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Original Article

Prevalence of antibiotic-resistant bacteria isolated from cultured and wild *Clarias gariepinus* in Awka, Nigeria

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A B S T R A C T

The presence of antimicrobial resistance in fish poses a serious risk to human health. The study evaluated the prevalence of antibiotic-resistant bacteria in cultured and wild Clarias gariepinus in Awka. A total number of forty-two live adult C. gariepinus were obtained from three aquaculture farms and three sampling points in Ebenebe river. Bacterial isolates were obtained from the muscles of the fish samples and identified through morphological and biochemical tests. The isolates were then tested for antibiotic susceptibility to Tetracycline, Ciprofloxacin, Amoxicillin and Azithromycin using the disc diffusion method. The results revealed that a total of 38 bacterial isolates belonging to five different bacterial species were recovered from the fish muscles. The most prevalent bacteria isolated in cultured and wild C. gariepinus were Staphylococcus aureus, (26.31%), followed by Echerichia coli (21.05%), Aeromonas hydrophila and Pseudomonas aeruginosa (18.42%) while Salmonella enterica was the least prevalent bacteria (15.79%). The antibiotic susceptibility test of the fish samples in this study revealed that bacteria isolated from cultured and wild C. gariepinus showed various levels of resistance to all the tested antibiotics. Bacterial isolates from the study showed high resistance to Tetracycline (60.53%), moderate resistance to Ciprofloxacin (23.68%) and Amoxicillin (13.16%) and low resistance to Azithromycin (7.89%). However, bacteria isolated from cultured C. gariepinus exhibited higher levels of resistance to antibiotics compared to those isolated from wild C. gariepinus. It is therefore recommended that antibiotic resistance patterns in fish should be regularly monitored to help protect the health of fish and human.

K E Y W O R D S : *Antibiotics, Antimicrobial resistance, Aquaculture, Clarias gariepinus, Wild fish*

INTRODUCTION

Fisheries and aquaculture sector has been experiencing a surging increase in fish production since 2020 which led to a 4.4 percent increase in 2022 (FAO, 2024). While capture fisheries production remained stable since the late 1980s, aquaculture has surpassed capture fisheries as the main producer of aquatic animals. Despite the growth in aquaculture, capture fisheries remain an essential source of aquatic animal production (FAO, 2024). The need to meet the growing animal-source protein demand by the teeming global population has culminated in global aquaculture expansion (Okon *et al.*, 2022). The rapid growth in aquaculture production in recent decades has been facilitated by a transition from extensive to intensive

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farming (Schar *et al.*, 2020). With the development of intensive farming patterns followed by the emergence of disease outbreak, large quantities of antibiotics are currently being used in aquaculture (Gao *et al*., 2023).

Antibiotics are naturally occurring, semi-synthetic, and synthetic compounds with antimicrobial activity that can be administered to treat and prevent disease. (Menkem *et al*., 2019; Okeke *et al.*, 2022). The most commonly used antibiotics in aquaculture worldwide are Tetracycline, Fluoroquinolones, Sarafloxacin, Enrofloxacin, Amoxicillin, Erythromycin, Sulfadimethoxine, Ormetoprim and Florfenicol (Lulijwa *et al*., 2020; Sun *et al*., 2020). The consequences of the use of antibiotics in aquaculture is the presence of residues of the drug in the edible tissues of the treated fish, as well as the emergence of antibiotic-resistant bacteria (Monteiro *et al.*, 2018). Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic rendering the drugs ineffective in treating infections. This phenomenon is as a result of the improper usage of antibiotics in aquaculture. Du *et al*. (2022) reported that the misuse of antibiotics has led to the rapid development of bacterial resistance, bacterial infections and increased healthcare costs in human.

