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Microbiota of gills and antibiotic susceptibility patterns of bacteria isolates from *Clarias gariepinus* in different holding facilities

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Abstract

Gill is a key respiratory and excretory organ in fish as it provides oxygen need for survival and excretes waste products. However, gills can be infected with pathogenic and opportunistic bacteria leading to increasing fish morbidity and mortality. This study was carried out to isolate, estimate and identify bacteria on the gills of *Clarias gariepinus* reared in different holding facilities. The susceptibility patterns of the bacteria were also studied using 10 antibiotics commonly used in pisciculture in Nigeria. A total of 84 bacteria belonging to 12 genera were isolated from the gills of 75 *Clarias gariepinus.* Gram-negative bacteria isolated included *Salmonella* species (3.6%), *Pseudomonas* species (7.1%), *Aeromonas* species (2.4%), *Escherichia coli* (13.1%), *Proteus* species (11.9%) *Klebsiella* species (3.6%), *Citrobacter* species (4.8%), and *Shigella* species (3.6%). Gram-positive *Corynebacterium* species (3.6 %), *Staphylococcus* species (20.3%), *Bacillus* species (19.0%) and *Streptococcus* species (7.1%) were also isolated. The result showed varying bacteria species when considering the different holding facilities. Greater than 50 % of Gram-positive and Gram-negative bacteria isolated were resistant to 5 and 6 different antibiotics respectively while greater than 80 % of all the bacteria were resistant to ≥ 3 antibiotics. The presence of these bacteria in fish predict subsequent impediment in pisciculture and may lead to socioeconomic losses, environmental contaminations and high public health risk. This study calls for concern and an urgent intervention on antibiotic stewardship among fish farmers.

Keywords: Antibiotics resistance, *Clarias gariepinus*, Fish farms, Gills microbiota, Kaduna state

Introduction

Fish production through aquaculture is one of the fastest growing agricultural enterprises in the world with a great capacity to meet the increasing demand for food supply (Hossain *et al*., 2014; FAO, 2018). Fish

and fish products make up more than 60 % of the total protein intake in adults and children especially in rural areas where they are widely accepted and form a much-cherished delicacy that cuts across all strata of the population. They have played a significant role in food security especially in developing countries like Nigeria (Adedeji & Onwenefah, 2013). In bridging the demand and supply gap of fish, *Clarias gariepinus* (*C. gariepinus*) is a suitable and the most preferred choice for fish production in Africa particularly in Nigeria due to its hardy nature, wide acceptability, consumers' delicacy and commands of good price (Tsutsui *et al*., 2011).

C. gariepinus can be reared in different holding facilities that can retain water such as earthen ponds concrete, plastic, wooden, metal, glass, and fibre tanks (Ozigbo *et al*., 2014). However, with an increase in commercial fish production, disease outbreak has become a major setback in production. Bacteria are one of the most important etiological agents and are of great risk in aquaculture worldwide (Wamala *et al*., 2018). There are numerous bacterial organisms in an aquatic environment that affect the health of the cultured fish and are also known to affect the post-harvest quality of fish (Al-Harbi & Uddim, 2008).

The knowledge of the bacteria of gills of *C. gariepinus* remains relatively poor. Gills play very important roles in gas exchange, ion-regulation, osmoregulation, acid–base balance, ammonia excretion, hormone production, modification of circulating metabolites and immune defense (Brauner & Rombough, 2012).

Moreso, the gill is in constant contact with the aquatic environment and thus represent an important target organ of dissolved pollutants and microorganisms. The gills may also provide an ideal portal of entry to all kind of pathogens that may play critical roles in overall fish health (Asmaa *et al*., 2015). Microbiological evaluation of the gills of fish from commercial fish farms would therefore be essential to assure the farmers and consumers about the quality and safety of fish products (Al-Harbi & Uddim, 2008; Pal *et al*., 2016).

