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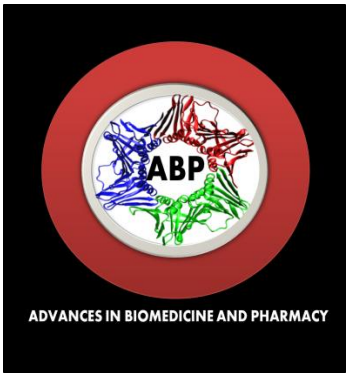

Original Article

Bacterial Flora of Wild and Cultured *Clariasgariepinus* (African Catfish) and their Public Health Implications.

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Abstract

The microbial quality of food fish is of public health importance. The microbiological safety of both wild and cultured *Clariasgariepinus* (African Catfish) randomly caught from different aquatic environment locations in Ibadan city was evaluated in the present study. The bacterial load of ninety-six (96) skin and stomach samples randomly harvested from fish specimen were examined and compared. The resistance of bacterial isolates towards commonly used antibiotics was also studied. All the samples were contaminated by bacteria ranging from $Log_{10}CFU/cm^2$ 13.07 to $Log_{10}CFU/g$ 13.16 which is largely above recommended level of $Log_{10}CFU/cm^2$2.0-7.0 (skin) and$Log_{10}CFU/g$ 3.0-9.0 (stomach) of fish. The bacterial species isolated from skin and stomach were: *Bacillus, Proteus, Pseudomonas, Klebsiella, Streptococcus, Staphyloccus, Micrococcus, Serratia* and *Escherichia*. The degree of resistance shown by the isolates to the antibiotics ranged from 50-87.5%, with multiple drugs resistance to 4-8 antibiotics. The Isolate (Gram-ve) showed 100% resistant to Nalidixic acid and Cotrimozazole. The public health implications of the findings arising from this study include emergence of drug resistant strains and risk of untreatable food poisoning conditions caused by these drug resistant food borne pathogens.

Keywords: Antibiotic sensitivity, Bacterial flora, Catfish, Public health.

Introduction

In the past decade, there has been rapid expansion in aquaculture and artisanal fish production. In the fisheries sub-sector, as in animal production, farming is replacing hunting as the primary food production strategy. In future, farmed fish will be ever important sources of protein foods, than they are today and the safety for human consumption of products from aquaculture is of public health significance.

Food and Agricultural Organization (FAO) [1] asserted that fish contributes about 60% of the world supply of protein and that 60% of the developing world derives more that 30% of their animal protein from fish. Fish allows for protein improved nutrition in that it has a high biological value and in terms of high protein retention in the body, low cholesterol level and presence of essential amino acids [2, 3]. Despite of the fact that fish are generally regarded as safe, nutritious and beneficial, aquaculture products have been associated sometimes with certain food safety issues [4]. Public health issues can be considered as those of direct importance to both producers and consumers of fish and include broader issues of food production, processing and delivery systems. Bacterial agents are among the highly encountered causes of diseases in warm water aquaculture, where stressful conditions play important roles in establishment and aggravation of the bacterial diseases in fish farms [5, 6]. Linkages have been made between fish food and human health [7]. In different studies on Lagos Lagoon, Otamiri river, Ele river, different

bacterial species with potential for causing high proportion of death and ill-health among the populations that are dependent on the water bodies for water related resources had been identified [8-10].

This study investigated the bacterial flora from the skin and stomach of *Clariasgariepinus* captured from natural water bodies and rearing ponds in Ibadan metropolis Southwest, Nigeria. The bacterial isolates were evaluated to determine their resistance to the commonly recommended antibiotics in Nigeria.

Materials and Methods

A total of ninety six (96) tissues samples (skin and stomach) harvested from twenty four (24) *Clariasgariepinus* (*African catfish*) consisting of twelve (12) wild fish and twelve (12) reared fish were randomly sampled from different aquatic environments in Ibadan metropolis during a period of four (4) months. Fish were caught by a local fishing gear and by cast net. Sampling was done between 8.00am and 10.00am in each occasion at periodic interval of seven (7) days. The specimen was brought to the laboratory in ice-box at temperature below 4^{o}C within two (2) hours of sampling.