Recently, quite a number of studies have confirmed the contributions of antibiotic usage to antimicrobial resistance in fish (Bondad-Reantaso *et al.*, 2023). Ayukekbong *et al.* (2017) noted that antibiotic consumption profiles in developing countries are greatly influenced by the gross misuse of antibiotics due to their availability over the counter and through unregulated supply chains. Disease treatment, enhanced growth and productivity are factors increasing the excessive use of antimicrobials in fish production (Menkem *et al*., 2019; Okon *et al*., 2022). Studies have confirmed that antibiotics are not effectively metabolized by fish (Sun *et al*., 2020). Pathmalal (2018) reported that antimicrobials that are not efficiently metabolized by fish are eliminated through urine and feces into the pond water. Many of these antibiotics remained biochemically active after been excreted from fish which can create antibiotic-resistant bacteria. Okon *et al*. (2022) reported that this leaching of antimicrobial compounds may enhance the spread of antibiotic-resistant pathogens during fish production into nearby aquatic environments. Due to the indiscriminate use of antibiotics in fish farming, many disease-causing microbes in fish have acquired resistance to commonly used antibiotics (Okeke *et al*., 2022). Common bacterial diseases occurring in aquaculture, such as furunculosis (*Aeromonas salmonicida*) and edwardsiellosis (*Edwardsiella tarda*), are becoming harder to treat due to an increase in antimicrobial resistance (AMR) (Bondad-Reantaso *et al.*, 2023).

Currently, some antimicrobial agents commonly used in aquaculture are only partially effective against select fish pathogens due to the emergence of resistant bacteria (Miller and Harbottle, 2018). Emerging antibiotic-resistant bacteria (ARB) in aquaculture have been reported such as antibiotic-resistant *Escherichia coli*, *Acinetobacter* spp., *Aeromonas* spp., *Salmonella* spp., *Edwardsiella* spp, and *Streptococcus* spp. (Lulijwa *et al*., 2020). Antibiotics are always active against these microbes, but when the microorganisms display resistance or low sensitivity, it necessitates an antibiotic concentration that is higher above the normal to have effects on them (Zaman *et al*., 2017). Antibiotic resistance is of great public health concern because the antibiotic-resistant bacteria associated with the fish may be pathogenic to humans, easily transmitted to humans through food chains, and widely disseminated in the environment through fish wastes (Manyi-Loh *et al.,* 2023). Rising incidence of antimicrobial resistant pathogens of fish production increases treatment failure rates, undermining sustainable fish production (Schar *et al*., 2020). Gao *et al*. (2023) reported that the ingestion of drug resistance genes carried by fish can lead to an imbalance of the normal flora in the human body, increase the resistance of pathogenic bacteria and pose a serious threat to human health. Thus, this study was aimed to evaluate the prevalence of antibiotic-resistant bacteria in *Clarias gariepinus* obtained from from the wild and aquaculture facilities in Awka, Anambra State.

MATERIALS AND METHOD

Sample Collection and Preparation

A total number of forty-two live adult *Clarias gariepinus* of mean weight of 959.23±0.18 g and mean length of 38.54±1.03 cm was purchased randomly from three fish farms in Awka South Local Government Area and three sampling points (upstream, midstream and downstream) in Ebenebe River located in Awka North Local Government Area both in Anambra State, with seven fish collected from each location. Upon collection, the fish samples were transported live in clean plastic bags containing water to the laboratory. In the laboratory, the fish were humanely killed by a blow to the head, and 10 grams of fish muscles were collected for bacteriological examinations.

