

Antibiotic Sensitivity Pattern of Pathogenic Bacterial Isolates From Diseased *Clarias gariepinus* From Selected Ibadan And Ikorodu Farms

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Abstract: This study was carried out to isolate, characterise and identify bacteria from diseased *Clarias gariepinus* and also assess the occurrence of resistance to antimicrobial in isolated bacteria. Samples of diseased African Catfish were collected for a period of six weeks from consenting farms in Ibadan and Ikorodu in Nigeria and examined for clinical signs of disease. Pond water samples along with organs such as gills, skin, intestine, kidney and lungs from these fish were analyzed microbiologically using differential and selective media. Bacteria enumeration, identification and biochemical characterization were carried out and the physicochemical parameters of the water samples determined. All isolates were subjected to antibiotic sensitivity test using the standard Kirby-Bauer disc diffusion method. The total bacterial load for the organs ranged between 3.0×10^4 (lungs sample) and 6.0×10^7 cfu/g (gill sample). The gills had the highest average total bacterial count, while lungs had the least. Morphologically unique bacterial isolates obtained included *Salmonella* (14 isolates), *Pseudomonas* (4 isolates), *Aeromonas* (2 isolates), *Edwardsiella* (3 isolates) and *Shigella* (3 isolates). These isolates displayed antibiotic resistance profile to the following: Cefotaxime (38%), Cefuroxime (77%), Gentamicin (37%), Cefixime (73%), Ofloxacin (23%), Augmentin (66%), Nitrofurantoin (58%) and Ciprofloxacin (15%). Two *Salmonella* isolates had multi-drug resistance pattern. This study showed that indiscriminate use of unlicensed or unapproved antibiotics for aquaculture portends significant hazards to public health therefore disease prevention is preferable through good culture and health management to ensure optimum yields and wholesome products.

Keywords: Aquaculture, Fish disease, Bacteria, Antibiotics resistance, Antimicrobial susceptibility.

INTRODUCTION

Aquaculture is farming of high-protein, aquatic organisms including fish, molluscs, crustaceans and aquatic plants under controlled or semi-controlled environment to enhance productivity, i.e. stocking, feeding, and protection from predators [1]. Aquaculture is extremely valuable in developing countries. It serves as a chief source of protein to population, income generation, employment and collateral for obtaining loans. Over the past three decades, aquaculture has intensified and diversified leading to heavy movement of animal and animal products such as broodstock, fingerlings (seeds) and feeds which are largely responsible for the introduction and spread of pathogen and disease into aquaculture system [2].

Diseases in fish can be classified as infectious and non-infectious. Infectious diseases are contagious diseases caused by pathogens (bacteria, fungi, virus, protozoa and parasites) while the non-infectious diseases can be broadly characterized as environmental and nutritional [3]. Bacterial infections

are the most recurrent type of disease problem in all types of aquaculture production followed closely by fungal diseases [4]. Aquaculture in Nigeria hangs essentially on catfish culture and its development which will enhance fish supply [5]. Although aquaculture is growing rapidly, disease prevention and treatment practices are far from standardization or regulation [6]. Fish and fishery products are in the forefront of food safety and quality improvement because they are among the most internationally traded food commodities. However, the use of antimicrobial agents in aquaculture and the possibility of antibiotic resistance among bacterial flora from fish have been identified [7]. The use of antimicrobial agent in aquaculture can therefore result in increased prevalence of resistant bacteria which can cause a direct spread of resistance from aquatic environments to humans [8] via consumption of aquaculture food products, direct contact with culture water or aquatic organisms or through the handling of aquaculture food products [9]. Bacterial species of public health importance include: *Aeromonas*, *Edwardsiella tarda*, *Escherichia coli*, *Plesiomonas*, *shigelloides*, *Salmonella*, *Shigella*, *Vibrio cholera*, *V. Parahaemolyticus* and *V. vulnificus* [10, 11]. The risk of administering antibiotics that may select for not only drug-resistant pathogenic bacteria of fish but also pathogens of

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human health significance has led to a rising concern over worldwide aquaculture and fish health management practices. Developed countries are especially vigilant in this domain, and the resulting debates have led legislations to dictate requirements, clearly limiting the veterinary use of antimicrobial drugs in aquaculture [12]. Some antibiotics have been prohibited from use in food producing animals in some countries (e.g. fluoroquinolones in the USA). The aims of this study were to assess the physicochemical parameters of culture water, determine microbial load of culture water and organs of diseased *Clarias gariepinus* from farms in Ibadan and Ikorodu: isolate and characterise bacterial pathogens from the diseased *Clarias gariepinus* samples and evaluate the antibiotic susceptibility patterns of the bacterial pathogens isolated from diseased *Clarias gariepinus*.