Antibiotics are currently utilized to treat fish infected by bacteria, as well as preventing the establishment of pathogenic bacteria within fish farms (Miranda *et al.*, 2018). Knowledge of etiological agents of infections and their sensitivities to available drugs is of great value for rational selection and development of appropriate decision on prescription in the use of antimicrobial agents in order to avoid the occurrence of antimicrobial resistance (Abubakar, 2009). Therefore, the aim of this study was to determine the microbiota of the gills of *C. gariepinus* and their antibiotic susceptibility patterns obtained from different holding facilities from selected commercial fish farms in Kaduna State.

Materials and Methods

Study location

The study was carried out in Kaduna State, which is located on latitude 10° 36' 33.54'' N and longitude 7° 25' 46.2144'' E. It occupies an area of approximately 48,473.2 square kilometers and has a population of more than 6 million people (KSGC, 2015).

Study design

The cross-sectional study involved multistage random sampling of 15 active farms comprising of five earthen ponds, five concrete and five plastic tanks from four Local Government Areas of Kaduna State. Four farms each from Sabon Gari, Kaduna North, Kaduna South, and 3 farms in Zaria Local Government Areas were selected for the study. The fish farms with concrete and plastic tanks were intensively managed and sourced their water from borehole while the earthen ponds were semi intensively managed with water supplementation from natural water bodies around the farms. The fish farms had different stages of *C. gariepinus* (fingerlings, growers, adults and broodstocks) stocked in the farms. The management practises were assessed and evaluated on the spot on the fish farms. Information on the antibiotic usage on the fish farms was recorded and used for the selection of the antibiotics for the susceptibility test.

Fish selection and processing

A total of 75 live *Clarias gariepinus* (5 fish per farm) were randomly collected from the selected fish farms. *C. gariepinus* of 4 - 8 months of age, with a total length of ≥12-33cm and a weight of 0.5-1kg, fed with commercial feed and reared in monoculture system were included in the study. The fish was caught at about 8.00 am using a fishnet of the farm and placed in a plastic bucket containing the pond water to ensure the survival of the fish samples. This was later transported to the Microbiology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for further processing within 2 hours of collection.

Each live fish was sacrificed (by brain spiking to minimize suffering) and placed on a clean stainless tray dorsally and sterile cotton wool swab soaked in 70% alcohol was used to clean the fish from the operculum to the abdominal area to reduce bacterial load and surface contamination. The operculum of

the fish was lifted to expose the gills and the sterile surgical scalpel blade was used to cut out the gill, which was placed in a sterile petri dish for bacterial isolation and estimation.

Bacteria population culture, isolation, estimation, and identification

Standard bacterial population, isolation, estimation, and identification as described by Barrow & Feitham (2003) were strictly followed. One gram of the fish gills was macerated aseptically in 10 ml of sterile distilled water (10% w/v), then, a 10-fold dilution of this was carried out. Subsequently, 0.1ml of the tube containing 10^{-10} dilution factor was inoculated on nutrient agar and MacConkey agar (MCA) (Oxoid, UK) in duplicate using the spread plate method. The plates were then incubated for 18-24 hrs at 37 °C. The colony forming unit (CFU)/gram of the gills was determined (APHA, 1993). After incubation, discrete isolates from the plates were picked with sterilized loop and streaked again on a nutrient agar plate for the isolation and purification of bacteria colonies and on MacConkey agar plate for growing of Gram-negative organisms and to differentiate between lactose fermenters and non-lactose fermenters. Eosin methylene blue agar (Oxoid, UK) was used for the isolation of *E. coli*, *Citrobacter* species and *Klebsiella* species. *Salmonella Shigella* Agar (Oxoid, UK) was used for isolation of *Salmonella* and *Shigella* species. The agar plates were then incubated for 18-24 hrs at 37 °C. The bacteria were identified using morphological characteristics, Gram staining, and biochemical tests such as motility test, oxidase test, catalase test, triple sugar iron (TSI), indole test, urease test, citrate utilization test, methyl red test, oxidative fermentation test, Voges Proskauer test nitrate, reduction test and gelatine liquefaction test (Barrow & Feitham 2003; Daodu *et al.,* 2017).

