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Research Article

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Isolation, Characterization and Antibiotic Resistance Profile of Bacteria from the Gut of African Catfish; *Clarias Gariepinus*

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Abstract *Clarias gariepinus*' gut is not a sterile environment as it contains hosts of microbial flora. Though beneficial, some of these microorganisms can be pathogenic and resistant to therapeutic agents which increase fish mortality with unpredicted long term effect on public health. This study was conducted to isolate, characterize and determine the antibiotic resistance pattern of bacterial species isolated from the gut of African Catfish, *Clarias gariepinus*. Five fish farms were selected for this study and a total of 22 samples (composite samples) were obtained; catfish guts were used as test samples while fishpond water served as control. Standard APHA methods were used to isolate and characterize the bacterial isolates present in the samples. Also, Kirby-Bauer disc diffusion method was used to determine their antibiotic resistance pattern. Results indicated that all the samples (test and control) contained bacterial species, though the test samples had less microflora than control. In all, 126 isolates were obtained and through series of biochemical characterization, 50 isolates belonging to six bacteria genera; Escherichia, Bacillus, Salmonella, Shigella, Staphylococcus and Pseudomonas were identified. Bacillus spp were the most occurring isolate (22% occurrence) while Shigella spp were the least with only 8% occurrence. The isolates showed varying degree of resistance to the test antibiotics. However, with the exception of Shigella spp, all the isolates were highly resistant to trimethoprim/sulfamthoxazole but highly susceptible to roceptrin, ciprofloxacin and pefloxacin. From this study, it can be concluded that *Clarias gariepinus*' gut harbours microorganisms some of which are antibiotic resistant and can pose serious problems in the management of fish diseases.

Keywords Antibiotic resistance, Bacterial isolates, Cat fish, *Clarias gariepinus*, Fish farm

1. Introduction

African catfish; or African sharp tooth cat fish is a specie of catfish found in the family Clariidae, normally colored black or dark gray on its back and fading to white on its belly [1]. Adult C. gariepinus can reach a maximum length of 1.7m and up to 60kg in weight (Figure 1). It is a nocturnal fish and feeds on living as well as dead organic matter [1]. The rearing of African catfish dates back to the 1970s in central and West Africa. Catfish rearing or farming is considered lucrative because of the numerous agricultural by-products and nutritional components such as vitamins, minerals, proteins, and saturated fat and very low carbohydrate accruing from such [2]. Studies have shown fish to possess bacterial populations on and in their skin, digestive tract, gills and in their internal organs (kidney, liver, and spleen) including Salmonella typhi, Serratia spp, Shigella spp, Streptococcus sp, Enterococcus spp, Staphylococcus spp, Escherichia coli, Pseudomonas spp, Klebsiella spp, Shigella spp, Proteus spp, Vibrio cholerae, Acinetobacter, Aeromonas, Enterobacteriaceae, Bacillus, Lactobacillus and Micrococcus [3,4]. The bacterial population of fish is not entirely useless as they

play important roles especially in metabolism [4]. Other roles include; production of friction preventing polymers essential for the fish to move through water column, degradation of complex molecules such as cellulose, chitin and collagen by intestinal bacteria amylase as well as the production of vitamins [4]. However, fish microflora such as Pseudomonas has been implicated to play significant role in fish spoilage through the production of histamines and other nitrogenous compounds during fish storage under oxic and refrigeration conditions. Antibiotic resistance in fish microflora can be attributed to the irrational use of antibiotics in fish farming such as in treatment of fish diseases. Antibiotic resistance in livestock and poultry farming has been attributed to irrational use of various classes of antibiotics including penicillins, tetracyclines and sulphonamides [5]. Bacterial mechanisms of antibiotic resistance to antibiotics include altered permeability to the antibiotic, inactivation of the antibiotic, and modification of the target site of the drug [6]. Antibiotic resistance in microorganisms have associated with it severe consequences including; prolonged illnesses due to reduced efficacy of antibacterial agents against resistant pathogens and increased risk of death [7]. Potential human pathogens such as Shigella, Staphylococcus aureus, Salmonella, Yersinia enterocolitica, Listeria monocytogenes and Vibrio cholerae have been isolated from the digestive tract of Clarias gariepinus. Pathogenic bacteria in fish spread to humans via numerous routes. For instance, pathogenic bacteria from fish can be acquired by fish farmers during fish harvesting and processing, or ingested directly by humans via consumption of improperly cooked fish or fish products. This study aimed to isolate and characterize bacteria from catfish gut as well as determine the antibiotic resistance profile of the isolates.