Culture, Isolation and Identification of Bacteria in Fish Muscles

Bacterial culture of the fish muscles was performed following the methods of Saengsitthisak *et al.* (2020). The fish muscles were aseptically cut into 10 g portions and spread over the surface of blood agar and MacConkey agar (HiMedia). The blood agar was used as a general-purpose medium that supports the growth of wide range of bacteria while the MacConkey agar was used as a selective medium for gram-negative bacteria. These agar plates were then incubated at 37°C for 24 h to allow for bacterial growth. The bacterial isolates were identified based on their morphological characteristics and subjected to biochemical tests following the methods described by Wamala *et al.* (2018) and Afolabi *et al.* (2020). The colony morphology of the isolates, including shape, color, pigmentation, and size, was carefully examined, and the isolates were grouped accordingly. Gram staining, motility testing, and various biochemical tests such as oxidase, catalase, indole, urea slants, citrate slants, methyl red, and glucose fermentation tests were performed to further characterize the isolates. Bacteria prevalence was evaluated as the number of samples detected positive for *Staphylococcus spp.*, *Echerichia coli*, *Aeromonas spp*, *Salmonella spp.* and *Pseudomonas spp.* isolated from the fish samples analyzed (Marijani, 2022).

Antimicrobial Susceptibility Assays

The identified bacterial isolates were tested for antibiotics susceptibility using the disc diffusion method according to the procedures described by Ogbonna and Mandu, (2018). The isolates were tested against Tetracycline (TE), Ciprofloxacin (CIP); Amoxicillin (AMX) and Azithromycin (AZM). Standard antibiotic disks (UK, Oxoid Limited) were used in the following concentrations: tetracycline (30 µg), ciprofloxacin (5 µg), amoxicillin (10 µg) and azithromycin (15 µg). The procedure involved spreading a suspension of the bacterial isolates corresponding to 0.5 McFarland standards (Marijani, 2022) on Muller-Hinton agar (Oxoid Limited), followed by placing the antibiotic discs on the agar surface using sterile forceps. The plates were then incubated at 37°C for 24 h to allow for the formation of zones of inhibition around the antibiotic discs. The diameter of the zone of inhibition was measured in millimeters using a ruler and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines as susceptible, intermediate, or resistant bacteria.

Data Analysis

Descriptive statistics were used to determine the prevalence of antibiotic-resistant bacteria isolated from cultured and wild *C. gariepinus.* The antibiotic susceptibility pattern for each antibiotic was determined by calculating the number of bacteria isolates that were resistant, intermediate, or sensitive to the antibiotic, divided by the total number of bacteria isolates tested in percentage. The rate of prevalence of bacterial resistance was also calculated for each tested antibiotic, expressed as a percentage. Multidrug resistance was defined as resistance to three or more antibiotics. One-way analysis of variance (ANOVA) was also used to compare the prevalence of antibiotic resistance of the bacteria isolates obtained from cultured and wild fish samples at a significance level of 0.05. All statistical analyses were performed in Stata 18 software.

RESULTS AND DISCUSSION

Morphology and Biochemical Characteristics of Bacteria isolates of Fish Samples

Result of isolation and identification of bacterial species revealed the presence of five bacteria species in the muscle of cultured and wild *Clarias gariepinus* in Awka. These isolates include one gram-positive coccal bacteria (*Staphylococcus aureus*) and four gram-negative rods bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica and Aeromonas hydrophila*) as shown in Table 1. Similar observation was reported by Ogbonna and Mandu, (2018) and Afolabi *et al.* (2020). *Escherichia coli* in this present study was positive in the catalase test and motility test but negative in the coagulase and citrate tests while *Pseudomonas aeruginosa* was positive in catalase and oxidase test but negative in indole and Methyl red test. *Salmonella enterica* and *Aeromonas hydrophila* showed positive reaction to catalase test and fermented glucose but were negative to indole, urease and citrate test. However, *Aeromonas hydrophila* was positive to coagulase test. Staphylococcus *aureus* showed a positive reaction to the catalase and coagulase tests, but a negative reaction to the indole test.