Antibiotic susceptibility test

Antibiotic susceptibility test was carried out on each of the isolates using Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates (MHA) (Oxoid Basingstoke, UK) with inocula adjusted to an optical density of 0.5 McFarland standard units (CLSI, 2010). The antibiotics used were ampicillin (10 µ*g*), chloramphenicol (10 µ*g*), ciprofloxacin (5 µ*g*), gentamycin (10 *ug*), oxacillin (5 *ug*), oxytetracycline (30 *ug*), penicillin (10 *iu*), streptomycin (10 µ*g*), tetracycline (30 µ*g*) and vancomycin (30 µ*g*) (Oxoid, UK). The susceptibility test followed the procedure described by Clinical Laboratory and Standards Institute, CLSI (2015).

Data analysis

Data of the isolates were initially entered into Microsoft Office Excel version 2010 for the determination of absolute frequencies and percentages. The mean ± standard deviation of the total bacterial count of the gills from the different holding facilities were calculated and compared using one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA) for windows version 22.0. The values of $p < 0.05$ were considered statistically significant.

Results

The total bacteria load from the gills, from the different holding facilities varies from 2.44 ± 0.77 x 10^4 to 5.20 ± 0.76 x 10^6 colony forming unit/gram of fish gill (Table 1). The bacterial load of gills of *C. gariepinus* from concrete tanks ranged between 2.81 ± 0.99 x 10^4 and 3.80 ± 0.73 x 10^4, while the value from earthen ponds ranged between 3.70 ± 1.75 x 10^5 and 5.20 ± 0.76 x 10^6 and values from plastic tanks ranged between 2.4 ± 0.77 x 10^4 and 4.06 ± 0.37 x 10^4. There was significant difference in the total bacterial load of gills at $P < 0.05$ between earthen ponds and both concrete and plastic tanks. However, there was no significant difference within the farms from the different holding facilities except for earthen ponds between farm 5 and the other farms.

A total of 84 bacteria belonging to 12 genera were isolated from gills of 75 *Clarias gariepinus* studied. The isolates comprised of 4 Gram-positive bacteria (*Streptococcus* species, *Bacillus* species, *Staphylococcus* species, and *Corynebacterium* species) and 8 Gram-negative bacteria known to be pathogenic to fish (*Escherichia coli, Proteus* species, *Citrobacter* species, *Pseudomonas* species, *Aeromonas* species, *Salmonella* species, *Shigella* species and *Klebsiella* species). Gills of fish cultured in concrete tank harboured the highest number of bacteria isolates with 30/84 (35.7%) followed by those cultured in earthen ponds with 28/84 (33.3%) and plastic tanks with 26/84 (31.0%) (Table 2).

Generally, there were 6 common bacteria genera/species isolated from the gills of fish cultured in any of the holding facilities and these were

Table 1: Bacteria population in gills of *Clarias gariepinus* (average) from some selected fish farms in Kaduna State

Holding Facilities	Farms	Bacterial population	
		Mean ± SD (x 10^4) CFU/gram of gill (within holding facilities)	Mean ± SD (x 10^4) CFU/gram of gill (between holding facilities)
Concrete tanks	1	3.40 ±1.98x$10^{4\,a}$	
	2	3.80 ± 0.73 x$10^{4\,a}$	
	3	3.71 ±2.29 x$10^{4\,a}$	3.4 ± 1.4x$10^{4\,a}$
	4	3.23 ± 0.61 x$10^{4\,a}$	
	5	2.81 ± 0.99 x$10^{4\,a}$	
Earthen ponds	1	3.70 ±1.76 x$10^{5\,a}$	
	2	3.90 ± 0.51 x$10^{5\,a}$	
	3	4.19 ± 0.80 x$10^{5\,a}$	1.4 ± 2.0 x$10^{6\,b}$
	4	4.48 ± 0.85 x$10^{5\,a}$	
	5	5.20 ± 0.76 x$10^{6\,b}$	
Plastic tanks	1	2.91 ±1.04 x$10^{4\,a}$	
	2	3.30 ± 0.23 x$10^{4\,a}$	
	3	2.44 ± 0.77 x$10^{4\,a}$	3.1 ± 0.9 x$10^{4\,a}$
	4	2.98 ± 1.25 x$10^{4\,a}$	
	5	4.06 ± 0.37 x$10^{4\,a}$	

