antibiotics and consequent generation of sufficient genomic selective pressure that enable microorganisms to adapt and acquire resistance (Witte, 2001).

There is strict regulation on the use of antibiotics in food animals in the developed countries of Europe and America, but it is not so in developing countries such as Nigeria where access to antibiotics by livestock farmers is unrestricted and as a result, indiscriminate administration of antibiotics without veterinary prescription is common practice (Kabir *et al.*, 2004). The potential for disease problems associated with intensive fish culture would increase the probability of the use of a number of antimicrobial drugs in their management. It has been demonstrated that indiscriminate use of antibacterial drugs and other synthetic chemotherapeutic agents to treat fish diseases and/or as feed additives has resulted in an increase in populations of antibiotic resistant bacteria as well as resistance plasmids in food-producing animals, fish and water microflora (Walter and Vennes, 1985). The prominently affected bacteria are members of the Enterobacteriaceae and related Gram-negative rods (Ogbondeminu and Olayemi, 1993).

Plasmids are extra-chromosomal pieces of DNA, which are capable of replicating independently of the genome, and are particularly important in the spread of antibiotic resistance genes. Plasmids are not considered to be life like viruses (Sinkovics and Horak, 1998). Plasmids can be transmitted from one bacterium to another via conjugation, translocation and transduction. The size of plasmids varies from 1 to over 1000 kilobase pairs (kbp), and the number of identical plasmids in a single cell can range from one to thousands under some circumstances (Thomas and Summers, 2008).

Plasmids have been directly implicated in the acquisition of resistance to many antibiotics (Johnsen *et al.*, 2002; Furuya and Lowry, 2006). This particularly poses great problem since plasmids can cross many species and genus barriers, and the rate of plasmid transfer has even been shown to increase in more heterogeneous communities (Dionisio *et al.*, 2002). Plasmids thus allow resistance to spread and persist in niches that are not necessarily subject to antibiotics (Hughes and Datta, 1983). This study aimed at isolating and determining the prevalence of antibiotic resistant bacteria obtained from catfish in some locations in Ibadan metropolis, Oyo State, Nigeria.

2. MATERIALS AND METHODS

Sample Collection and Processing

Fresh Catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis* sp.) species fish samples were collected at Eleyele, Asejire and University of Ibadan fish farm. Smoked Catfish and Tilapia fish samples were also obtained and were placed in sterile polythene bags. The fresh fish samples were collected in clean plastic containers and transported to the Postgraduate laboratory of the Department of Microbiology, University of Ibadan for analysis. The fresh fish samples were killed by hitting the head with the handle of a sterile knife. The fish samples were aseptically placed on sterile foil paper with sterile hand gloves and the flesh was swabbed with 90% ethanol. The fish samples were carefully dissected with sterile scissors and blade to reveal the flesh, the gills and the gut. Sterile scissors was also used to carefully cut the desired grams that would be used for serial dilution**.**

Isolation and Characterization of Isolates

Serial dilutions were carried out on the flesh, gills and guts were estimated as follows; 1 g of flesh, gut and gills were weighed and then placed into 9 ml of sterile distilled water in McCartney bottles and it served as the stock solution. 1 ml of the appropriate dilution was plated out using the standard pour plate technique of Harrigan and McCance. The pour plates were used using Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), MacConkey Agar (MAC) and Salmonella/Shigella Agar (SSA) for total aerobic counts and total Enterobacteriaceae counts respectively. The plates were incubated at 37 ^{0}C for 24 hours. The discrete colonies were then sub-cultured unto fresh agar plates aseptically to obtain pure colonies of isolates.

Antibiotic Susceptibility Test

The bacterial isolates were investigated using Gram Positive discs (Abtek Biological Ltd) containing the following: Gentamycin (Gen), Ceftriaxone (Ctr), Erythromycin (Ery), Cloxacillin (Cxc), Ofloxacin (Ofl), Augmentin (Aug), Ceftazidime (Caz), and Cefuroxime (Crx); Gram negative discs from (Rapid Lab Ltd) containing the following: Gentamycin (Gen), Ciprofloxacin (Cpr), Ofloxacin (Ofl), Augmentin (Aug), Nitrofurantoin (Nit), Ampicillin (Amp), Ceftazidime (Caz), and Cefuroxime (Crx). Two single discs broad spectrum antibiotics were obtained from (Oxoid Lab Ltd).

24 hour old culture was standardized to 0.5 McFarland standard. This was done by picking an inoculum with sterile inoculating loop and transferring it into 1ml of sterile normal saline in test tube. The vortex mixer was then used to mix the solution. The turbidity was then compared with the McFarland standard. Sterile swab stick was then used to pick the solution from the test tube and was streaked unto fresh Nutrient Agar. The antibiotics disc was then placed unto the agar with the sterile forceps. The plates were incubated for 18-24 hours after which the results were read (Bauer *et al.*, 1966). The result was read by measuring the zone of inhibition and was interpreted by CLSI, 2011 given a range within which they can be Susceptible, Resistant or Intermediate.

Plasmid Extraction

Plasmid profile analysis was done for twelve isolates and plasmids of different sizes were found for ten isolates. The plasmid profile analysis was carried out at the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. The plasmid extraction was done for both Gram-negative and Gram-positive bacterial isolates using the modified method of Ehrenfeld and Clewell, 1987.