Table 1: Morphology and Biochemical Characteristics of Bacteria isolates of Fish Samples

GST	Cat	Oxi	Ind	Ure	Coa	Citr	Mob	Met	Glu	Shape	Morphology	Probable bacteria
-	+	+	+	-	-	-	+	+	+	Rod	Medium pink colonies	*E. coli*
+	+	+	-	-	+	+	-	+	-	Cocci	Large yellowish colonies	*Staphylococcus aureus*
-	+	+	-	-	-	-	+	-	+	Rod	Small colorless colonies	*Salmonella enterica*
-	+	-	-	-	+	-	+	+	+	Rod	Small grey mucoid colony	*Aeromonas hydrophila*
-	+	+	-	+	-	-	+	-	+	Rod	Large green-colored colonies	*Pseudomonas aeruginosa*

+=Positive; − =Negative; GST = Gram Staining test; Cat= Catalase; oxi = Oxidase, Ind = Indolase; Ure = Urease; Coa = Coagulase; Citr= Citrate; Mob = Mobility; Met = Methyl red; Glu = glucose fermentation test.

Prevalence of Bacteria Isolates in the Muscles of Cultured and Wild *C. gariepinus*

The results of the prevalence of bacteria isolates from the muscle of the cultured and wild *C. gariepinus* in Table 2 revealed that a total of 38 bacterial isolates belonging to five different bacterial species were recovered from the fish muscles. This study revealed that *Staphylococcus aureus* was the most prevalent bacteria (26.31%), followed by *Escherichia coli* (21.05%), *Aeromonas hydrophila* and *Pseudomonas aeruginosa* (18.42%) while *Salmonella enterica* was the least prevalent bacteria (15.79%). The identified bacteria isolate from this study were similar to the work of Afolabi *et al.* (2020) who identified seven bacterial species which were *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Micrococcus luteus, Escherichia coli* and *Streptococcus* spp. in *C. gariepinus* obtained from cultured and natural habitats in Ondo State. Ogbonna & Mandu, (2018) also identified *Escherichia coli, Pseudomonas putida, Salmonella sp, Shigella sp,* and *Staphylococcus aureus* in catfish obtained from ponds and rivers in Port Harcourt, Nigeria. A review of antimicrobial resistance in fish and other aquatic sectors in Africa by Moffo *et al*. (2024) reported that the most commonly isolated bacteria were *Escherichia coli, Salmonella spp., Staphylococcus spp., Aeromonas spp.* and *Klebsiella spp.* Additionally, fish farm 2 (FF2) had the highest bacterial load (21.05%) followed by fish farm 1 (FF1), and fish farm 3 (FF3) both of which had the same prevalence (18.42%). Ebenebe sampling point 2 (SP2) and Ebenebe sampling point 3 (SP3) recorded a prevalence of 15.79% respectively. Least bacteria load was observed in Ebenebe sampling point 1 (10.53). *Salmonella spp.* was not detected in the fish samples obtained from Ebenebe sampling point 1, while *Pseudomonas spp*. was not detected in fish samples obtained from Ebenebe sampling point 2. It was also observed that *C. gariepinus* (57.89%) recorded higher prevalence of bacteria isolates than those of wild *C. gariepinus* (42.10%). However, there was no significant difference ($p > 0.05$) in the prevalence of the different bacteria isolates between the cultured and wild *C. gariepinus*. This observation agrees with the work of Afolabi *et al*. (2020) who reported that the total bacterial loads in cultured habitat were higher than the total bacterial load recorded in natural habitat. Furthermore, Wamala *et al*. (2018) also reported that ponds have a higher prevalence of bacteria compared to the wild water.