Different alphabets (a, b) connote significant differences ($p < 0.05$) within and between holding facilities

Table 2: Distribution of different bacteria isolates from gills of *Clarias gariepinus* in different holding facilities from selected fish farms in Kaduna State

Bacteria	Concrete Tanks (%)	Plastic Tanks (%)	Earthen Ponds (%)	Total (%)
Gram-positive isolate	15 (50.0)	15 (57.7)	12 (42.9)	
Bacillus species	5 (16.7)	6 (23.1)	5 (17.9)	16 (19.0)
Corynebacterium species	2 (6.7)	1 (3.8)	0 (0.0)	3 (3.6)
Staphylococcus species	6 (20.0)	7 (26.9)	4 (14.3)	17 (20.3)
Streptococcus species	2 (6.7)	1 (3.8)	3 (10.7)	6 (7.1)
Gram-negative isolates	15 (50.0)	11 (42.3)	16 (57.1)	
Aeromonas species	1 (3.3)	1 (3.8)	0 (0.0)	2 (2.4)
Citrobacter species	0 (0)	2 (7.7)	2 (7.1)	4 (4.8)
Escherichia coli	4 (13.3)	3 (11.5)	4 (14.3)	11 (13.1)
Klebsiella species	2 (6.7)	0 (0.0)	1 (3.6)	3 (3.6)
Proteus species	4 (13.3)	3 (11.5)	3 (10.7)	10 (11.9)
Pseudomonas species	3 (10)	1 (3.8)	2 (7.1)	6 (7.1)
Salmonella species	1 (3.3)	0 (0.0)	2 (7.1)	3 (3.6)
Shigella species	0 (0)	1 (3.8)	2 (7.1)	3 (3.6)
Total number of isolates	30 (100.0)	26 (100)	28 (100)	

Staphylococcus species, *Streptococcus* species, *Bacillus* species, *Escherichia coli*, *Proteus* species and *Pseudomonas* species (Table 2). In concrete tanks, 10 bacteria genera/species were isolated in the absence of *Citrobacter* species and *Shigella* species while 10 bacteria genera/species obtained in earthen ponds fish gill in the absence of *Corynebacterium* species and *Aeromonas* species. *Staphylococcus* species were the highest prevailing isolates among bacteria genera/species obtained from gills of fish cultured in concrete tanks with 6/30 (20%) and plastic tanks with 6/26 (26.9%) while *Bacillus* species with 5/28 (17.9%) was the highest prevailing bacteria in gills of fish cultured in earthen pond. Among Gram-negative bacteria, *Escherichia coli* and *Proteus* species were most prevalent among bacteria genera obtained from fish gills cultured in the concrete tanks with 4/30 (13.3 %) and plastic tanks with 3/30 (11.5%) while only *E. coli* remains at the peak in gills of fish cultured in the earthen pond (Table 2). Table 3 described the patterns for Gram-positive bacteria. Based on the 10 antibiotics used, resistant percentage ranges include *Corynebacterium* species (33.3 – 100%), *Bacillus* species (25 - 75.0%), *Staphylococcus* (16.7 - 83.3%). The Gram-positive bacteria antibiotic resistant pattern followed the order vancomycin > penicillin >