3.RESULTS AND DISCUSSION

Isolation of samples

A total of Ninety (90) isolates were obtained from the different sampling site; Asejire, Eleyele and University of Ibadan fish farm. The isolates were identified as *Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp., *Acinetobacter baumanii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Moraxella catarrhalis*, *Klebsiella pneumonia*, *Klebsiella oxytoca* and *Klebsiella ozaenae*.

Frequency of occurrence of isolates

Table 1 shows the frequency of occurrence of bacteria isolates with *Shigella* sp. 25 (27.78) being the highest which was closely followed by *Acinetobacter baumanii* 22(24.44) and then *Salmonella* sp., and the least is *Proteus vulgaris* and *Citrobacter freundii* 1(1.11) and *Klebsiella pneumonia* and *Escherichia coli* 2 (2.22).

Antibiotic susceptibility pattern of isolates

The Antibiotic Susceptibility pattern of gram negative isolates obtained from different fish samples at Asejire is shown in Table 2.1a. A total of 15 isolates were subjected to Antibiotic susceptibility test, the antibiotics included; Gentamycin, Ciprofloxacin, Ofloxacin, Augmentin, Nitrofurantoin, Ampicillin, Ceftazidime, Cefuroxime, Amikacin and Ticarcillin. Multi drug resistance to more than three antibiotics was observed for in 3 isolates namely; *Salmonella* spp, *Shigella* spp and *Proteus vulgaris*. High resistance of above 30 percent was observed in Asejire for Augmentin, Ampicillin, Cefuroxime and Ticarcillin and this may be linked to human activities around the water body such as Industrial effluent and sewage disposal.

High level of resistance of above 30 percent was observed in Eleyele for Augmentin, Ampicillin, Cefuroxime and Ticarcillin. High level of resistance of above 50 percent was found in the University of Ibadan Fish Farm for Augmentin, Ampicillin and Ticarcillin.

Percentage frequency of antibiotic susceptibility of isolates

Table 2.1b shows the percentage frequency of Antibiotic Susceptibility of Asejire gram negative isolates. The table revealed that high level of susceptibility of above 70 percent was observed for in Gentamycin, Ciprofloxacin, Ofloxacin, Nitrofurantoin, Ceftazidime and Amikacin. High level of resistance of above 30 percent was observed for Augmentin, Ampicillin, Cefuroxime and Ticarcillin.

Table 3.1a shows the Antibiotic Susceptibility pattern of gram negative isolates from different fish obtained from University of Ibadan Fish farm. A total of 44 isolates were subjected to Antibiotics Susceptibility test, the antibiotics includes Gentamycin, Ciprofloxacin, Ofloxacin, Augmentin, Nitrofurantoin, Ampicillin, Ceftazidime, Cefuroxime, Amikacin and Ticarcillin. Multi drug resistance to 3 or more antibiotics was recorded for 19 isolates namely; *Salmonella* spp, *Shigella* spp, *Acinetobacter baumaii*, *Klebsiella oxytoca*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Moraxella catarrhalis* and *Enterobacter aerogenes*.

Table 3.1b shows the percentage frequency of Antibiotic Susceptibility of University of Ibadan gram negative isolates. The table revealed that high level of susceptibility of above 70 percent was also observed for gram negative isolates from University of Ibadan Fish Farm in Gentamycin, Ciprofloxacin, Ofloxacin, Nitrofurantoin, Ceftazidime, Cefuroxime and Amikacin. High level of resistance of above 50 percent was found for Augmentin, Ampicillin and Ticarcillin.

Table 4.1a shows the Antibiotic Susceptibility pattern of gram negative isolates obtained from different fish sample at Eleyele. A total of 26 isolates were subjected to Antibiotics Susceptibility test, the antibiotics includes Gentamycin, Ciprofloxacin, Ofloxacin, Augmentin, Nitrofurantoin, Ampicillin, Ceftazidime, Cefuroxime, Amikacin and Ticarcillin. Multi drug resistance to more than three antibiotics was observed in 10 isolates namely; *Acinetobacter baumanii*, *Klebsiella ozaenae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Shigella* spp, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Table 4.1b shows the percentage frequency of Antibiotic Susceptibility of Eleyele gram negative isolates. The table indicates that for the gram negative isolates from Eleyele, high level of above 70 percent susceptibility was observed in Gentamycin, Ciprofoxacin, Ofloxacin, Nitrofurantoin, Ceftazidime and Amikacin whereas high level of resistance of above 30 percent was observed for Augmetin, Ampicillin, Cefuroxime and Ticarcillin in *Salmonella* spp, *Klebsiella ozaenae*, *Citrobacter freundii* and *Klebsiella pneumoniae* had 100 percent resistance to Augmentin.

Table 5.1a shows the Antibiotic susceptibility pattern of gram positive isolates obtained from Catfish samples in Eleyele. Two isolates were subjected to antibiotics screening, the antibiotics were Gentamycin, Ceftriaxone, Erythromycin, Cloxacillin, Ofloxacin, Augmentin, Ceftazidime, Cefuroxime, Amikacin and Ticarcillin. One isolate, *Staphylococcus aureus* exhibited multidrug resistance to more than four antibiotics.

Table 5.1b shows the Antibiotic Susceptibility Pattern for gram positive isolates from different fish samples from University of Ibadan Fish farm. Three isolates were subjected to antibiotics screening, the antibiotics were Gentamycin, Ceftriaxone, Erythromycin, Cloxacillin, Ofloxacin, Augmentin, Ceftazidime, Cefuroxime, Amikacin and Ticarcillin. Two of the isolates exhibited multi drug resistance to more than four antibiotics, and the isolates were both *Staphylococcus aureus*.