Table 2: Number (percentage) of Bacteria Species Isolated in the Muscles of Cultured and Wild *C. gariepinus*

	E. coli **n(%)**	***S. aureus*** **n(%)**	***Salmonella spp*** **n(%)**	***P. aeruginosa*** **n(%)**	***Aeromonas spp*** **n(%)**	**Total n(%)**
FF1	1(12.5)	2(20)	1(16.67)	2(28.57)	1(14.29)	7 (18.42)
FF2	2(25)	2(20)	1(16.67)	1(14.29)	2(28.57)	8 (21.05)
FF3	1(12.5)	2(20)	1(16.67)	2(28.57)	1(14.29)	7 (18.42)
SP1	1(12.5)	1(10)	ND	1(14.29)	1(14.29)	4 (10.53)
SP2	2(25)	2(20)	1(16.67)	ND	1(14.29)	6 (15.79)
SP3	1(12.5)	1(10)	2(33.33)	1(14.29)	1(14.29)	6 (15.79)
Total	8 (21.05)	10 (26.31)	6 (15.79)	7 (18.42)	7 (18.42)	38 (100)

FF1 = Fish farm 1, FF2 = Fish farm 2, FF3 = Fish farm 3, SP1 = Ebenebe sampling point 1, SP2 = Ebenebe sampling point 2 and SP3 = Ebenebe sampling point 3. ND - Not Detected.

Antibiotic Resistance Pattern in Cultured and Wild *Clarias gariepinus*

The antibiotic sensitivity test of the analysed fish samples as shown in Table 3 revealed that bacteria isolated from cultured and wild *C. gariepinus* showed various forms of resistance to all the tested antibiotics. It was observed that 60.53% of the bacteria isolates were resistant to Tetracycline, followed by bacteria that were susceptible (26.32%), while only 13.16% were intermediate. 50% of the isolates were susceptible to Ciprofloxacin, with 26.32% and 23.68% intermediate and resistant bacteria respectively. Additionally, 71.05% of isolates were susceptible to Amoxicillin, with 15.79% intermediate and 13.16% resistant. Furthermore, 81.58% of the bacteria isolates were susceptible to Azithromycin, followed by bacteria that were intermediate 10.53% with 7.89% resistant bacteria. Bacteria isolates from this study showed high resistance to Tetracycline (60.53%), moderate resistance to Ciprofloxacin (23.68%) and Amoxicillin (13.16%) and low resistance to Azithromycin (7.89%). This study is comparable to the work of Tiamiyu *et al*. (2015) who reported high resistance of bacteria isolates (79.17%) to Tetracycline in fish obtained in Ibadan. However, bacteria isolate in this study showed lower resistance to Amoxicillin (13.16%) when compared to 75.0% reported by Tiamiyu *et al*. (2015). Unlike this study, Miranda *et al*. (2024) reported high resistance of bacteria isolates to Amoxicillin (64.8%), moderate resistance to Oxytetracycline (23.2%) and lowest resistance to Ciprofloxacin (0.7%). Saengsitthisak *et al.* (2020) also recorded a high resistance to amoxicillin (93.75%) and oxytetracycline (79.69%) but moderate resistance to ciprofloxacin (40.63%) in ornamental fish sold in Thailand. However, Krahulcová *et al*. (2023) did not observe Ciprofloxacin resistance in any antibiotic-resistant isolates while Ogbonna & Mandu, (2018) reported that Ciproflaxacin were highly susceptible to most of the bacteria isolates from catfish in Port Harcourt. The antibiotic susceptibility test in this study showed that high levels of resistance were expressed by all bacteria isolates to Tetracycline. Ogbonna & Mandu, (2018) and Krahulcová *et al*. (2023) also recorded high antibiotic-resistance to Tetracycline in analysed fish samples. The antibiotics susceptibility test between the cultured and wild *Clarias gariepinus was* significantly different at $p < 0.05$. Furthermore, bacteria isolated from cultured *C. gariepinus* exhibited higher levels of resistance to antibiotics compared to those isolated from wild *C. gariepinus.* Similar observation was confirmed by Wamala *et al*. (2018) who reported that resistance phenotypes were observed more in farmed fish compared to wild fish in Uganda.