oxacillin > ampicillin > oxytetracycline > tetracycline > streptomycin > chloramphenicol > gentamycin > ciprofloxacin. Generally, the study showed that > 50% of Gram-positive isolates (n= 42) were resistant to oxytetracycline, oxacillin, ampicillin, penicillin and vancomycin (Table 3). For Gram-negative bacteria isolates, resistant percentage ranges include *Aeromonas* species (25 – 100.0%), *Citrobacter* species (0.0 – 100.0%), *E. coli* (18.2 - 90.9%), *Klebsiella* species (33.3 – 100.0%), *Proteus* species (20.0 – 90.0%), *Pseudomonas* species (16.7 - 83.3%), *Salmonella* species (33.3 - 100.0%) and *Shigella* species (33.3 – 100.0%) (Table 4). Gram-negative antibiotic resistant pattern followed the order penicillin > oxacillin > vancomycin > ampicillin > oxytetracycline > tetracycline > streptomycin > chloramphenicol > gentamycin > ciprofloxacin. Generally, the study showed that ≥ 50% of Gram-negative isolates (n= 42) were resistant to tetracycline, oxytetracycline, oxacillin, vancomycin, penicillin and ampicillin.

Discussion

There was variation in the bacterial load obtained from gills in the different holding facilities sampled in this study. This may be due to the difference in pond type, sources of water and management practices in the different fish farms leading to the different levels of organic content consequently, resulting in the

Table 3: Antibiotic susceptibility patterns of Gram-positive bacteria isolates from gills of *Clarias gariepinus* from selected fish farms in Kaduna State

Antibiotic	Reaction pattern	*Staphylococcus* species *n* = 17 (%)	*Streptococcus* species n= 6 (%)	*Corynebacterium species* n = 3 (%)	*Bacillus species* n = 16 (%)	Total n = 42 (%)
Ampicillin	Sensitive	2 (11.8)	1 (16.7)	0 (0.0)	3 (18.8)	6 (14.3)
	Intermediate	4 (23.5)	1 (16.7)	1 (33.3)	3 (18.8)	9 (21.4)
	Resistant	11 (64.7)	4(66.7)	2 (66.7)	10 (62.5)	27 (64.3)
Chloramphenicol	Sensitive	8 (47.1)	3 (50.0)	2 (66.7)	7 (43.8)	20 (47.6)
	Intermediate	2 (11.8)	1 (16.7)	0 (0.0)	3 (18.8)	6 (14.3)
	Resistant	7 (41.2)	2 (33.3)	1 (33.3)	6 (37.5)	16 (38.1)
Ciprofloxacin	Sensitive	10 (58.8)	4 (66.7)	2 (66.7)	9 (56.3)	25 (59.5)
	Intermediate	2 (11.8)	1 (16.7)	0 (0.0)	3 (18.8)	6 (14.3)
	Resistant	5 (29.4)	1 (16.7)	1 (33.3)	4 (25.0)	11 (26.2)
Gentamycin	Sensitive	6 (35.3)	3 (50.0)	3 (100.0)	9 (56.3)	21 (50.0)
	Intermediate	4 (23.5)	1 (16.7)	0 (0.0)	3 (18.8)	8 (19.0)
	Resistant	7 (41.2)	2 (33.3)	0 (0.0)	4 (25.0)	13 (31.0)
Oxacillin	Sensitive	3 (17.6)	1 (16.7)	1 (33.3)	2 (12.5)	7 (16.7)
	Intermediate	4 (23.5)	0 (0.0)	0 (0.0)	2 (12.5)	6 (14.3)
	Resistant	10 (58.8)	5 (83.3)	2 (66.7)	12 (75.0)	29 (69.0)
Penicillin	Sensitive	1 (5.9)	0 (0.0)	0 (0.0)	1 (6.3)	2 (4.8)
	Intermediate	4 (23.5)	2 (33.3)	0 (0.0)	4 (25.0)	10 (23.8)
	Resistant	12 (70.6)	4 (66.7)	3 (100.0)	11 (68.8)	30 (71.4)
Streptomycin	Sensitive	4 (23.5)	2 (33.3)	1 (33.3)	3 (18.8)	10 (23.8)
	Intermediate	4 (23.5)	2 (33.3)	1 (33.3)	7 (43.8)	14 (33.3)
	Resistant	9 (52.9)	2 (33.3)	1 (33.3)	6 (37.5)	18 (42.9)
Tetracycline	Sensitive	4 (23.5)	1 (16.7)	1 (33.3)	3 (18.8)	9 (21.4)
	Intermediate	4 (23.5)	3 (50.0)	1 (33.3)	6 (37.5)	14 (33.3)
	Resistant	9 (52.9)	2 (33.3)	1 (33.3)	7 (43.8)	19 (45.2)
Oxytetracycline	Sensitive	4 (23.5)	1 (16.7)	0 (0.0)	2 (12.5)	7 (16.7)
	Intermediate	4 (23.5)	1 (16.7)	0 (0.0)	4 (25.0)	9 (21.4)
	Resistant	9 (52.9)	4 (66.7)	3 (100.0)	10 (62.5)	26 (61.9)
Vancomycin	Sensitive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Intermediate	4 (23.5)	1 (16.7)	1 (33.3)	4 (25.0)	10 (23.8)
	Resistant	13 (76.5)	5 (83.3)	2 (66.7)	12 (75.0)	32 (76.2)