Isolation

Nutrient Agar (NA) and MacConkey Agar (MCA) (Micro Master Thane, India) were prepared according to manufacturer's instruction. Ten milliliters of each media sterilized at 121^{o}C for 15 minutes in autoclave (Fisher's Scientific, U.S.A) was poured into sterile disposable petri dishes. Bacterial isolates from each specimen were obtained aseptically from the skin (1cm squared area) and stomach (1g portion). Each sample was homogenized in 10ml distilled water, one milliliter of stock solution was serially diluted and then 0.1ml of each of the homogenized samples was placed on appropriate media and was incubated at 37^{o}C for 18-24 hrs. The resulting isolates were purified and single colonies of bacteria were identified based on morphological and biochemical characteristics as described by [11].

Antibiotic Sensitivity Test

The antibiotic sensitivity of the isolates was determined using gram positive disc (Abtek Biological Ltd) containing the following; Cotrimoxazole (Cot), 25µg; Gentamycin (Gen) 10µg; Nalidixic acid (Nal) 30µg; Ofloxacin (Ofl) 30 µg; Chloramphenicol (Chl), 10 µg; Augmentin (Aug), 30 µg; Amoxycillin (Amx) 25 µg; and tetracycline (tet) , 10 µg; and poly disc Gram negative, Mutti susceptibility disc (Poly-tesMed Laboratoratories) containing the following; Nitrofuratin (Nit),100 µg; Ciprofloxacin (Cip),5µg; Tetracycline (Tet) 50µg; Norfloxacin (Nfl), 10 µg; Amoxicillin (Amx),30µg; Ofloxacin (Ofl), 5µg; chloroamphenol (chl),10µg; Cefuroxime(Cfx), 30µg; Ampicillin (Amp), 10 µg; and Gentamycin (Gen), 10µg.

The commercial antibiotics were placed on nutrient agar plates previously seeded with 18-24 hr culture of the test organism using sterile glass spreader. The plates were incubated at 37^{o}C for 48hr, after which zones of inhibition were examined and interpreted accordingly [12]. The resistance pattern of each isolate was constructed from the antibiogram. Earlier, the potencies of all the antibiotics used in the study were confirmed using susceptible *Escherichia coli* strains.

Statistical Analysis

All the readings were taken in duplicates and the P value was determined using t-test. P value of <0.05 was considered as significant.

Results and discussion

The mean total bacteria load of skin and stomach of the fish samples is presented in Table1. All the fish samples were found to be contaminated with the microbial load (log_{10}Cfu/cm^{2}or g) in the range of 13.07-13.16. The density of total aerobic bacteria found in the skin and stomach of African catfish sampled from natural body water environment (wild) and rearing pond (cultured) were not different, however the total bacteria count measured were higher in the stomach of both types of fish studied compared to the load obtained from the skin. The result of enterobacteriaceae count on the skin and stomach of the fish samples are presented in Table 2. The (log_{10}Cfu / cm^{2}or g) varied from 12.18-13.04. The highest count was also obtained from the stomach of both wild and cultured fish samples studied. A significant higher count was obtained in the stomach of wild catfish compared to the skin ($p<0.001$). This could be due to mass pollution of the environment where the fish were caught. Generally lower count of bacteria observed on the skin of Africa catfish compared from stomach could be due to the lack of scales on the skin of Africa catfish that can harbor feed matter suitable for the survival of the bacteria and /or skin of Africa catfish with thick slime that contain several antibacterial components or probably the voracious eating habit of the Africa catfish which make it to consume much from polluted environment. Generally, the bacteria load of all the skin and stomach of the fish samples from different aquatic environment were generally high with log_{10}CFU/g from 12-13. The permissible count of heterotrophic bacteria in the 1cm^2 of the skin, ranged from log_{10}CFU/cm^{2}2-7[13] and log_{10}CFU/g3-9 of digestive tract

[13, 14] or bacteria of $\log_{10}$Cfu/cm^2≤ 5.70 according to International Commission on the Microbiological Specification of Foods [15]. Therefore, the bacterial load found in this study ranging from($\log_{10}$CFU/g 13.07-13.16 was beyond the standard value which indicated public health risk to the handlers and consumers of fish caught from these environments. High microbial load found on the skin and in the stomach of fish samples studied could be due to a variety of factor having influence on the qualitative and quantitative composition of the bacteria including environmental (contamination, temperature, oxygen saturation, pH) diet type, development stage of fish, species and fish condition[16]. Predominant organisms identified on the fish samples studied include species of bacteria of the following genera: *Bacillus, Proteus, Pseudomonas, Klebsiella, Streptococcus, Staphylococcus, Micrococcus, Serratia* and *Escherichia*. Many bacteria species encountered are no doubt potentially pathogenic in different species under certain condition as reported for *Pseudomonasanguilliseptica* [17]. *Streptococcus*sp [18], *Pseudomonas*sp, and *Staphylococcus*sp [19]. *Bacillus* sp, *E. coli*, *Salmonella* sp, *Streptococcus* sp and *S. aureus* were also implicated in fish borne [20] and shrimp-borne [21] diseases of humans.