Table 3: Antibiotic Susceptibility Pattern in Cultured and Wild *C. gariepinus*

Analytes	Susceptible Bacteria			Intermediate Bacteria			Resistant Bacteria		
	Cultured (n)	Wild (n)	Total (%)	Cultured (n)	Wild (n)	Total (%)	Cultured (n)	Wild (n)	Total (%)
TC	5	5	26.32	3	2	13.16	14	9	60.53
CIP	10	9	50	6	4	26.32	6	3	23.68
AMX	16	11	71.05	3	3	15.79	3	2	13.16
AZM	18	13	81.58	2	2	10.53	2	1	7.89
Total	49	38		14	11		25	15	

TC= Tetracycline; CIP= Ciprofloxacin; AMX= Amoxicillin; AZM= Azithromycin; n = Number of bacteria isolates resistant, susceptible or intermediate.

Prevalence of Resistance Bacterial Isolates n (%) in Cultured and Wild *C. gariepinus*

Table 4 shows the prevalence of resistant bacteria isolates in the analysed fish samples. In this study, *Staphylococcus spp.* showed 60% resistance to Tetracycline, 20% resistance to Amoxicillin and 10% resistance to Ciprofloxacin and Azithromycin respectively. In a study on the antimicrobial resistance of bacteria isolated from marine and freshwater fish in Tanzania, Marijani (2022) reported that 31% of *Staphylococcus* isolates were resistant to Tetracycline while both studies recorded low resistance to Ciprofloxacin. Furthermore, Tiamiyu *et al.* (2015) also reported that *Staphylococcus* spp. isolated from fish samples in Ibadan recorded 100% and 66.67% resistance to Amoxicillin and Tetracycline respectively. In addition, a total of 50%, 37.5% and 12.5% of *Echerichia coli* isolates were resistant to Tetracycline, Ciprofloxacin and Amoxicillin respectively. *Echerichia coli* in this present study recorded high antimicrobial resistance to Ciprofloxacin compared to the work of Marijani (2022) who recorded resistant rate of 10% for Ciprofloxacin and 15% for Azithromycin. However, *Echerichia coli* in this study showed no resistance to azithromycin. Additionally, Tiamiyu *et al.* (2015) reported 83.33% resistance of *Echerichia coli* to both Tetracycline and Amoxicillin respectively. Salmonella *spp*. in this present study exhibited 66.67% resistance to Tetracycline and 16.67% resistance to Ciprofloxacin and Azithromycin respectively. Marijani (2022) reported 27% resistance of *Salmonella* spp. to Tetracycline while Tiamiyu *et al.* (2015) reported 100% resistant to Tetracycline and only 33.33% to Amoxicillin. *Aeromonas* spp. in this present study exhibited resistance to only two antibiotics which were Tetracycline (71.43%) and amoxicillin (28.57%). Similarly, Saengsitthisak *et al.* (2020) recorded 79.69% resistance of *Aeromonas* spp to oxytetracycline but higher resistance percentage (93.75%) to Amoxicillin than the result of this study. For *Pseudomonas* spp. isolates, 57.14%, 28.57% and 14.29% were resistant to Tetracycline, Ciprofloxacin, and Azithromycin respectively. Mhenni *et al.* (2023) reported resistance rate of *Pseudomonas* spp greater than 50% to Tetracycline and Amoxicillin in fresh fish fillet in Italy.

Table 4: Prevalence of Resistance Bacteria Isolates in Cultured and Wild *C. gariepinus*

ARB	Disc µg	*Echerichia spp* n(%)	*Staphylococcus spp* n(%)	*Salmonella spp* n(%)	*Pseudomonas spp* n(%)	*Aeromonas spp* n(%)
TC	30	4(50)	6(60)	4(66.67)	4(57.14)	5(71.43)
CIP	5	(3)37.5	1(10)	1(16.67)	2(28.57)	0
AMX	10	1(12.5)	2(20)	0	0	2(28.57)
AZM	15	0	1(10)	1(16.67)	1(14.29)	0