Table 5.1c shows the percentage frequency of Antibiotic Susceptibility of gram positive isolates from Eleyele and University of Ibadan fish farm. The table revealed that hundred percent of susceptibility was observed for Gentamicin, Ofloxacin, Ceftazidime and Amikacin among Eleyele gram positive isolates. Meanwhile, hundred percent susceptibility was also observed for Ceftriaxone, Ofloxacin, Ceftazidime and Amikacin.

The table also shows that hundred percent resistance was found for Cloxacillin among gram positive isolates across University of Ibadan Fish Farm and Eleyele. Hundred percent resistance was also observed for Ticarcillin amongst gram positive isolates from

University of Ibadan Fish farm and resistance of above 60 percent for Augmentin and Cefuroxime. While resistance of above 50 percent was observed for Erythromycin, Augmentin, Cefuroxime and Ticarcillin amongst Eleyele isolates namely *Staphylococcus aureus*. Hundred percent (100%) resistance was found for Cloxacillin among Gram-positive isolates across University of Ibadan Fish Farm and Eleyele, this may be due to misuse of such antibiotics. Hundred percent resistance was also observed for Ticarcillin amongst Gram-positive isolates from University of Ibadan Fish farm, and this may be due to the feeds being fed the fishes which may have been treated with antibiotics because they claimed not to feed fishes with antibiotics, and resistance of above 60 percent for Augmentin and Cefuroxime. While resistance of above 50 percent was observed for Erythromycin, Augmentin, Cefuroxime and Ticarcillin amongst Eleyele isolates. Generally, multiple drug resistance was observed among the isolates and this is an extremely serious public health problem according to Prescott, (1999) and multi drug resistance has been found to be associated with the outbreak of major epidemics throughout the world according to Canton,(2003); Levy, (2001). The spread of multidrug resistance constitutes a main impediment to the control of infectious diseases globally (So *et al.*, (2012).

Generally, different level of resistance was observed by among the isolates. This could be so because different strains of the same microorganisms isolated from different sources may present diverse level of resistance as a means of surviving which is in agreement with the report of Lateef, (2004). According to Lateef (2005), several multidrug resistance has been reported in *Staphylococcus aureus*, *Klebsiella* species, *Escherichia coli*, *Enterobacter* species and *Pseudomonas aeruginosa* and this was also observed in this research work. According to Kadlec (2011), plasmid profile have been reported to be useful in tracing the epidemiology of antibiotic resistance. Plasmid profiling for 12 isolates that showed the highest multidrug resistance and plasmids of different sizes were found for 10 of the isolates (figure 1). Resistance was found in those in which plasmids were present as well as in those who had no plasmids. The molecular weight of the isolates range from 14.52 kb to 19.52 kb which was lower compared to that of coliforms (≤56.4 kb) isolated from clinical samples in the work of Karbasizaed *et al.*, (2003)

TABLE 1: The frequency of occurrence of bacterial isolates in the fish samples

Isolates	**No (%)**	**Fish parts (%)**			**Sample site location (%)**		
		Gills	Gut	Flesh	Asejire	Eleyele	U.I Fish Farm
Staphylococcus aureus	6 (6.67)	4 (66.67)	1 (16.67)	0(0.00)	0(0.00)	3 (50)	3 (50)
***Salmonella* sp.**	13 (14.44)	7 (53.85)	2 (15.38)	4 (30.77)	3 (23.08)	3 (23.08)	7 (53.85)
***Shigella* sp.**	25 (27.78)	14(56.00)	6 (24.00)	5(20.00)	8 (32.00)	4 (16)	13 (52.00)
Pseudomonas aeruginosa	3 (3.33)	1 (33.33)	0 (0.00)	2 (66.67)	0(0.00)	1 (33.33)	2 (66.67)
Klebsiella pneumonia	2 (2.22)	1 (50.00)	1 (50.00)	0 (0.00)	0 (0.00)	1 (50.00)	1 (50.00)
klebsiella ozaenae	4 (4.44)	2 (50.00)	1 (25.00)	1 (25.00)	0 (0.00)	1 (25.00)	3 (75.00)
Klebsiella oxytoca	3 (3.33)	2 (66.67)	1 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)	3 (1000
Acinetobacter baumanii	22 (24.44)	11(50.00)	5 (22.73)	6(27.27)	2 (9.09)	12 (54.55)	8 (36.36)
Escherichia coli	2 (2.22)	2 (100)	0 (0.00)	0 (0.00)	0 (0.00)	1 (50)	1 (50)
Proteus vulgaris	1(1.11)	0 (0.00)	0 (0.00)	1 (100)	1 (100)	0 (0.00)	0 (0.00)
Citrobacter freundii	1 (1.11)	0 (0.00)	1 (100)	0 (0.00)	0 (0.00)	1 (100)	0 (0.00)
Enterobacter aerogenes	5 (5.56)	-	2 (40.00)	3 (60.00)	2 (40.00)	1 (20.00)	2 (40.00)
Moraxella catarrhalis	3 (3.33)	2 (66.67)	1 (33.33)	-	-	-	3 (100)
Total	90 (100)	46(51.11)	21(23.33)	23(25.56)	16(17.78)	28 (31.11)	46 (51.11)

TABLE 2.1a: Antibiotics susceptibility pattern of Gram-negative isolates obtained from different fish samples at Asejire with the zone of inhibition in millimeters (mm)