MATERIALS AND METHODS

Study Area

Ibadan and Ikorodu were selected as the study locations based on the aggregations of fish farms in Ibadan, while Ikorodu has a 34-hectare fish farm estate at Odogunyan, Ikorodu. Ibadan is located in Oyo state, its geographical coordinates are 7°23'16" North, 3°53'47" East while Ikorodu is geographically located in Lagos State with geographical coordinates 6°37'0" North, 3°31'0" E in Nigeria.

Sample Collection and Processing

Samples were collected over a period of six weeks from a total of eight consenting farms depending on availability of diseased fish [five farms from Ikorodu Fish Farm estate (A-E) and three from Ibadan (I-III)]. Samples of water (eight) were collected in sterile plastic 1L containers from each pond (transported on ice in a cooler box), while moribund diseased fish (fifty fish samples) were collected in clean sterile cooler containing ice pack and then transported to Fish Disease Diagnostic Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan. Fish samples were examined for clinical disease and skin, gill, lungs, intestine and kidney samples were obtained using sterile scalpel [13]. One gram of each sample was weighed and mixed with 9ml of 0.1% sterile peptone water (Oxoid, CM9) placed in a sterile stomacher bag (Stomacher 400, Seward medicals) and homogenized in a stomacher blender (Seward Medical, United Kingdom) Ten-fold dilutions of the homogenates were made and plated [14].

Bacteria Isolation, Enumeration and Identification

All the chemicals and reagents used were of analytical grade. Media used in this study were Nutrient Agar (NA), Nutrient broth (NB), Peptone water (PW), *Salmonella- Shigella* Agar (SSA), Eosin Methylene Blue Agar (EMB) and Tryptone Soy Agar (HiMedia Lab. Pvt. Mumbai, India). All media were prepared according to manufacturer's specification and sterilized at 121°C for 15mins. From the ten-fold serial dilutions of homogenates, 1ml aliquots of homogenates were plated in replicates on different media, using the pour plate method [15]. The plates were incubated at 37°C for 18-24hrs. The total viable aerobic bacteria count was performed on Nutrient agar. Colonies were counted using the Lapiz® digital colony counter (Mumbai, India) and expressed as Colony forming unit per gramme of suspension (Cfu/g). Discrete colonies were sub-cultured onto fresh agar plates by streaking aseptically to obtain pure culture of the isolates. Pure cultures of bacterial organisms were identified using standard colony morphological responses on the different chromogenic media along with other microscopic and biochemical procedures [16].

ANTIBIOTIC SENSITIVITY TEST

Commercially available antibiotics disc from Abtek® Biologicals, Liverpool, United Kingdom, was used. Pure bacteria colonies were picked from broth using a sterile wire loop and transferred to tubes each containing 5ml of sterile saline. The suspension was vortexed and adjusted to match 0.5 McFarland turbidity standard. Sterile cotton swab was then dipped, rotated and pressed firmly on the tube walls above the culture to remove excess inoculum from the swabs. This was then evenly swabbed on the dried surface of Mueller-Hinton agar (HiMedia Lab. Pvt. Mumbai, India) plates ensuring even distribution of the bacterium. The antimicrobial loaded discs were placed on the bacteria plates using sterile forceps and incubated at 37°C for 18 to 24hrs. Interpretations of results were done using the zones diameters [17]. The eight antibiotics used had the following concentrations; Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg), Ofloxacin (5µg), Augmentin (30µg), Nitrofurantoin (300µg) and Ciprofloxacin (5µg).

WATER QUALITY ASSESSMENT

Hach® water quality test kit (Hach company, USA) was used to determine levels of nitrite, alkalinity, dissolved oxygen, pH, total hardness, dissolved carbon

dioxide while a thermometer (Donggian Instrument, China) was used to determine temperature.

RESULTS AND DISCUSSION

Clinical Signs of Diseased Fish from the Different Fish Farms

Fingerlings from Ibadan farm I were off feed for 3 days. They had distended abdomen filled with slightly opaque fluid (Figure 1) and there was high mortality in the pond from where they were obtained.



Figure 1: Farm I in Ibadan showing mass mortality with fingerlings having fluid-filled distended abdomen.

In farm II, there were mortalities without visible clinical signs except that the fingerlings were off feed. Juveniles from farm III had ulcers at the base of the fins and accumulation of bloody fluid in the abdomen (Figure 2).



Figure 2: Juveniles from Farm III in Ibadan with haemorrhages in the skin tissue and bloody discharges from the vent.

At Ikorodu, fish from farm A had severe hyperaemic ulcers on the skin while those from farm B had hyperpigmentation, severe ulceration and necrosis of the skin and musculature. Fish from farm C presented with pustules and ulceration of the musculature, necrosis of the tail fin and parietal part of the head. Those from Farm D had greenish discoloration, severe cutaneous ulceration and necrosis (Figure 3).