The public flora of Nigeria fish species have not been adequately defined, mainly due to the mode of preparation which involved cooking for considerable length of time. The heat would have eliminated most, if not all the bacterial flora [22]. However the presence of potential human pathogens suggest that fish improperly handled, undercooked or consumed raw, may cause various disease to susceptible individuals [23].

The results of the antibiotic sensitivity tests were interpreted and presented as the resistance of the bacteria isolates to the antibiotics as shown in Table 3 for Gram negative isolates and Table 4 for Gram positive isolates. High levels of resistance were obtained among the bacterial isolates to the antibiotics (50-87.5%). None of the antibiotics tested had 100% activity against all the bacterial isolates; however Gentamycin showed 100% activity against all the Gram negative isolates. Ciprofloxacin and tetracycline were not active against all Gram negative isolates; however tetracycline showed 25% activity against all Gram positive isolates. Nalidixic acid and Cotrimoxazole were not active against any Gram positive isolates obtained from the fish samples. The total resistance of the bacteria strains to the antibiotics ranged from 50% for Ampicillin, Amoxycillin, chloramphenicol and Nitrofurantoin to 100% for Ciprofloxacin and tetracycline. The total resistance of the bacteria ranged from 50% for Gentamycin and Ofloxacin to 100% Cotrimoxazole and Nalidixic acid. The cumulative effectiveness of the antibiotics as obtained in the study is Gentamycin > Ampicillin = Amoxycillin = Chloramphenicol=Nitrofuratoin>Norfloxacin>Ofloxacin> Cefuroxime >Ciprofloxacin =Tetracycline as shown in Table 3 and Gentamycin =Ofloxacin>Nitrofuratoin = Tetracycline >Amoxycillin = Augmentin >Cotrimoxazaole = Nalidixic acid as shown in Table 4. The relative high level of resistance to antimicrobial agent is of public health importance as the emergence of drug resistance strains of organisms from products can infect human consumers leading to untreatable infections [24]. Environmental factors like previous exposure, the type of antibiotic used in the respective locality and incidence of plasmids in the isolates may have resulted to the antibiotics resistance [25]. Such plasmid could be pheromones – responsible or broad host-range [26]. The resistance to antibiotics can also be as a result of the selective antibiotics pressure [27-29] or integrons and other insertion elements [30].

The misuse of antibiotics in the environment is also contributory factor towards antibiotic resistance [31-35]. Antibiotic prescriptions in hospitals are given without clear evidence of infection or adequate medical indication. Broad spectrum antibiotics are sometimes given in place of narrow spectrum drugs as a substitute for culture and sensitivity testing, with the consequent risk of dangerous side effects, super infections, and the selection of drug resistant mutants [36]. The long standing practice of using low doses of antibiotics for a long period of time for growth promotion and arbitrary use of antibiotics in animal husbandry is the strong contributors to the development of the antibiotics resistant bacteria in the environment. Five patterns of multiple drug resistance with the number of antibiotics ranging from 4 to 8 among the bacteria isolates obtained (Table 5) and fall within the range obtained by earlier workers [37-39]. Multiple drug resistance is an extremely serious public health concern [36] and it has been found to be associated with the outbreak of major epidemics throughout the world [40, 41]. Thus, the multiple drug resistance shown by these bacteria is annoying because of the public health implications. Handling and consumption of fish materials of this kind can expose the handler and consumer to certain health risks.

Table 1: Total Bacteria Load of skin and stomach of *Clariasgariepinus*

	Body parts		t- value
Sources	Skin	Stomach	
Wild	121.08± 8.84 (13.08)*	137.33 ±17.22 (13.14)**	-1.078
Cultured	117.33 ±11.55 (13.07)*	144.50 ±22.39 (13.16)**	-0.840

*Log_{10}CFU/cm^2skin; ** Log_{10}CFU/g stomach.

*each value is a mean of duplicate reading of 12 fish samples.