Isolates code	Gentamycin	Ciprofloxacin	Ofloxacin	Augmentin	Nitrofurantoin	Ampicillin	Ceftazidime	Cefuroxime	Amikacin	Ticarcillin
ACFF MAC FLESH 3	- (R)	16.5 (I)	18.5 (S)	18 (R)	20 (S)	- (R)	23.5 (S)	- (R)	22 (S)	20 (S)
ACFF EMB FLESH 6	23 (S)	22 (S)	15 (I)	11 (R)	11 (R)	- (R)	22.5 (S)	15.5 (R)	23 (S)	19 (S)
ACFF EMB FLESH 8	17 (S)	25 (S)	23.5 (S)	- (R)	- (R)	- (R)	23.5 (S)	- (R)	21 (S)	- (R)
ACFF EMB GUT 3	18 (S)	22.5 (S)	17 (S)	- (R)	- (R)	- (R)	24.5 (S)	- (R)	20 (S)	- (R)
ACFF SSA GILL 2	18.5 (S)	22.5 (S)	20 (S)	- (R)	- (R)	- (R)	28 (S)	- (R)	20 (S)	- (R)
ASCF SSA FLESH 2	23.5 (S)	24 (S)	24 (S)	22.5 (S)	29 (S)	- (R)	23 (S)	23 (I)	21.5 (S)	6 (R)
ATSF NA GUT 2	23 (S)	22 (S)	24 (S)	29 (S)	23 (S)	30 (S)	25 (S)	27.5 (S)	22 (S)	36 (S)
ATSF NA GUT 3	30 (S)	25 (S)	25 (S)	36 (S)	34 (S)	32 (S)	20 (S)	30 (S)	31 (S)	34 (S)
ATFF MAC GUT 1	29 (S)	22 (S)	19 (S)	21 (S)	32 (S)	16. 5 (I)	13 (R)	- (R)	28 (S)	16 (I)
ATFF EMB GILL 1	29 (S)	21 (S)	18 (S)	21 (S)	30 (S)	16 (I)	15 (R)	- (R)	22 (S)	20 (S)
ATFF EMB GILL 3	27.5 (S)	27.5 (S)	27.5 (S)	- (R)	27.5 (S)	- (R)	27.5 (S)	27.5 (S)	20 (S)	23 (S)
ATFF SSA FLESH 1	33 (S)	33 (S)	33 (S)	21 (S)	36 (S)	22.5 (S)	32 (S)	33 (S)	23 (S)	20 (S)
ATFF SSA FLESH 2	31 (S)	31 (S)	31 (S)	33 (S)	35.5 (S)	20 (S)	32.5 (S)	30 (S)	21 (S)	24 (S)
ATFF SSA GILL 1	36 (S)	36 (S)	36 (S)	21 (S)	27.5 (S)	16.5 (I)	36 (S)	36 (S)	25 (S)	14 (R)
ATFF SSA GUT 2	35 (S)	35 (S)	35 (S)	23 (S)	35 (S)	21.5 (S)	37 (S)	33 (S)	24 (S)	- (R)

KEY ACFF= Asejire Catfish Fresh, ASCF= Asejire Smoked Catfish, ATSF= Asejire Tilapia Smoked Fish, ATFF= Asejire Tilapia Fresh fish, NA= Nutrient Agar, EMB= Eosin Methylene Blue Agar, MAC= MacConkey Agar, SSA= Salmonella Shigella Agar, -= No zone of inhibition.

TABLE 2.1b: Percentage Frequency of Antibiotic Susceptibility Test of Asejire gram negative Isolates

ANTIBIOTICS	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
	←	%	→
GENTAMYCIN	93.33	-	6.67
CIPROFLOXACIN	93.33	6.67	-
OFLOXACIN	93.33	6.67	-
AUGMETIN	60	-	40
NITROFURANTOIN	73.33	-	26.67
AMPICILLIN	33.33	20.0	46.67
CEFTAZIDIME	86.67	-	13.33
CEFUROXIME	46.67	6.67	46.67
AMIKACIN	100	-	-
TICARCILLIN	53.33	6.67	40

KEY

-= No percentage frequency of occurrence

TABLE 3.1a: Antibiotic Susceptibility pattern of Gram negative isolates of University of Ibadan Fish farm with the zone of inhibition in millimeters (mm)