African catfish from Farm E had accumulation of yellowish fluid in the intestine with ulcers at the base of the fins (Figure 4).

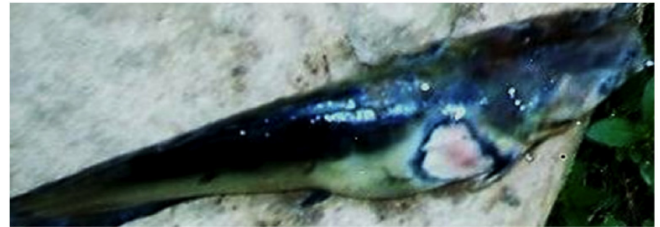


Figure 3: Fish from Farm D in Ikorodu showing greenish discoloration, severe cutaneous ulceration and necrosis.



Figure 4: African catfish from Farm E in Ikorodu with accumulation of yellowish fluid in the intestine and with ulcers at the base of the fins.

Farm II fish samples had more bacterial load than other farms (Table 1). This should not be taken in isolation because further results confirmed that farm II pond water also had higher levels of bacterial load (Table 2). The physico-chemical parameters of pond II revealed a high pH, high dissolved CO₂, high alkalinity and total hardness above the recommended standards (Table 3). These factors predispose fish to stress, which encourages development of disease. The poor water quality of farm II could be the primary cause of the mortalities observed with the bacterial infection

Table 1: Microbial Load of Diseased *Clarias gariepinus* Fingerlings from Farms I and II in Ibadan

Source	MEDIA (Cfu/g).		
	SSA	EMB	NA
Farm I	2.6x10 ⁵	3.5x10 ⁵	5.3x10 ⁶
Farm II	9x10 ⁴	1.3x10 ⁵	2.4x10 ⁵

SSA –*Salmonella-Shigella* Agar, EMB-Eosin Methylene Blue Agar, NA - Nutrient Agar.

being opportunistic. The bacterial load of various fish organs from the different farms is presented as Tables 4, 5, 6, 7 and 8.

Table 2: Microbial Load of Water Samples from Ponds where Diseased *Clarias gariepinus* were Obtained

LOCATION	FARM	MEDIA (Cfu/ml)		
		SSA	EMB	NA
IBADAN	FARM I	5.5X10 ⁵	9.2X10 ⁵	1.4X10 ⁵
	FARM II	7.4X10 ⁵	5.6X10 ⁵	3.5X10 ⁶
	FARM III	1.2X10 ⁷	7.6X10 ⁵	2.0X10 ⁷
	FARM A	1.2X10 ⁵	7.4X10 ⁵	3.2X10 ⁵
IKORODU	FARM B	3.4X10 ⁵	8.5X10 ⁵	4.5X10 ⁴
	FARM C	1.2X10 ⁵	8.7X10 ⁵	8.2X10 ⁵
	FARM D	1.1X10 ⁵	8.2X10 ⁵	7.4X10 ⁵
	FARM E	1X10 ⁷	8.6X10 ⁵	1.7X10 ⁸

SSA-Salmonella-Shigella Agar, EMB-Eosin Methylene Blue Agar, NA-Nutrient Agar.

Generally in the adult fish, the gills had more bacteria load than all the other organs, which is not surprising considering the fact that gills filter the water to obtain oxygen and as such is exposed to environmental contaminants [18]. Also it serves as the first point of entry for most pathogens; hence it can be used as a biomarker of microbial contamination in live fish [19]. It was also observed that water samples from Farms I and Farm II containing fingerlings had higher bacteria load than those of the adult fish. Fingerlings have higher metabolic rate and are therefore fed more frequently which could lead to poor water quality hence increase in bacteria growth [20, 21]. Additionally, death in fingerlings are usually more common due to the fact that their immune system are not as developed as in the adult fish so any change in environmental conditions leading to stress could be detrimental [22].

A total of 153 isolates were obtained from fish and water samples of which 57 unique cultures were selected based on their morphology and stored on

Table 3: Physiochemical Parameters of Pond Water from the Various Farms

Location	Farm	Temp (°C)	pH	DO (mg/L)	Nitrite (mg/L)	Dissolved CO ₂ (mg/L)	Alkalinity (mg/L)	Total Hardness (mg/L)	Age
Ibadan	I	27	6.5	3.0	0.00	180.0	256.5	30.0	4weeks
	II	25	7.5	18.0	0.132	295.0	239.4	427.5	5weeks
	III	26	7.0	2.0	0.99	287.5	205.2	171.0	10weeks
Ikorodu	A	26	6.5	5.0	0.792	155.0	85.5	32.2	4months
	B	26	6.0	2.0	0.594	190.0	68.4	34.2	5months
	C	26	5.5	7.0	0.99	125.0	51.3	34.2	4months
	D	25	5.5	15.0	0.66	110.0	34.4	17.1	5months
	E	26	6.0	1.0	1.32	130.0	171.0	102.6	3months
Standard Value		25-30	6.5-8.5	>5.0	0.1	0-15	20-200	20-100	Not Applicable