Table 4: Antibiotic susceptibility patterns of Gram-negative bacteria isolates from gills of *Clarias gariepinus* from selected fish farms in Kaduna State

Antibiotic		*E. coli* n=11	*Salmo* n= 3	*Pro* n= 10	*Pseu n*= 6	*Kleb* n= 3	*Aero* n= 2	*Citro* n= 4	*Shige* n= 3	Total n= 42
Ampicillin	S	1 (9.1)	0 (0.0)	1 (10.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (7.1)
	IN	4 (36.4)	1	3 (30.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (25.0)	1 (33.3)	11 (26.2)
	R	6 (54.5)	2 (66.7)	6 (60.0)	5 (83.3)	2 (66.7)	2 (100.0)	3 (75.0)	2 (66.7)	28 (66.7)
Chloramphenicol	S	5 (45.5)	2 (66.7)	5 (50.0)	5 (83.3)	2 (66.7)	2 (100.0)	3 (75.0)	2 (66.7)	26 (61.9)
	IN	2 (18.2)	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (9.5)
	R	4 (36.4)	1 (33.3)	3 (30.0)	1 (16.7)	1 (33.3)	0 (0.0)	1 (25.0)	1 (33.3)	12 (28.6)
Ciprofloxacin	S	7 (63.6)	2 (66.7)	6 (60.0)	4 (66.7)	2 (66.7)	2 (100.0)	4 (100.0)	2 (66.7)	29 (69.0)
	IN	2 (18.2)	1 (33.3)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (11.9)
	R	2 (18.2)	0 (0.0)	2 (20.0)	2 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	1 (33.3)	8 (19.0)
Gentamicin	S	7 (63.6)	2 (66.7)	5 (50.0)	5 (83.3)	2 (66.7)	2 (100.0)	2 (50.0)	3 (100.0)	28 (66.7)
	IN	1 (9.1)	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (7.1)
	R	3 (27.3)	1 (33.3)	3 (30.0)	1 (16.7)	1 (33.3)	0 (0.0)	2 (50.0)	0 (0.0)	11 (26.2)
Oxacillin	S	2 (18.2)	0 (0.0)	1 (10.0)	2 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	6 (14.3)
	IN	2(18.2)	0(0.0)	1 (10.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (33.3)	5 (11.9)
	R	7 (63.6)	3(100.0)	8 (80.0)	4 (66.7)	2 (66.7)	1 (50.0)	4 (100.0)	2 (66.7)	31 (73.8)
Penicillin	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.8)
	IN	1 (9.1)	0 (0.0)	1 (10.0)	2 (33.3)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	5 (11.9)
	R	10 (90.9)	3(100.0)	9 (90.0)	4 (66.7)	1 (33.3)	2 (100.0)	3 (75.0)	3 (100.0)	35 (83.3)
Streptomycin	S	2 (18.2)	1 (33.3)	2 (20.0)	3 (50.0)	1 (33.3)	0 (0.0)	1 (25.0)	1 (33.3)	11 (26.2)
	IN	3 (27.3)	1 (33.3)	3 (30.0)	2 (33.3)	1 (33.3)	2 (100.0)	1 (25.0)	0 (0.0)	13 (31.0)
	R	6 (54.5)	1 (33.3)	5 (50.0)	1 (16.7)	1 (33.3)	0 (0.0)	2 (50.0)	2 (66.7)	18 (42.9)
Tetracycline	S	3 (27.3)	1 (33.3)	0 (0.0)	1 (16.7)	1 (33.3)	0 (0.0)	1 (25.0)	1 (33.3)	8 (19.0)
	IN	1 (9.1)	1 (33.3)	5 (50.0)	3 (50.0)	1 (33.3)	0 (0.0)	1 (25.0)	1 (33.3)	13 (31.0)
	R	7 (63.6)	1 (33.3)	5 (50.0)	2 (33.3)	1 (33.3)	2 (100.0)	2 (50.0)	1 (33.3)	21 (50.0)
Oxytetracycline	S	1 (9.1)	1 (33.3)	1 (10.0)	2 (33.3)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	6 (14.3)
	IN	3 (27.3)	0 (0.0)	4 (40.0)	1 (16.7)	1 (33.3)	1 (50.0)	1 (25.0)	1 (33.3)	12 (28.6)
	R	7 (63.6)	2 (66.7)	5 (50.0)	3 (50.0)	2 (66.7)	1 (50.0)	2 (50.0)	2 (66.7)	24 (57.1)
Vancomycin	S	2 (18.2)	0 (0.0)	2 (20.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (11.9)
	IN	4 (36.4)	1 (33.3)	1 (10.0)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (16.7)
	R	5 (45.5)	2 (66.7)	7 (70.0)	4 (66.7)	3 (100.0)	2 (100.0)	4 (100.0)	3 (100.0)	30 (71.4)