Isolates code	Gentamycin	Ciprofloxacin	Ofloxacin	Augmentin	Nitrofurantoin	Ampicillin	Ceftazidime	Cefuroxime	Amikacin	Ticarcillin
UTFF NA FLESH 2	36 (S)	36 (S)	36 (S)	36 (S)	36 (S)	36 (S)	36 (S)	36 (S)	25 (S)	14 (R)
UTFF NA FLESH 3	19.5 (S)	25 (S)	25 (S)	30 (S)	23 (S)	25 (S)	25 (S)	36 (S)	36 (S)	36 (S)
UTFF NA GILL 1	33 (S)	33 (S)	33 (S)	17 (R)	32 (S)	18 (S)	32 (S)	33 (S)	24 (S)	11 (R)
UTFF NA GILL 2	24 (S)	31 (S)	31 (S)	27.5 (S)	31 (S)	23 (S)	30 (S)	29 (S)	23 (S)	28 (S)
UTFF NA GILL 3	22.5 (S)	30 (S)	30 (S)	14 (R)	27.5 (S)	- (R)	28 (S)	- (R)	21 (S)	- (R)
UTFF NA GILL 5	22.5 (S)	30 (S)	27.5 (S)	10 (R)	31 (S)	- (R)	31 (S)	- (R)	24 (S)	24 (S)
UTFF NA GILL 7	30 (S)	30 (S)	30 (S)	- (R)	11 (R)	- (R)	28 (S)	- (R)	30 (S)	13 (R)
UTFF MAC GILL 1	16 (S)	23 (S)	23 (S)	17 (R)	33.5 (S)	16 (I)	27.5 (S)	30 (S)	26 (S)	13 (R)
UTFF MAC GILL 2	15 (S)	24.5 (S)	26 (S)	19 (R)	29 (S)	- (R)	27.5 (S)	23.5 (S)	21 (S)	5 (R)
UTFF MAC GILL 3	13 (I)	22.5 (S)	29 (S)	18.5 (R)	28.5 (S)	11 (R)	29 (S)	22 (S)	21 (S)	- (R)
UTFF MAC GILL 4	11 (R)	32 (S)	31 (S)	13 (R)	29 (S)	- (R)	31.5 (S)	23 (I)	22 (S)	6 (R)
UCFF MAC GILL 2	25 (S)	35 (S)	30 (S)	19 (R)	30 (S)	12.5 (R)	30 (S)	30 (S)	27 (S)	- (R)
UCFF MAC GILL 4	17.5 (S)	32.5 (S)	32.5 (S)	13.5 (R)	30 (S)	- (R)	32.5 (S)	32 (S)	23 (S)	27 (S)
UCFF MAC GILL 5	15.5 (S)	27.5 (S)	28 (S)	15 (R)	21 (S)	- (R)	22.5 (S)	25.5 (S)	26.5 (S)	7.5 (R)
UTFF EMB FLESH 2	27.5 (S)	24.5 (S)	25.5 (S)	33.5 (S)	32.5 (S)	28 (S)	30 (S)	34 (S)	33 (S)	33 (S)
UTFF EMB GILL 2	22 (S)	30 (S)	27.5 (S)	17 (R)	- (R)	- (R)	27.5 (S)	27.5 (S)	23 (S)	20 (S)
UTFF EMB GILL 3	22.5 (S)	14 (R)	16.5 (S)	31 (S)	20 (S)	20 (S)	18.5 (I)	20 (R)	26 (S)	21 (S)
UTFF EMB GILL 4	25.5 (S)	23 (S)	26.5 (S)	20.5 (S)	32 (S)	30 (S)	30.5 (S)	35 (S)	27 (S)	21 (S)
UTFF EMB GILL 5	20 (S)	30 (S)	32 (S)	16 (R)	14 (R)	17.5 (S)	13 (R)	9 (R)	26 (S)	20 (S)
UTFF EMB GILL 7	26 (S)	27 (S)	23.5 (S)	14.5 (S)	27.5 (S)	- (R)	32 (S)	- (R)	24 (S)	22 (S)
UCFF EMB GILL 2	25 (S)	32 (S)	31.5 (S)	21 (S)	33 (S)	25 (S)	33 (S)	30 (S)	28 (S)	17 (I)
UCFF EMB GILL 3	21.5 (S)	27 (S)	25 (S)	18.5 (R)	30 (S)	17 (S)	32.5 (S)	30.5 (S)	24 (S)	- (R)

UCFF EMB GILL 6	24 (S)	22 (S)	25 (S)	19.5 (R)	33 (S)	- (R)	33 (S)	33 (S)	27 (S)	8 (R)
UCFF MAC GUT 2	19 (S)	27 (S)	28 (S)	16 (R)	31 (S)	- (R)	32 (S)	225 (S)	23 (S)	11 (R)
UCFF MAC GUT 3	17 (S)	27 (S)	26 (S)	22.5 (S)	24 (S)	16 (I)	24 (S)	22 (I)	19 (S)	23 (S)
UCFF MAC GUT 4	18.5 (S)	27 (S)	27 (S)	16 (R	- (R	- (R)	29.5 (S)	- (R)	20 (S)	15 (I)
UTFF SSA FLESH 1	25 (S)	32 (S)	28 (S)	22 (S)	33 (S)	16 (I)	31 (S)	32 (S)	28 (S)	27 (S)
UTFF SSA GUT 1	24 (S)	25 (S)	31 (S)	19 (R)	33 (S)	- (R)	32 (S)	32 (S)	28 (S)	27 (S)
UTFF SSA GILL 2	23 (S)	32.5 (S)	37.5 (S)	13.5 (R)	33 (S)	14 (I)	32 (S)	32 (S)	28 (S)	- (R)
UTFF SSA GILL 3	24 (S)	32 (S)	24 (S)	17 (R)	32 (S)	10 (R)	27.5 (S)	25 (S)	24.5 (S)	9 (R)
UTFF SSA GILL 4	26.5 (S)	32 (S)	33.5 (S)	17 (R)	32 (S)	16 (I)	27.5 (S)	27.5 (S)	24 (S)	11 (R)
UCFF SSA GILL 1	33 (S)	33 (S)	33 (S)	20 (S)	31 (S)	19 (S)	33 (S)	33 (S)	23 (S)	- (R)
UCFF SSA GILL 2	21.5 (S)	27.5 (S)	26.5 (S)	19 (R)	23 (S)	- (R)	32.5 (S)	30 (S)	22 (S)	20 (S)
UCFF SSA GILL 3	34 (S)	34.5 (S)	34 (S)	28.5 (R)	35 (S)	24 (S)	34 (S)	32 (S)	29 (S)	9 (R)
UCSF MAC FLESH 4	27.5 (S)	25 (S)	28.5 (S)	30 (S)	- (R)	22 (S)	25.5 (S)	25 (S)	28 (S)	28 (S)
UCLF EMB GUT 1	35 (S)	40 (S)	35 (S)	35 (S)	35 (S)	35 (S)	35 (S)	25 (S)	32 (S)	55 (S)
UTFF EMB FLESH 1	37 (S)	45 (S)	39 (S)	40 (S)	35 (S)	35 (S)	35 (S)	35 (S)	33 (S)	45 (S)
UTFF MAC FLESH 3	32 (S)	33 (S)	35 (S)	15 (R)	37.5 (S)	13 (R)	35 (S)	35 (S)	25 (S)	9 (R)
UCFF NA GUT 5	15 (S)	27.5 (S)	28.5 (S)	19 (R)	30 (S)	11 (R)	28 (S)	22.5 (I)	21 (S)	10 (R)
UCFF MAC GILL 6	21 (S)	21. 5 (S)	24 (S)	14 (R)	20 (S)	- (R)	22 (S)	25.5 (S)	23 (S)	21 (S)
UCFF NA GILL 3	17.5 (S)	27 (S)	26.5 (S)	16 (R)	33.5 (S)	- (R)	31 (S)	31 (S)	22 (S)	7 (R)
UTFF NA GUT 1	23 (S)	30 (S)	31 (S)	22 (S)	- (R)	- (R)	33 (S)	- (R)	23 (S)	- (R)
UCFF NA GILL 1	25 (S)	15 (R)	21 (S)	25 (S)	30 (S)	15 (R)	25 (S)	28 (S)	27 (S)	16 (S)
UCFF NA GILL4	12.5 (S)	30 (S)	30 (S)	25.5 (S	31 (S)	- (R)	31 (S)	31 (S)	24 (S)	14 (R)