Table 4: Microbial Load of Skin Obtained from Diseased *Clarias gariepinus* in Different Farms

SOURCE	MEDIA (Cfu/g)		
	SSA	EMB	NA
Ibadan Farm III	2.2 x 10 ⁵	4.3x10 ⁵	4.2x10 ⁵
Ikorodu Farm A	4.0 x 10 ⁴	3.7x 10 ⁷	1.5x 10 ⁶
Ikorodu Farm B	1.6 x 10 ⁵	5.0x10 ⁴	2.9x10 ⁶
Ikorodu Farm C	3.1x10 ⁵	1.7x10 ⁵	2.6x10 ⁷
Ikorodu Farm D	1.0x10 ⁵	1.0x10 ⁵	4.1x10 ⁷
Ikorodu Farm E	1.0x10 ⁵	1.7x10 ⁵	4.3x10 ⁷

SSA -Salmonella Shigella Agar, EMB - Eosin Methylene Blue Agar, NA -Nutrient Agar.

Table 5: Microbial Load of Gills Obtained from Diseased *Clarias gariepinus* in Different Farms

SOURCE	MEDIA (Cfu/g)		
	SSA	EMB	NA
Ibadan Farm III	5.6×10^5	7.8×10^5	1.3×10^7
Ikorodu Farm A	3.0×10^6	7.0×10^5	3.6×10^6
Ikorodu Farm B	2.0×10^5	7.0×10^5	6.3×10^6
Ikorodu Farm C	4.2×10^5	2.5×10^5	4.4×10^5
Ikorodu Farm D	6.0×10^6	3.5×10^5	1.3×10^5
Ikorodu Farm E	5.0×10^7	7.3×10^5	6.0×10^7

SSA -*Salmonella-Shigella* Agar, EMB - Eosin Methylene Blue Agar, NA -Nutrient Agar.**Table 6: Microbial Load of Lungs Obtained from Diseased *Clarias gariepinus* in Different Farms**

SOURCE	MEDIA (Cfu/g)		
	SSA	EMB	NA
	4.5×10^5	2.2×10^5	3.7×10^4
Ikorodu Farm A	8.0×10^4	3.0×10^4	2.7×10^6
Ikorodu Farm B	3.9×10^6	4.8×10^6	5.3×10^6
Ikorodu Farm C	2.5×10^5	2.3×10^3	3.8×10^5
Ikorodu Farm D	2.7×10^5	4.2×10^5	5.0×10^4
Ikorodu Farm E	3.8×10^5	8.7×10^5	6.5×10^5

SSA -*Salmonella-Shigella* Agar, EMB - Eosin Methylene Blue Agar, NA -Nutrient Agar.**Table 7: Microbial Load of Kidney Obtained from Diseased *Clarias gariepinus* in Different Farms**

SOURCE	MEDIA (Cfu/g)		
	SSA	EMB	NA
Ibadan Farm III	1.6×10^5	4.6×10^5	7.1×10^5
Ikorodu Farm A	1.7×10^5	7.5×10^5	1.6×10^6
Ikorodu Farm B	3.0×10^5	1.1×10^5	4.5×10^6
Ikorodu Farm C	3.4×10^5	1.7×10^5	2.3×10^5
Ikorodu Farm D	3.3×10^5	2.4×10^5	4.5×10^5
Ikorodu Farm E	2.6×10^5	5.0×10^5	3.3×10^5

SSA -*Salmonella-Shigella* Agar, EMB - Eosin Methylene Blue Agar, NA -Nutrient Agar.**Table 8: Microbial Load of Intestine Obtained from Diseased *Clarias gariepinus* in Different Farms**

SOURCE	MEDIA (Cfu/g)		
	SSA	EMB	NA
Ibadan Farm III	7.0×10^4	1.5×10^5	4.3×10^5
Ikorodu Farm A	5.0×10^5	2.1×10^5	7.3×10^6
Ikorodu Farm B	2.6×10^5	2.0×10^5	2.1×10^6
Ikorodu Farm C	1.8×10^5	1.9×10^5	1.5×10^5
Ikorodu Farm D	4.3×10^5	3.3×10^5	2.9×10^5
Ikorodu Farm E	3.6×10^5	4.9×10^5	5.4×10^5

SSA -*Salmonella-Shigella* Agar, EMB - Eosin Methylene Blue Agar, NA -Nutrient Agar.