Key: E. coli- *Escherichia coli,* Pro - *Proteus* species, Pseu- *Pseudomonas* species, Kleb- *Klebsiella* species, Aero- *Aeromonas* species, Salmo- *Salmonella* species, Citro - *Citrobacter* species, Shige- *Shigella* species. S: Sensitive; IN: Intermediate, R: Resistant

proliferation of the bacterial load observed in the gills (Sule *et al*., 2016). It was found that the bacterial load was higher in gills of *C. gariepinus* cultured in earthen ponds compared with plastic tanks. The higher values could be due to sources of water used in earthen ponds. Water in earthen ponds is usually untreated surface water sourced from dams, streams, rivers, lakes and runoff water while, the underground water source is used in most of the concrete and plastic tanks (Moshood, 2017). Also, the rate of change of the water in the different holding facilities affected the microbial load, especially in concrete and plastic tanks where water is completely drained and refilled with fresh water while in earthen ponds topping system is practiced by adding more water to maintain specific volume or water levels. Moreso, excessive feed and faecal wastes are known to increase bacteria load in fish holding facilities which occur more in earthen ponds and can cause stress, weakness of the immune system with subsequent precipitation of diseases in earthen ponds (Olojo *et al*., 2010).

Gram-negative bacteria known to be pathogenic to the fish were the most prevalent bacteria isolated from the gills in this study, which was similar to the finding of Oni *et al*. (2013) and Njoku *et al*. (2015). The different genera of bacteria isolated in this study are similar to those reported by Njoku *et al*. (2015) and Sule *et al*. (2016). Most of the genera of bacteria isolated from the gills of *C. gariepinus* in this study have the tendency to cause serious diseases in fish

and have high public health significance as they are zoonotic. The number of bacteria genera isolated in this study are similar to the report of Uddim & Al-Harbi (2012), who identified 12 possible bacteria genera from fish gills. However, this study differs from the reports of Fatuyi *et al.* (2014), Subhash *et al*. (2015), Abu & Uwadirioha (2016), and Wamala *et al.* (2018), who isolated 14, 13, 5, and 15 different bacteria genera respectively, in a similar study. Varying bacteria genera reported might be due to difference in locations, sampling and isolation methods. These genera are associated with tropical freshwater environment and have been isolated from water, sediments, planktons, invertebrates and digestive tracts of many aquatic animals (Austin & Austin, 2007).