KEY

UCFF= University of Ibadan Fish Farm Catfish fresh, UTFF= University of Ibadan Tilapia Fresh Fish, UCSF= University of Ibadan Smoked Catfish, UCLF= University of Ibadan Laboratory Smoked Catfish, NA= Nutrient Agar, EMB= Eosin Methylene Blue Agar, SSA= Salmonella Shigella Agar, MAC= MacConkey Agar, -= No zone of inhibition.

TABLE 3.1b: Percentage Frequency of Antibiotic Susceptibility Test of University of Ibadan Fish Farm Gram-negative Isolates

ANTIBIOTICS	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
	←	%	→
GENTAMYCIN	95.46	2.27	2.27
CIPROFLOXACIN	95.45	-	4.55
OFLOXACIN	100	-	-
AUGMENTIN	38.64	-	61.36
NITROFURANTOIN	86.36	-	13.64
AMPICILLIN	34.09	11.36	54.55
CEFTAZIDIME	95.46	2.27	2.27
CEFUROXIME	72.73	9.09	18.18
AMIKACIN	97.73	-	2.27
TICARCILLIN	40.91	6.82	52.27

KEY -= No percentage frequency of occurrence

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TABLE 4.1a: Antibiotic susceptibility pattern of Gram-negative isolates obtained from different fish samples at Eleyele with the zone of inhibition in millimeters (mm)