Table 9: Colony, Morphology and Biochemical Characteristics of Bacterial Isolates

Isolate	Identified Bacteria	Organ and Source	Pigmentation	Gram Status	VP	MR	Mannitol	Lactose	Indole	Haemolysis	Oxidase	Nitrate reduction	Glucose (acid prod)	Motility	H ₂ S	Citrate
1	<i>Salmonella</i> Sp.	LUNG A	Black: underneath	-	-	+	+	-	-	-	-	+	+	+	+	+
2	<i>Salmonella</i> Sp.	SKIN A	Colourless colonies with black centre.	-	-	+	+	-	-	-	-	+	+	+	+	+
3	<i>Salmonella</i> Sp.	GILL A	Black: surface	-	-	+	+	-	-	+	-	+	+	+	+	+
4	<i>Salmonella</i> Sp.	GILL A	Black: surface	-	-	+	+	-	-	-	-	+	+	+	+	+
5	<i>Salmonella</i> Sp.	LUNG A	Black: surface	-	-	+	+	-	-	-	-	+	+	+	+	+
6	<i>Shigella</i> Sp.	GILL A	Dull yellow: surface	-	-	-	-	-	-	-	-	+	+	-	-	-
7	<i>Salmonella</i> Sp.	LUNG A	Black centre	-	-	+	+	-	-	+	-	+	+	+	+	+
8	<i>Aeromonas</i> Sp.	SKIN A	Cream: surface	-	+	-	+	+	+	+	+	+	+	+	+	+
9	<i>Aeromonas</i> Sp.	SKIN A	Cream: surface	-	+	-	+	+	+	+	+	+	+	+	+	+
10	<i>Salmonella</i> Sp.	GILL B	Black: surface	-	-	+	+	-	-	+	-	+	+	+	+	+
11	<i>Edwardsiella</i> Sp.	KIDNEY B	Cream: surface	-	-	+	-	-	-	+	-	+	+	-	-	-
12	<i>Shigella</i> Sp.	GILL B	Colourless colonies.	-	-	-	-	-	-	+	-	+	+	-	-	-
13	<i>Salmonella</i> Sp.	WATER II	Clear zone with black centre: surface	-	-	+	+	-	-	-	-	+	+	+	+	+
14	<i>Shigella</i> Sp.	WATER II	Cream with brown centre: surface	-	-	-	-	-	-	-	-	+	+/-	-	-	+
15	<i>Salmonella</i> Sp.	WATER II	Black: surface	-	-	+	+	-	-	-	-	+	+	+	+	+
16	<i>Pseudomonas</i> Sp	JUVENILE III	Greenish cream: surface	-	-	-	+	+	-	+	+	+	+	+	-	+
17	<i>Pseudomonas</i> Sp.	WATER D	Greenish cream: surface	-	-	-	+	+	-	-	+	+	+	+	-	+
18	<i>Pseudomonas</i> Sp.	SKIN D	Greenish cream: surface	-	-	-	+	+	-	+	+	+/-	+	+	-	+
19	<i>Salmonella</i> Sp.	WATER E	Black: under the plate	-	-	-	+	-	-	-	-	+	+	+	+	+
20	<i>Edwardsiella</i> Sp.	GILL E	Cream: surface	-	-	+	-	-	-	-	-	+	+	-	-	-
21	<i>Salmonella</i> Sp.	WATER E	Black: under the plate	-	-	+	+	-	-	-	-	+	+	+	+	+
22	<i>Salmonella</i> Sp.	INTESTIN E E	Black: surface	-	-	+	+	-	-	-	-	+	+/-	+	-	+
23	<i>Pseudomonas</i> Sp.	WATER G	Greenish cream: surface	-	-	-	+	+	-	-	+	+	+	+	-	+
24	<i>Salmonella</i> Sp.	GILL G	Black: surface	-	-	+	+	-	-	-	-	+	+	+	+	+
25	<i>Edwardsiella</i> Sp.	GILL H	Cream: surface	-	-	+	-	-	-	+	-	+	+/-	-	-	+
26	<i>Salmonella</i> Sp.	WATER H	Black: under the plate	-	-	+	+	-	-	+	-	+	+	+	+	+

slants (Table 9). Positively identified pathogens included 14 *Salmonella* (24.6%), 4 *Pseudomonas* (7%), 2 *Aeromonas* (3.5%), 3 *Edwardsiella* (5.3%) and 3 *Shigella* (5.3%) species.