Table 2: Enterobacterial count of skin and stomach of *Clariasgariepinus*

	Body parts		t-value
Sources	Skin	Stomach	
Wild	66.75+ ±7.11 (12.81)*	110.42±11.47 (13.04)**	3.235***
Cultured	66.08±5.51 (12.82)*	75.33±10.64 (12.86)**	-0.77

*Log_{10}CFU/cm^2skin;** Log_{10}CFU/g stomach; ***significant difference at ($p<0.001$);*each value is a mean of duplicate reading of 12 fish samples.

Table 3: The antibiogram of some strains of *Salmonella* sp and *E. coli* obtained from the fish samples.

Isolates	Nit	Gen	Amp	Cfx	Chl	Ofl	Amx	Nfx	Tet	Cip	****%
***Ec**	-	+	-	-	-	+	-	+	-	-	**70**
***Ec**	+	+	-	-	-	-	-	+	-	-	**70**
****Ec**	+	+	-	-	-	-	-	+	-	-	**70**
****Ec**	+	+	-	-	-	-	-	-	-	-	**80**
***S_A**	+	+	+	-	+	-	+	-	-	-	**50**
***S_A**	-	+	+	+	+	-	+	-	-	-	**50**
****S_A**	-	+	+	-	+	-	+	-	-	-	**60**
****S_A**	-	+	+	-	+	+	+	-	-	-	**50**
%*	**50**	**0**	**50**	**87.5**	**50**	**75**	**50**	**62.5**	**100**	**100**	

Antibiotics abbreviations as defined under materials and methods; Ec, *E. coli*; SA, *Salmonella* sp; +, susceptible; -, resistance; *, wild catfish; ** cultured catfish; ***,% resistance of the isolates to each antibiotics; ****, cumulative % resistance of each bacterium to all the antibiotics.

Table 4: The antibiogram of some strains of *Staphylococcusaureus* and *Streptococcus*sp obtained from the fish samples.

Isolates	Nal	Gen	Ofl	Tet	Aug	Nit	Cot	Amx	****%
***St**	-	+	-	-	-	-	-	-	**87.5**
***St**	-	+	-	+	+	-	-	-	**75.0**
****St**	-	+	+	-	-	-	-	-	**62.5**
****St**	-	+	+	+	-	-	-	+	**50.0**
***Sp**	-	-	-	-	-	+	-	-	**87.0**
***Sp**	-	-	+	-	-	-	-	-	**87.0**
****Sp**	-	-	-	-	-	+	-	-	**87.0**
****Sp**	-	-	+	-	-	-	-	-	**87.0**
%*									

antibiotics abbreviations as defined under materials and methods; Sp, *Streptococcus* sp; St, *Staphylococcus aureus*; +, susceptible; -, resistance; *, wild catfish; ** cultured catfish; ***,% resistance of the isolates to each antibiotics; ****, cumulative % resistance of each bacterium to all the antibiotics.

Table 5: The resistant pattern of some of the bacterial isolates.

No of antibiotics	Resistance pattern	Bacterial Isolates.
4	Aug,CotNal Nit	**St
5	Cip, Cfx, Nfx, Ofl, Tet Amx, cot, Nal, Nit, Tet, Cip, Nfx, Nit, Ofl, Tet Cip, Cfx, NfX, Nit, Tet	*S_A **St *S_A **S_A
6	Amx, Aug, Cot, Nat, Nit, Ofl Cfx, Cip, Ofl, Nfx, Nit, Tet	*S_C **S_A
7	Amx, Aug, Cot Nal, Nit,Ofl, Tet Amp, Aug, Cot, Gen, Nal, Ofl, Tet Amx, Aug, Cot, Gen, Nal, Nit, Tet Amp, Amx, Cfx, Chl, Cip, Nit, Tet Amp, Amx, Cfx, Chl, Cip, Ofl, Tet	*St *Sp,**Sp *Sp **Sp *Ec *Ec, *Ec
8	Amp, Amx, Cfx, Chl, Cip, Nfx, Ofl, Tet	**Ec

Ec, *E. coli*; St, *S. aureus*;Sp, *Streptococcus*; $S_{A,,}$*Salmonella;*. *,isolate from wild catfish; **, isolate from cultured catfish.

Conclusion

Keeping in view the arbitrary use of antibiotics and sub-therapeutic doses for growth promotion in fishery should be totally discouraged and sanitary conditions under which fish are reared or cultured in ponds should be improved by following standard or good aquaculture practice such as use of good quality water, use of feed with high microbial quality, regular draining of pond water after specific period of time, closure of ponds to the public, 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.

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Conflict of interest

Authors declare that there is no conflict of interest to reveal.

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