Isolates code	Gentamycin	Ciprofloxacin	Ofloxacin	Augmentin	Nitrofurantoin	Ampicillin	Ceftazidime	Cefuroxime	Amikacin	Ticarcillin
ECFF NA GUT 3	23 (S)	15 (R)	21.5 (S)	15 (R)	30 (S)	- (R)	13 (R)	15 (R)	21 (S)	15 (I)
ECFF NA GILL 2	22 (S)	24 (S)	30 (S)	13.5 (R)	33 (S)	- (R)	29 (S)	27 (S)	21 (S)	- (R)
ECFF NA GILL3	25 (S)	28.5 (S)	32.5 (S)	14.5 (R)	34 (S)	16 (I)	32.5 (S)	32 (S)	22 (S)	- (R)
ECFF NA FLESH 1	- (R)	21 (S)	23 (S)	24.5 (S)	7.5 (R)	18.5 (I)	7 (R)	18.5 (R)	29.5 (S)	- (R)
ECFF NA FLESH 2	25 (S)	32 (S)	28 (S)	19 (R)	26 (S)	- (R)	30 (S)	30 (S)	22 (S)	- (R)
ECFF NA FLESH 3	23.5 (S)	28 (S)	27.5 (S)	12.5 (R)	29 (S)	- (R)	26.5 (S)	23 (I)	21 (S)	9 (R)
ECFF NA FLESH 4	22 (S)	25 (S)	22 (S)	34.5 (S)	26 (S)	32.5 (S)	25 (S)	28 (S)	22.5 (S)	40 (S)
ESCF NA GILL 1	- (R)	25 (S)	20 (S)	19 (R)	18.5 (S)	10 (R)	- (R)	- (R)	- (R)	17 (I)
ESCF NA GUT 1	- (R)	23 (S)	23 (S)	21 (S)	- (R)	14.5 (I)	- (R)	- (R)	- (R)	26 (S)
ESCF NA FLESH 1	- (R)	26.5 (S)	26 (S)	20 (S)	- (R)	17.5 (I)	- (R)	15.5 (R)	8 (R)	28 (S)
ECFF EMB FLESH 1	28 (S	30 (S)	30 (S)	13.5 (R)	19 (S)	17 (I)	24 (S)	24 (S)	23.5 (S)	20.5 (S)
ECFF EMB FLESH 4	30 (S)	33 (S)	33 (S)	14.5 (R)	27 (S)	16 (I)	29 (S)	- (R)	23 (S)	- (R)
ECFF EMB GILL 1	23 (S)	24.5 (S)	24 (S)	7 (R)	29 (S)	16.5 (I)	23.5 (S)	26 (S)	23.5 (S)	20 (S)
ECFF EMB GILL 3	25 (S)	23 (S)	26 (S)	13 (R)	28 (S)	- (R)	30 (S)	24 (S)	24 (S)	- (R)
ECFF EMB GILL 4	25 (S)	25 (S)	27.5 (S)	29 (S)	12.5 (R)	16 (I)	17.5 (I)	17 (R)	25 (S)	17 (R)
ECFF EMB GILL 5	- (R)	30 (S)	27 (S)	24 (S)	11.5 (R)	- (R)	7 (R)	15 (R)	- (R)	- (R)
ECFF EMB GUT 3	- (R)	24 (S)	- (R)	- (R)	16 (I)	- (R)	- (R)	13 (R)	8 (R)	- (R)
ECFF EMB GUT 4	- (R)	28 (S)	22.5 (S)	23 (S)	- (R)	- (R)	5 (R)	11 (R)	8 (R)	- (R)
ECFF EMB GUT 5	6 (R)	30 (S)	22.5 (S)	6 (R)	13 (R)	- (R)	8 (R)	15.5 (R)	- (R)	- (R)
ECFF EMB GUT 6	22.5 (S)	27.5 (S)	27.5 (S)	13.5 (R)	30 (S)	- (R)	30.5 (S)	27.5 (S)	25 (S)	- (R)
ESCF EMB FLESH 1	- (R)	29 (S)	22 (S)	6 (R)	- (R)	- (R)	- (R)	- (R)	- (R)	22.5 (S)
ECFF MAC GILL 1	- (R)	28 (S)	19 (S)	14 (R)	- (R)	- (R)	-(R)	- (R)	- (R)	- (R)
ECFF MAC GILL 2	19.5 (S)	25 (S)	23 (S)	18 (R)	30 (S)	14 (I)	25 (S)	25 (S)	24 (S)	- (R)
ECFF MAC GUT 2	21 (S)	30 (S)	30 (S)	13 (R)	30 (S)	- (R)	31 (S)	26 (S)	- (R)	24 (S)
ECFF SSA GUT 1	25 (S)	27.5 (S)	27.5 (S)	15.5 (R)	32 (S)	- (R)	27.5 (S)	27.5 (S)	23 (S)	- (R)
ECFF SSA GILL 1	- (R)	22 (S)	22 (S)	- (R)	21.5 (S)	- (R)	27 (S)	- (R)	24.5 (S	15.5 (I)

KEY: ECFF= Eleyele Catfish Fresh, ESCF= Eleyele Smoked Catfish, NA= Nutrient Agar, SSA= Salmonella Shigella Agar, EMB= Eosin Methylene Blue Agar, MAC= MacConkey Agar, -= No zone of inhibition

TABLE 4.1b: Percentage Frequency of Antibiotic Susceptibility Test of Eleyele Gram-negative Isolates

ANTIBIOTICS	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
	←	%	→
GENTAMICIN	61.54	-	38.46
CIPROFLOXACIN	96.15	-	3.85
OFLOXACIN	96.15	-	3.85
AUGMENTIN	26.92	-	73.08
NITROFURANTOIN	61.54	3.85	34.62
AMPICILLIN	3.85	34.62	61.54
CEFTAZIDIME	53.85	3.85	42.31
CEFUROXIME	42.31	3.85	53.85
AMIKACIN	61.54	-	38.46
TICARCILLIN	34.62	15.38	50

KEY -= No percentage frequency of occurrence

TABLE 5.1a: Antibiotic susceptibility pattern of Gram-positive isolates obtained from Eleyele Catfish with the zone of inhibition in millimeters

Isolates code	Gentamycin	Ceftriazone	Erythromycin	Cloxacillin	Ofloxacin	Augmentin	Ceftazidime	Cefuroxime	Amikacin	Ticarcillin
ECFF NA GILL 1	29 (S)	32.5 (S)	16 (I)	13.5 (R)	31 (S)	25 (S)	32.5 (S)	32.5 (S)	24 (S)	21 (S)
ECFF MAC GILL 4	18 (S)	20.5 (I)	- (R)	- (R)	19 (S)	14.5 (R)	24.5 (S)	- (R)	25 (S)	12 (R)

KEY: NA= Nutrient Agar, MAC= MacConkey Agar, ECFF= Eleyele Catfish Fresh, = No zone of inhibition

TABLE 5.1b: Antibiotic Susceptibility pattern of Gram-positive isolates obtained from different fish samples at University of Ibadan UI) Fish Farm with the zone of inhibition in millimeters (mm)

Isolates code	Gentamycin	Ceftriazone	Erythromycin	Cloxacillin	Ofloxacin	Augmentin	Ceftazidime	Cefuroxime	Amikacin	Ticarcillin
UTFF NA GILL 6	22.5 (S)	27.5 (S)	- (R)	- (R)	30 (S)	17 (R)	28 (S)	9 (R)	24 (S)	8 (R)
UCFF NA FLESH 1	14.5 (I)	33 (S)	16 (I)	13 (R)	27.5 (S)	22.5 (S)	28.5 (S)	27.5 (S)	21 (S)	- (R)
UCFF NA GUT 1	18.5 (S)	32.5 (S)	14 (I)	13.5 (R)	30 (S)	- (R)	31 (S)	18.5 (R)	25 (S)	0.8 (R)