Antibiotics resistance is a significant public health issue. There have been many papers reporting a link between use in food animals, emergence of antibiotics resistance in *Salmonella*, *Escherichia coli*, Enterococci and campylobacter in treated animals and transfer to humans via food chain [23, 24]. The antibiotics used in the sensitivity test were mostly third and fourth generation antibiotics with the exception of Gentamicin, which is a second generation antibiotics. The result revealed a very disturbing trend as there was high resistance levels to the antibiotics by the isolates. The highest resistance was to Cefuroxime where 44 of the 57 isolates were totally resistant. Ciprofloxacin was the antibiotic to which the least resistance (15%) was shown (Figure 5). This information is similar to the findings of Akinbowale *et al.* [25] in which two strains of *Salmonella* species exhibited a multi-drug resistance pattern. Resistance to antibacterial drugs may be as a result of indiscriminate use of these drugs in aquaculture at less than optimum dosage and such action leads to resistance in exposed pathogens. On most farms, to prevent disease outbreaks chemotherapeutants of all kinds are used. These substances are applied prophylactically under

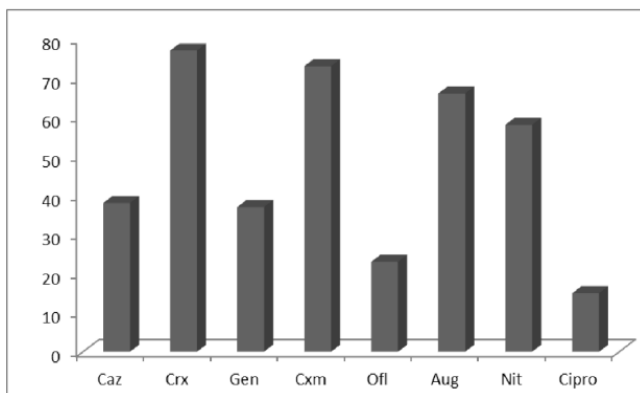


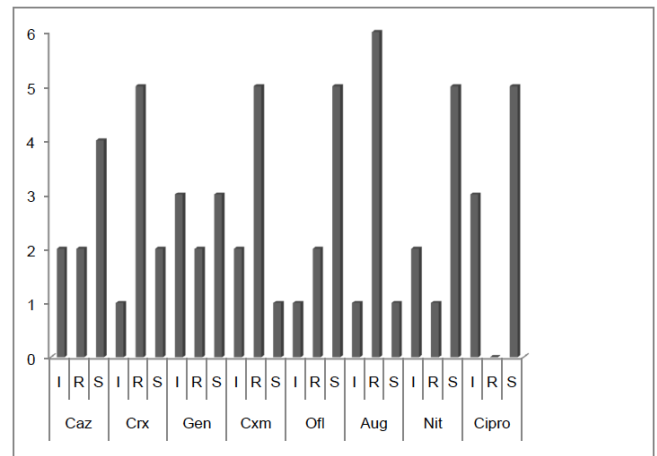
Figure 5: Resistance Pattern of Bacterial Isolates to Antibiotics (%).

Key

Antibiotics concentrations

Caz	Ceftazidime	30µg
Crx	Cefuroxime	30µg
Gen	Gentamicin	10µg
Cxm	Cefixime	5µg
Ofi	Ofloxacin	5µg
Aug	Augmentin	30µg
Nitr	Nitrofurantoin	300µg
Cipr	Ciprofloxacin	5µg

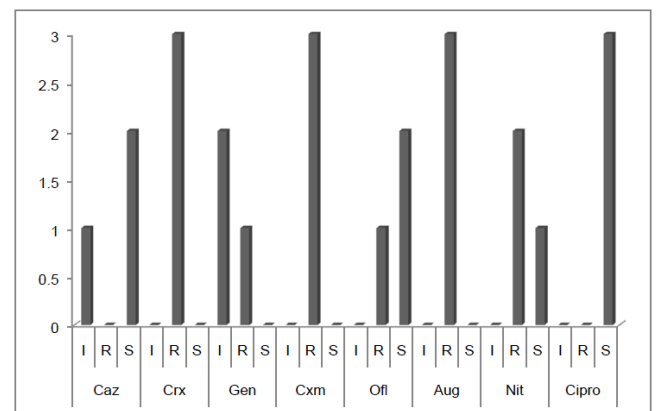
uncontrolled conditions [26]. One of the significance of this study is the establishment of the possibility of transfer of antibiotics resistant bacteria to human through fish and fish culture. In Farm I, the bacteria isolates were highly resistant to Augmentin, followed by Cefixime and Cefuroxime while they were highly susceptible to Ciprofloxacin, Nitrofurantoin, Ofloxacin, Ceftazidime and Gentamicin (Figure 6), which implies that there has been minimal abuse of those antibiotics on this farm. Farms II, III, A, B, C, D and E had higher levels of antibiotics resistance compared to Farm I (Figures 7, 8, 9, 10, 11, 12 and 13). In a previous study by Adewoye and Lateef [27], gram-negative bacteria



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

Resistant pattern: I-intermediate, R- resistance and S- sensitive.