TC= Tetracycline; CIP= Ciprofloxacin; AMX= Amoxicillin; AZM= Azithromycin

It was also observed that bacteria isolated from cultured and wild *C. gariepinus* showed multidrug resistance to three or more antibiotics. Similar observation was reported by Miranda *et al*. (2024) who recorded that high number of bacteria isolates from wild marine fish in Chile exhibited multi-drug resistance (MDR) to at least three classes of antimicrobials. This finding was contrary to the work of Krahulcová *et al*. (2023) who observed that all the isolated antibiotic-resistant bacteria were not multidrug resistant. Moffo *et al*. (2024) also reported that high multidrug resistance rate of bacteria to commonly used antimicrobial agents, such as tetracycline, ampicillin, cotrimoxazole, gentamicin, and amoxicillin were observed in Africa. In a study on antibiotic susceptibility patterns of bacteria isolated from fish in Kenya, Wanja *et al*. (2020) reported that all the bacteria including *Streptococcus, Proteus, Pseudomonas,* and *Serratia* except *Micrococcus, Escherichia*, and *Salmonella* species showed multiple drug resistance patterns. The presence of resistant bacteria observed in wild *C. gariepinus* in this study confirmed the report by Bondad-Reantaso *et al.* (2023) who noted that even in aquatic environments where exposure to antimicrobial agents is negligible, resistant bacteria have been discovered. Due to the inherent connections between aquacultural systems with open water bodies, such as rivers and lakes, ARB have been reported to have side effects in open water systems (Lulijwa *et al.*, 2020). These resistant bacteria can be transmitted to humans through food consumption and direct contact with animals and their environment (Manyi-Loh *et al.,* 2023). The long-term accumulation of these resistant bacteria tends to reduce the effectiveness of treating infectious diseases in humans (Zhou *et al*., 2021). Marijani (2022) suggested that susceptibility tests should be routinely performed to guide antibiotic treatment and policy.

CONCLUSION AND RECOMMENDATION

The occurrence of antibiotic-resistant bacteria in fish is of particular concern due to the potential for these bacteria to be transmitted to humans through the consumption of contaminated fish. This study revealed that bacteria isolated from cultured and wild *Clarias gariepinus* showed various levels of resistance to all the tested antibiotics. Antibiotic susceptibility test showed that high levels of resistance were expressed by all bacterial isolates to tetracycline, moderate resistance to Ciprofloxacin and Amoxicillin and low resistance to Azithromycin. However, bacteria isolated from cultured *C. gariepinus* exhibited higher levels of resistance to antibiotics compared to those isolated from wild *C. gariepinus.* This may be as a result of routine use of antibiotics in fish farms. In comparison to farmed fish, wild fish might be exposed to antibiotics through discharge from fish farms and industrial waste into aquatic habitats. The emergence of antibiotic resistance in bacterial isolates can be attributed to various factors, including the improper usage of antibiotics in aquaculture production.

Based on the findings of the study, several recommendations can be made to reduce the occurrence of antibiotic-resistant bacteria in fish. There is a need for increased surveillance of antibiotic use in aquaculture practices in Awka, Anambra State. This will help to identify areas where antibiotics are being overused and where resistance is likely to develop. Furthermore, there is a need for increased awareness among catfish farmers and consumers about the risks associated with antibiotic-resistant bacteria. Education programs should be implemented to inform farmers about the proper use of antibiotics and the potential consequences of antibiotic resistance.

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Authors' contribution

NCG conducted the fieldwork and sample collection for both cultured and wild *Clarias gariepinus,* in various locations in Awka, Anambra State. ICF and OPA performed the laboratory analysis, including bacterial isolation, identification, and antibiotic susceptibility testing. ICC and OKP were responsible for data analysis and interpretation. NCG and ICF designed the study and writing of the manuscript. All authors reviewed and approved the manuscript for submission.

Ethical Statement

All fish samples were handled and processed in accordance with standard laboratory protocols.

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