The numbers of bacteria associated with the gill's lamellae are reported to be actively maintained at low levels to avoid invasion of the fish (Koppang *et al*., 2015). However, the increasing intensive fish farming practice characterized by high stocking density, low water quality and increased human interference as observed in this study could increase stress on the fish making them prone to opportunistic infections (Lio-Po & Lim 2014).

The occurrence of *Salmonella* species and *Shigella* species may indicate contamination by livestock manure added to the fish ponds for pond fertilization. The isolation of *Salmonella* species*, Shigella* species, and *E. coli* is also an indication that the water in which the fish were reared was contaminated with faeces and as such could be a possible source of infection during handling by the farm workers (Traore *et al.,* 2015). Similar observation was made by Osungbemiro *et al.* (2014) who reported that *Salmonella* species and *Shigella* species existed on the skin, gills and intestine of *C. gariepinus*. Furthermore, considering the role of fish in meeting the protein needs of humans and animals (poultry ration) (Mona *et al*., 2011), the presence of *Salmonella* species in fish gills could be a possible source of salmonellosis in humans and poultry especially in inappropriately preserved crude fish (Fernandes *et al*., 2018). Aquatic environment is the major reservoir of the *Salmonella* spp. (Bibi *et al*., 2015), which is the second leading cause of food borne illness worldwide (Wong & Chen, 2013), with about 1.3 billion annual cases in humans seen as human gastroenteritis caused by the ingestion of undercooked fish (Awuor *et al*., 2011). Also, Fujioka (2001) reported that *Salmonella* species and *Escherichia coli* can survive for very long periods in tropical waters and once introduced may become indigenous to the environment.

The presence of *Bacillus* species and *Pseudomonas* species in gills has been incriminated in the fast deterioration of *C. gariepinus* as soon as they are taken out of the water from ponds (Olojo *et al*., 2010; Oni *et al*., 2013).).

Varying antibiotic resistance has been overtly reported in fish production (Akinbowale *et al*., 2006; Adedeji *et al.,* 2011) and these occurrences among different bacterial genera are very complex. Apart from natural resistance exhibited by some organisms, resistance has been reported to occur mainly as the consequences of the abuse of antimicrobial agents in aquaculture (WHO, 1999). Spanggard *et al.* (1993) suggested that bacterial groups that co-habit in a common environment may share a pool of R-factor plasmids and therefore have similar antibiotics resistant patterns. Beside these, other sources of resistant bacteria in aquaculture systems may be due to the rapid multiplication of few antibiotic-resistant organisms originally inhabiting the system or introduced from enteric tracts of fish. This multiplication is usually aided by the degradation of uneaten feed and organic manure used for pond fertilization. The high resistance of the Gram-negative and Gram-positive bacteria isolated in this study correlate with the findings of Samuel *et al. (*2011), Shah *et al.* (2012) and Ayandiran & Dahunsi (2017). Also as observed in this study, the same bacteria genera isolated from gills in the different holding facilities showed the varying level of antibiotic resistance (Ayandiran & Dahunsi, 2017). This might have resulted from the different environmental conditions, management systems and physiological mechanisms of survival. The emergence of antibiotic resistance of bacteria in fish production again calls for global concern as it poses a major public health threat (Magiorakos *et al*., 2012).

In conclusion, this study reported the presence of 12 bacteria genera in the gills of *C. gariepinus* with potential pathological implications on the fish and environment. Also, the presence of antibiotics resistance of bacteria in this study is suggestive of misuse and abuse of antibiotic in the fish production line in the study area. Thus, the presence of these bacteria in gills of *C. gariepinus* predict subsequent impediment to outstanding fish production processes from farm to table and carries notable socioeconomic losses, environmental contaminations and high public health risk (food borne disease). This study calls for concern and urgent intervention on antibiotic stewardship among fish farmers.

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Conflicts of Interest
The authors declare no conflict of interest.

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