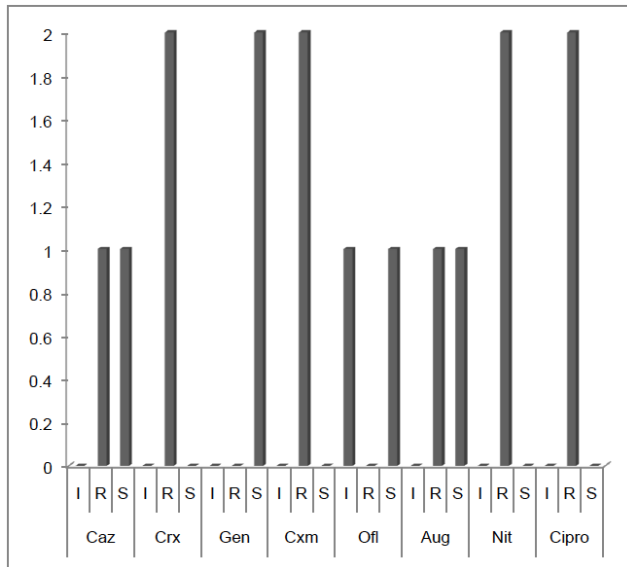
Figure 6: Antibiotics Susceptibility pattern of isolates from Farm I in Ibadan.



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

Resistant pattern: I-intermediate, R- resistance and S- sensitive.

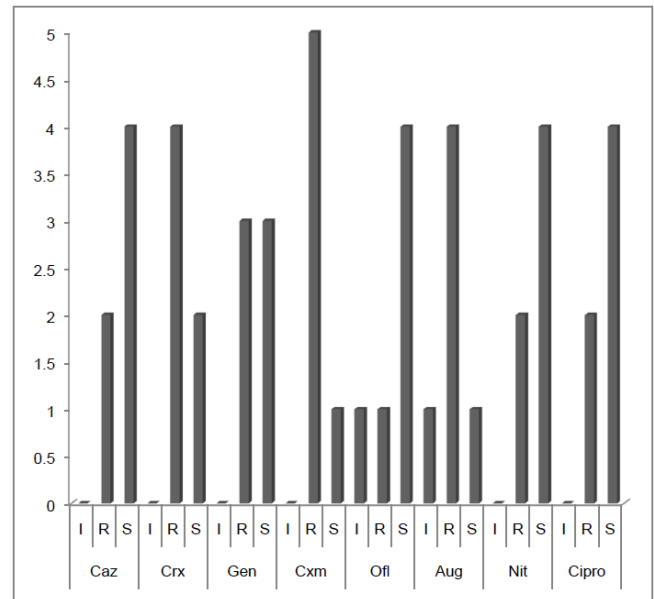
Figure 7: Antibiotics Susceptibility pattern of isolates from Farm II in Ibadan.



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

Resistant pattern: I-intermediate, R- resistance and S- sensitive.

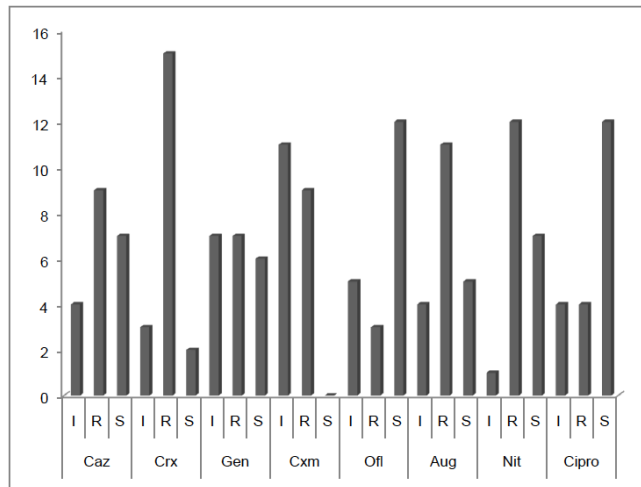
Figure 8: Antibiotics Susceptibility pattern of isolates from Farm III in Ibadan.



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

Resistant pattern: I-intermediate, R- resistance and S- sensitive.

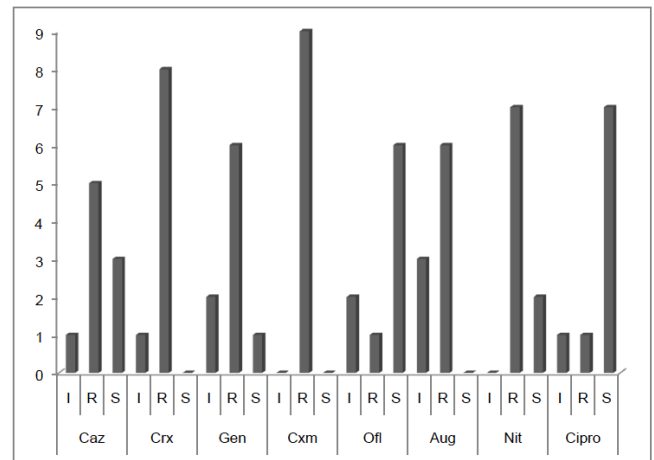
Figure 10: Antibiotics Susceptibility pattern of isolates from Farm B in Ikorodu.