Figure 1: Clarias gariepinus

2. Materials and Methods

2.1. Description of Study Area

Nsukka is a local government area in Enugu State, Nigeria situated at latitude 6.86°N and longitude 7.39° E longitude and covers a total land mass of 1,810km^2 (Figure 2).

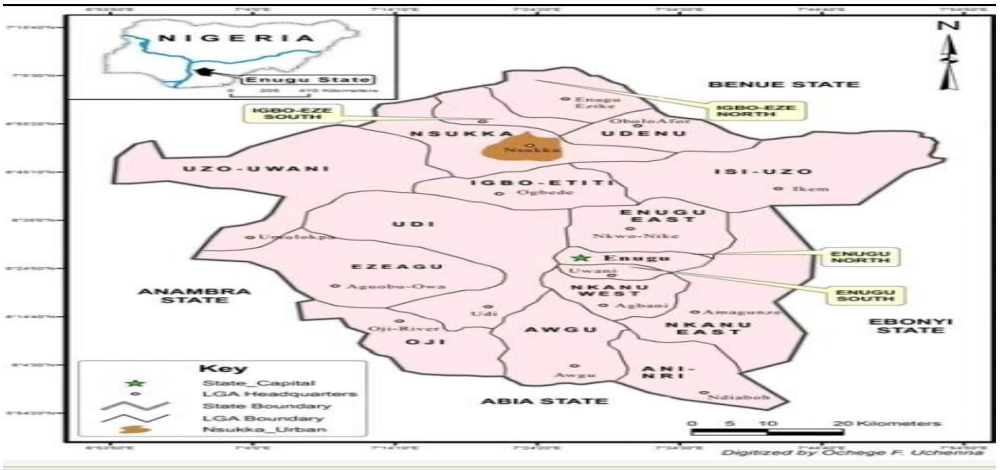

Figure 2: Map of Nsukka town (Adapted from Department of Geography Archives, UNN)

2.2. Sample collection

A total of 22 composite samples (10 fish pond water samples and 12 catfish samples) were obtained from five actively producing fish farms within University of Nigeria, Nsukka. Fish pond water samples were used as control. Catfish samples were obtained with the aid of cast nets and put into sterile polythene bags to avoid contamination. Also, the fishpond water samples (100ml) were collected in sterilized Durham bottles and immediately sent to the laboratory in ice packs for analyses.

2.3. Preparation of media

All media used were prepared following the manufacturer's instruction and sterilized for 15 at 121^{o}C in an autoclave.

2.4. Extraction of catfish gut

All samples were processed in accordance with the standard methods of the American Public Health Association [8]. The fish were sacrificed and cleaned with ethanol. By means of sterile scapel, the fish samples were aseptically dissected aseptically and approximately 2.5cm of the gut was excised using a sterile scalpel.

2.5. Sample Enrichment

Enrichment was carried out as described by Elhadi [9]. Using a sterile swab stick, the catfish gut samples were swabbed, rinsed into 10ml sterile tryptone soya broth contained in test tubes and incubated at 37^{o}C for 24 hours. Similarly, the fish pond water samples (1ml) were aseptically transferred into 10ml sterile tryptone soya broth contained in test tubes and incubated at 37^{o}C for 24 hours.

2.6. Isolation of bacteria from catfish gut and control

After enrichment, solidified nutrient agar and EMB contained in sterile petri dishes were aseptically inoculated with the enriched broth containing each sample by streaking and subsequently incubated for 24 hours at 37^{o}C. The swab collected on the sterile swab stick (which is the composite catfish gut sample) was aseptically introduced into 10ml sterile tryptone soya broth contained in test tubes and incubated for 24 hours for 37^{o}C [9].

2.6.1. Purification and stocking of isolates

After incubation, visible colonies on each plate were sub-cultured to obtain pure cultures, stocked in slant bottles and maintained at refrigeration temperature (4^{o}C).

2.7. Biochemical characterization of isolates

Biochemical tests such as catalase, coagulase, indole, lactose fermentation, citrate utilization and oxidase tests were employed to characterize the isolates according to Bergey's manual of systematic bacteriology [10].

2.7.1 Catalase test

This was done by placing 0.5ml of 3 % H_2O_2 on a 24hour old bacterial colony on glass slides after which the slides were observed for effervescence. Prompt effervescence indicates catalase production i.e. breakdown of H_2O_2 to release H_2O and O_2 by the organism.

2.7.2. Coagulase test

This was used to confirm the presence of Staphylococcus aureus species. Approximately 3 drops of sterile saline were placed on a clean glass slide. A loopful of 24hr old test colony was added to the slide followed by the addition of a drop of citrated rabbit plasma and checking for agglutination or clumping.