KEY: NA= Nutrient Agar, UTFF= UI Tilapia Fresh Fish, UCFF= UI Catfish Fresh, **=**No zone of inhibition

TABLE 5.1c. Percentage frequency of antibiotic susceptibility tests of Gram-positive isolates across Eleyele and University of Ibadan Fish Farm

ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENIC GRAM POSITIVE ISOLATES ACROSS TWO LOCATIONS

	ELEYELE			U.I FISH FARM		
ANTIBIOTICS	SUSCEPTIBLE	INTERMEDIATE %	RESISTANT	SUSCEPTIBLE	INTERMEDIATE %	RESISTANT
GENTAMYCIN	100	-	-	66.67	33.33	-
CEFTRIAXONE	50	50	-	100	-	-
ERYTHROMYCIN	-	33.33	66.67	-	50	50
CLOXACILLIN	-	-	100	-	-	100
OFLOXACIN	100	-	-	100	-	-
AUGMETIN	50	-	50	33.33	-	66.67
CEFTAZIDIME	100	-	-	100	-	-
CEFUROXIME	50	-	50	33.37	-	66.67
AMIKACIN	100	-	-	100	-	-
TICARCILLIN	50	-	-	-	-	100

KEY- = No percentage frequency of occurrence

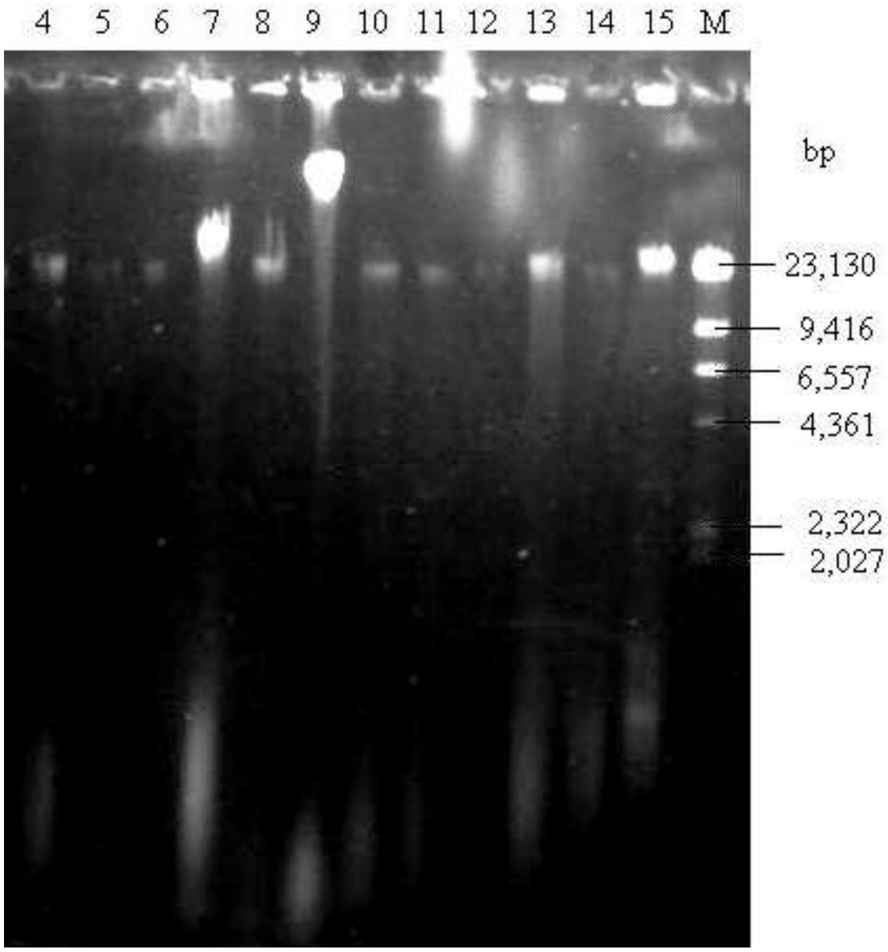

Figure 1. Plasmid profile analysis of isolates with highest multidrug resistance and their most probable identity
KEY

M = Marker
Lane 4 = *Staphylococcus aureus*
Lane 5 = *Staphylococcus aureus*
Lane 6 = *Staphylococcus aureus*
Lane 7 = *Acinetobacter baumanii*
Lane 8 = *Shigella* spp.
Lane 9 = *Shigella* spp.
Lane 10 = *Shigella* spp.
Lane 11 = *Klebsiella ozaenae*
Lane 12 = *Moraxella catarrhalis*
Lane 13 = *Pseudomonas aeruginosa*
Lane 14 = *Acinetobacter baumanii*
Lane 15 = *Salmonella* spp.

4. CONCLUSION

In conclusion, the removal of antibacterial in feed aquaculture could cause great losses; however, national regulations on the use of antibacterial should be introduced and enforced. The public should also be enlightened on the feeding of fresh fish parts such as gills and gut to dogs and poultry which should be discouraged since these animals can harbor the bacterial carrying R-plasmids, chromosomes or transposons. In the course of this research, multidrug resistance was observed in the majority of isolates. The spread of multidrug resistance pathogens has constituted a major impediment to the control of infectious diseases, and since this study has also shown that fish samples could be a reservoir of bacteria carrying R- plasmids as well as genes responsible for resistance, therefore, the indiscriminate use of antibiotics should be discouraged.

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