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

Resistant pattern: I-intermediate, R- resistance and S- sensitive.

Figure 9: Antibiotics Susceptibility pattern of isolates from Farm A in Ikorodu.



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

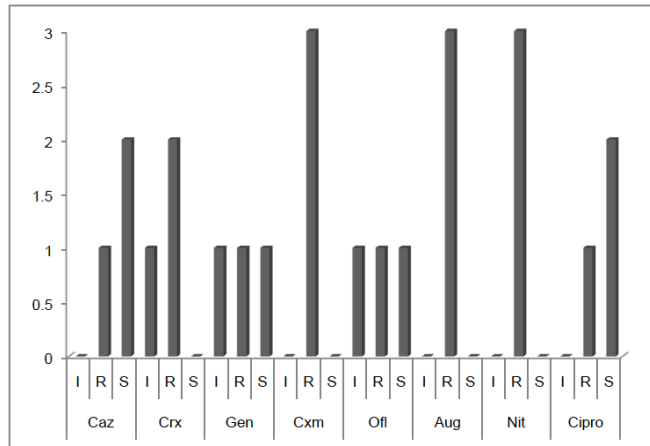
Resistant pattern: I-intermediate, R- resistance and S- sensitive.

Figure 11: Antibiotics Susceptibility pattern of isolates from Farm C in Ikorodu.

species isolated from the body surfaces of *C. gariepinus* in Nigeria were comparably identified as *Proteus*, *Pseudomonas*, *Serratia*, *Enterobacter* and *Escherichia*. The study also reported resistance of isolated bacteria to commonly used antibiotics in Nigeria as follows: 100% (augmentin, amoxicillin and cloxacillin); 85.71% (tetracycline), 80.95% (cotrimoxazole) and 71.43% (erythromycin).

Fujioka [28] reported that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced almost become indigenous to the environment. This is also possible in some other fish pathogens of food-borne importance. Therefore, there is the possibility that some bacterial species obtained from *Clarias gariepinus* can serve as potential

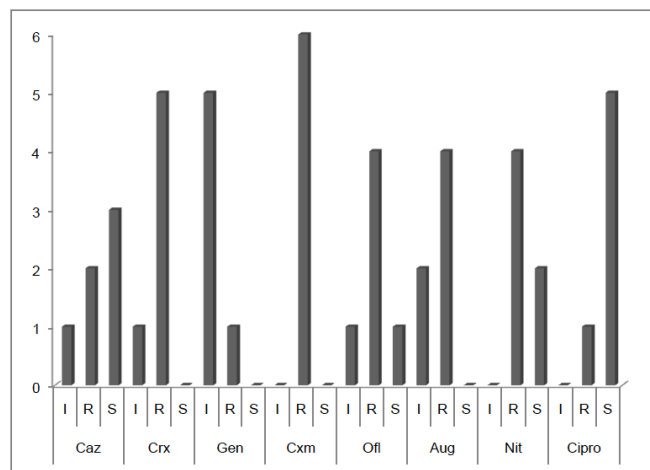
aetiological agents of infectious fish, environmental or human diseases of aquatic origin [29, 10].



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

Resistant pattern: I-intermediate, R- resistance and S- sensitive.

Figure 12: Antibiotic Susceptibility pattern of isolates from Farm D in Ikorodu.



Antibiotics: Caz-Ceftazidime, Crx- Cefuroxime, Gen- Gentamicin, Cxm-Cefixime, Ofi- Ofloxacin, Aug- Augmentin, Nit- Nitrofurantoin, Cipro- Ciprofloxacin.

Resistant pattern: I-intermediate, R- resistance and S- sensitive.

Figure 13: Antibiotics Susceptibility pattern of isolates from Farm E in Ikorodu.

CONCLUSION

Numerous diseases have emerged as serious economic or ecological problems in aquaculture species; meanwhile, very little work has been done on fish diseases in Nigerian aquaculture [26] which this work has addressed in this preliminary study. It is therefore necessary to build research and diagnostic capacities in the sub-Saharan African region to deal

with fish disease problems [30], since the rate and extent of emergence can be reduced by the application of biosecurity programmes designed to mitigate the risk factors for disease emergence [31, 32, 33].

The results obtained from this study showed that, for the control of fish bacterial diseases, the legislation of appropriate antimicrobial agent is very important to protect human health from potential hazards associated with antibiotic resistance from aquaculture. Disease prevention should be carried out by means of good culture and health management to ensure optimum yield and wholesome products.

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