2.7.3. Indole test

This was used to test for organisms that possessed tryptophanase which enables them to split the aromatic amino acid tryptophan into indole, pyruvic acid andammonia. The isolates were first inoculated into peptone water broth and incubated at 37oC for 48 – 96 hours. Kovac's reagent (0.5ml) was added, gently shaken and observed for colour changes.

2.7.4. Citrate utilization test

An 18-24hr old colony was inoculated into sterile simmon's citrate agar medium in bijou bottles, incubated for 48-72 hours and observed for colour change.

2.7.5. Oxidase test

Sterile glass rod was used to place 3 to 4 drops of freshly prepared oxidase reagent on a filter paper. A sterile glass slide was used to pick a test colony and smear it over a small area of the impregnated filter paper and observed for color change.

2.7.6. Lactose fermentation test

Phenol red lactose broth in a test tube was inoculated aseptically with a pure culture of the test organism. The tube was incubated at 37^{o}C for 24 hours and observed for color change.

2.8. Antibiotic susceptibility testing

This was carried out using Kirby-Bauer agar disc diffusion method [11]. A 24hr old culture was standardized to a turbidity of 0.5 MacFarland. A disc of blotting paper impregnated with known volume and concentration of antibiotics was placed on a plate of Mueller Hinton Agar (MHA) uniformly inoculated with the test organism and incubated at 37^{o}C for 24 hours. After incubation, diameters of the zone of inhibition produced by each isolate was measured in mm and interpreted according to the Clinical Laboratory Standard [12].

3. Results and Discussion

3.1. Biochemical Characterization of isolates

Based on characteristics specified in Bergey's Manual of Systematic Bacteriology, 50 out of 126 bacteria isolates were confirmed to belong to six genera; Bacillus, Salmonella, Staphylococcus, Escherichia, Pseudomonas and Shigella were identified as shown in Table 1.

Table 1: Biochemical Characterization of Isolates

S/N	LFT	OxT	GS	CT	CoT	InT	CiT	Probable organism
1.	+	-	+	+		-	+	Bacillus spp
2.	-		-	+		-	-	Salmonella spp
5.	+	-	+	+	+	-	+	Staphylococcus spp
4.	+	-	-	-		+	-	Escherichia spp
5.	-	+	-	+		-	+	Pseudomonas spp
6.	-	-	-	+		-	-	Shigella spp

Legend;

LFT-Lactose Fermentation Test; OxT-Oxidase test; GS-Gram Staining; CT-Catalase test; CoT- Coagulase test; InT-Indole test; CiT-Citrate test; + =Positive; - =Negative

3.2. Distribution/ Frequency of isolation of organisms from the test and control samples

The result from the 22 composite samples collected from the five fish farms shown in Table 2 and Figure 3 indicates that Bacillus spp had the highest frequency of occurrence (22%) while Shigella spp were the least isolated (8%). Farm 5 had the highest number of isolates while the lowest number of isolates was obtained from Farm 1. More so, the control samples NCWS had higher bacterial load than the test samples NCCS.

Table 2: Distribution/ Frequency of isolation of organisms from the test and control samples

Farm	NCWS	NCCS	NI/NCWS	NI/NCCS
1	2	1	6	2
2	2	2	8	3
3	2	3	6	3
4	0	3	4	2
5	4	3	11	5
Total	10	12	35	15

Legend:

NCWS = Number of Composite Water Samples

NCCS= Number of Composite Catfish gut Samples

NI/NCWS= Number of Isolates obtained from composite water samples

NI/NCCS=Number of Isolates obtained from composite catfish gut samples

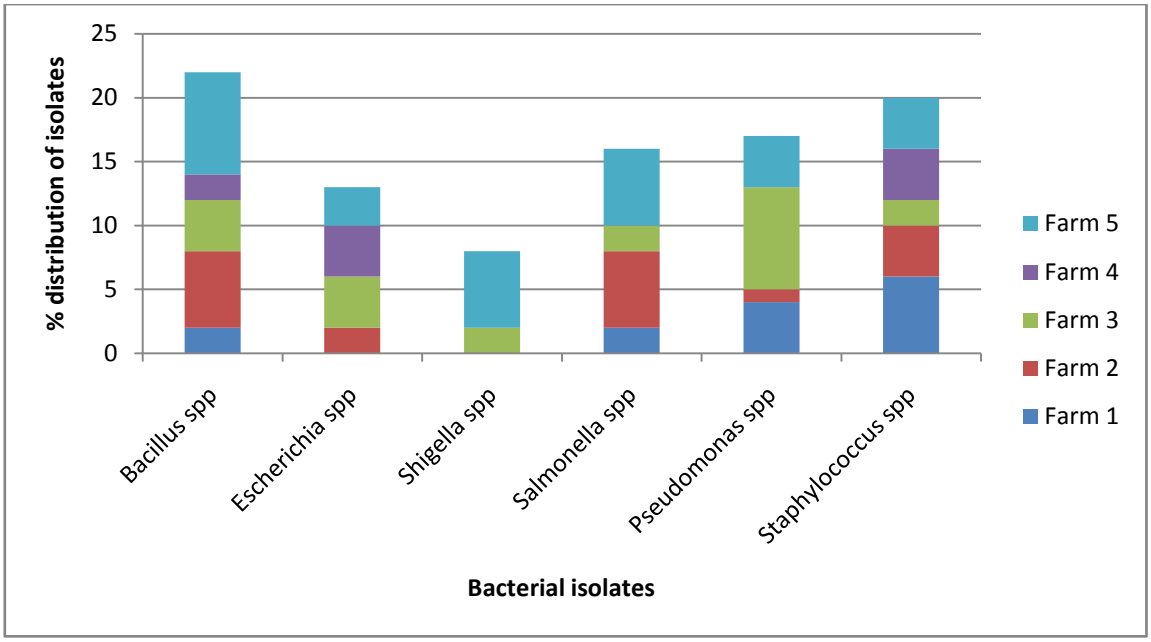

Figure 3: Percentage distribution of Isolates in the fish pond

3.3. Antibiotic susceptibility testing

The result of antibiotic sensitivity showed that all the isolates except Shigella spp were resistant to trimethoprim & sulfamthoxazole, the isolates were highly susceptible to roceptrin, ciprofloxacin and pefloxacin. While Pseudomonas spp had the highest multi- antibiotic resistance (MAR) index, Shigella spp had the least.

Table 3: Antibiotic Susceptibility Profile of Isolates

S/N	Organism	Resistant	Susceptible	MAR Index
1	Bacillus spp	SXT, AM	R, CPX, PEF	0.2
2	Salmonella spp	AU,CN, SXT	PEF, SP, CPX, AM	0.3
3	Staphylococcus spp	APX, AM, SXT	R, E, PEF, CPX, S, CN	0.3
4	Pseudomonas spp	AU, CN, OFX, S, SXT, CH	CPX	0.6
5	Shigella spp	-	S, SXT, CH, SP, CPX, PEF	0.0
6	Escherichia spp	AU, OFX, AM	PEF, CPX,	0.4

Legend:

CPX-Ciprofloxacin; PEF-Pefloxacin; E-Erythromycin; SP-Sparfloxacin; R-Roceptrin; APX-Ampiclox; OFX-Tarivid; S-Streptomycin; CH-Chloramphenicol; SXT-Trimethoprim & Sulfamthoxazole; AM-Amoxacillin; AU-Augmentine; CN-Gentamycin.

3.4. Distribution of Resistance According to Sample Source

Figure 4 indicates that bacterial isolates obtained from Farm 5 were the most resistant to the antibiotics used, this was followed closely by isolates obtained from Farm 3. As indicated, isolates from Farm 4 were the least resistant.

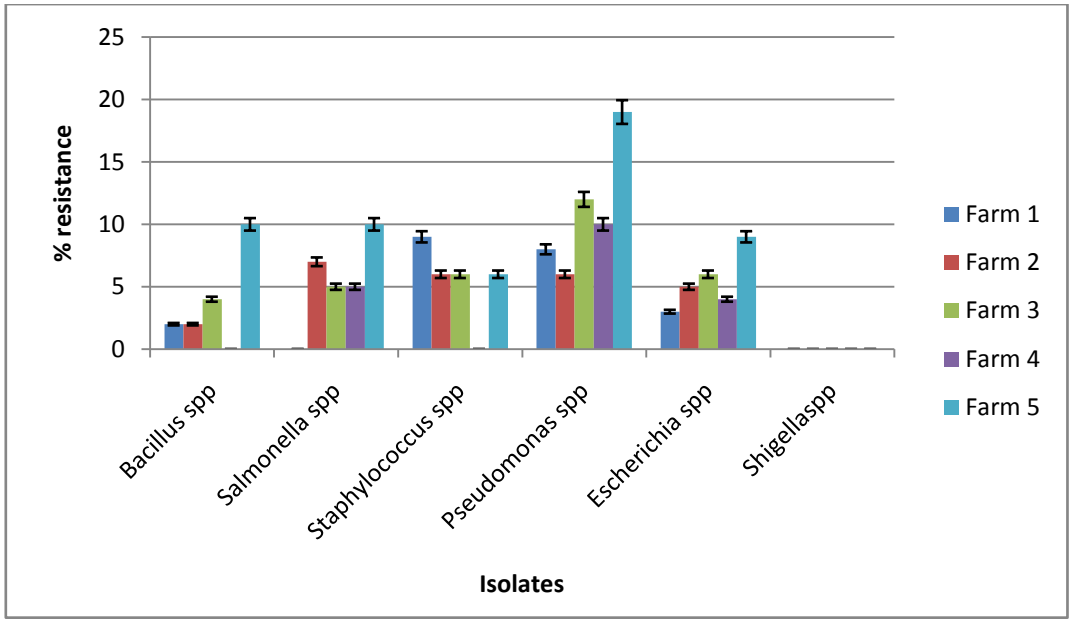

Figure 4: Distribution of Resistance According to Sample Source

Isolation of bacterial species from catfish is not novel as it has previously by reported by some researchers [1, 13]. In this study, 126 bacterial isolates were obtained but only 50 of the isolates belonging to the genera; Escherichia, Salmonella, Shigella, Pseudomonas, Bacillus and Staphylococcus were identified via series of biochemical tests. Due to the similarity in the taxa of isolates got from both Clarias gariepinus' gut (test) and fishpond water (control), it can be inferred that Clarias gariepinus' gut microflora depends on the microbial composition of the aquatic habitat where it is found. Some of the isolates obtained were identified to belong to specific groups of bacteria known as enteric bacteria, these include; Salmonella spp, Shigella spp and Eschericia spp. Enteric bacteria have been implicated as causative agents of various enteric diseases generally referred to as gastroenteritis [14, 15]. Fertilizing fish ponds using organic manure from commercial farms, especially poultry is a common practice among fish farmers in the study area, this is the most accountable source of enteric bacteria isolated from the study area. In a similar study conducted in Egypt, frequency of isolation of enteric pathogens; Salmonella spp and E. coli from water and fish raised in ponds receiving unfermented chicken manure significantly exceeded those that were unfertilized [16]. In addition, enteric bacteria in both water and Clarias gariepinus is as a result of contamination with faecal matter as these pathogens don't form part of fish normal flora but homoitherms. The use of organic materials as fertilizers in fish farming may not only harbour enteric pathogens but also confers resistance to antibiotics by transferring antibiotic residuals or resistant bacteria to fish especially if such manure was obtained from commercial farms that also use antibiotics [16]. Isolates obtained in this study with the exception of *Shigella* spp were absolutely (100%) resistant to trimethoprim & sulfamthoxazole. However, they were highly susceptible to roceptrin, ciprofloxacin and pefloxacin. In all, bacterial isolates from the farms where no antibiotic was used (Farms 1 and 4) demonstrated lower resistance to antibiotics compared to isolates from the farm where antibiotics were used to treat the fish (Farms 2, 3 and 5). Aside the use of antibiotics to treat fish diseases, antibiotic resistance can also be attributed to sources of water supply for the ponds (borehole, dug wells and taps) especially as those from polluted environments are also sources of bacterial flora in fish gut [17]. Though there was no history of antibiotics use in Farm 1 and Farm 4, isolated bacteria species still demonstrated resistance to antibiotics. This corroborates a study in Tanzania and Pakistan where, isolates from pond water sediments failed to show susceptibility to chloramphenicol, tetracycline, sulphamethoxazole/trimethoprim and amoxicillin despite no record of antibiotics use, they postulated that resistance genes in aquaculture might have arisen from integrated fish farming practices like the use of domestic farm wastes [18].

Furthermore, multi antibiotic resistant isolates may develop due to selective pressure resulting from the use multiple classes of antibiotics in fish farming. MAR was observed to be highest in Pseudomonas spp. Pseudomonas spp isolated in this study is a known fish pathogen which remains in fish even after processing. This poses a serious threat to public health as humans acquire such resistant bacterial pathogen via fish consumption which can result in high rates of morbidity and mortality since these organisms are capable of inactivating antibiotics use in therapeutics [19]. Hence, the observed trend of multi antibiotic resistant strains poses a major public health threat and sincere efforts should be made to overcome this challenge.

4. Conclusion

This research has shown that bacterial species including enteric pathogens can be isolated from catfish gut. As a result, people who eat improperly cooked catfish are at risk of contracting gastrointestinal diseases like typhoid, cholera and dysentery. More so, to prevent eminent outbreak of diseases by antibiotic resistant strains, farmers should be discouraged from irrational use of antibiotics in fish farming.

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