



## Enumeration, Identification and Antibigram of Some Pathogenic Bacteria Recovered from Diseased Farmed *Clarias gariepinus* (Burchell, 1822)

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### ABSTRACT

Prevalent etiological agents and treatment options are essential for diagnosing and managing fish diseases. Therefore, this study used standard microbiological procedures to enumerate and identify the total bacterial count in the water, skin, and gastrointestinal tract of 75 moribund cultured *Clarias gariepinus* from different culture facilities. Following that, the antibiotic susceptibility of the isolated bacteria to eight antibiotics was tested using the Kirby-Bauer disc diffusion method. Various clinical signs and pathological lesions were observed on the sampled moribund fish. Total bacterial count varied significantly ( $P < 0.05$ ) regardless of the types of culture facilities. Bacteria species from eight different genera were present, comprising three Gram-positive bacteria, *Bacillus subtilis*, *Streptococcus agalactiae*, and *Staphylococcus aureus*, and five Gram-negative bacteria, *Aeromonas hydrophila*, *Citrobacter freundii*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica*, in which Gram-negative bacteria (> 70%) were the most prevalent type of bacteria isolated. There was an association between the bacteria species isolated from the water and the different parts of the fish sampled regardless of the culture facilities. The antibiogram of the bacteria recovered revealed different sensitivity and resistance profiles. More than fifty percent of the Gram-negative bacteria were sensitive to ciprofloxacin, while the Gram-positive bacteria were susceptible to ciprofloxacin and gentamycin (> 40%). Amoxicillin, ampicillin oxytetracycline, and tetracycline resistance were high in the bacteria isolates. This study confirmed the presence of antibiotic-resistant pathogenic bacteria isolated with a multiple antimicrobial resistance (MAR) index of 0.63–0.75, indicating indiscriminate and irrational use of antibiotics.

**Keywords:** Bacteria loads, *Clarias gariepinus*, Fish disease, Multidrug resistance.

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### INTRODUCTION

The African catfish, *Clarias gariepinus* (Burchell, 1822) is one of the most cultured freshwater fish in the tropical region of the world due to their nutritional value, high resistance to disease, reproductive and growth rate (Afolabi *et al.*, 2020). It is a good source of cheap protein of high quality in the developing world (Shahbaa *et al.*, 2020; Adah *et al.*, 2021). The successful culturing of *C. gariepinus* in various recommended holding facilities, such as earthen ponds, concrete, and plastic tanks can be impeded by the occurrence of bacterial infections (Adah *et al.*, 2020).

Bacteria are ubiquitous and belong to three groups: gram-negative bacteria (the most prevalent),

gram-positive bacteria, and acid-fast bacteria (Irshath *et al.*, 2023). Generally, some of the pathogenic bacteria that can cause infections in *Clarias gariepinus* belong to genera of *Pseudomonas*, *Staphylococcus*, *Flavobacterium*, *Vibrio*, *Micrococcus*, *Bacillus*, and *Aeromonas*; some exist as microflora in pond water and on the fish part under normal conditions (Ogbukagu *et al.*, 2021).

Fish health is specifically correlated with the conditions in their immediate surroundings, which can have extremely high levels of microorganisms, with the fish intestines having the highest microbial load, followed by the skin and gills (Peřkala-Safińska, 2018). The microbial load typically varies from  $10^2$ – $10^7$

colony-forming units (CFU)/cm<sup>2</sup> on the skin and 10<sup>3</sup> and 10<sup>7</sup> cfu/g in the intestines and gills, respectively (Rathod et al., 2022).

Identifying pathogenic bacteria in fish or changes in the water's microbiota is a key sign of environmental contamination (Dissasa et al., 2022). The interaction between the fish bacteria, host, and changes in the prevailing aquatic environment determines the appearance and progression of fish disease, which are associated with the intensification of aquaculture farming practices. These practices exert huge stresses from high population density, change in temperature, and hypoxia, compromising the innate immune defenses of the fish, which can hasten the spread of pathogenic bacteria and result in major disease outbreaks (Ziarati et al., 2022). Bacteria can infect a fish's body through the water, skin, and digestive tract, threatening the fish's health and thereby impairing the fish's normal physiology, leading to clinical signs of disease (Pekala- Safińska, 2018).

Up to 50% mortality rate has been recorded in a population of *C. gariepinus* stocked in different holding facilities due to an outbreak of bacterial diseases (Austin and Austin, 2016; Clois-Fuentes et al., 2023). These bacterial diseases will significantly impact the economic sustainability of fish farms and protein production for human consumption at any stage of fish production (Irshath et al., 2023).

Currently, the widespread and inappropriate use of antibiotics for therapeutic or prophylactic purposes in fish raised for human consumption encourages the growth of resistant bacteria. Hence, a good grasp of disease-causing organisms and their susceptibility to available drugs is of great importance for choosing drugs, improving fish health, controlling diseases, and minimizing the incidence of antibiotic resistance (Prestinaci et al., 2015; Manyi-Loh et al., 2018).

Therefore, this study aimed to enumerate the total count and identify pathogenic bacteria from the water, skin, and gastrointestinal tract of diseased *C. gariepinus* and also to determine the antibiogram and multiple antimicrobial resistance (MAR) index values of the bacteria isolates in some commercial fish farms in Nigeria.

## MATERIALS AND METHODS

### Ethics committee approval

The authors declare that all applicable guidelines for sampling, care, and experimental use of animals in this study have been followed and were approved by the Ahmadu Bello University Animal Care and Use Committee (ABUACAUC) with reference number UREC/FVM/1545.

### Study design

The cross-sectional study employed a multistage random selection process to choose 15 active fish farms across four Local Government Areas in Kaduna State, Nigeria. Five earthen ponds, concrete and plastic tanks respectively were also included in this study. Water from boreholes was the water source for the intensively managed fish farms with concrete and plastic tanks. As opposed to the semi-intensively managed earthen ponds that were supplemented with water from surrounding water sources such as rivers and streams. *Clarias gariepinus* in various stages of development (fingerlings, growers, adults, and brood stocks) were stocked on the fish farms with standard varying stocking density ranging between 12- 15kg of fish per cubic metre and also based on the stage of development of the fish (Baßmann et al., 2023). On-site assessments and evaluations of the management procedures were assessed and details regarding the age of the fish and use of antibiotics in fish farms were used to select the antibiotics for the susceptibility test.

### Sample collection and processing

Water samples were randomly collected from various fish farms using 100 mL sterile sampling bottles. The pond water was first stirred, after which the water bottle was dipped to about 25 cm below the water surface to collect the water samples. A total of 45 properly labeled water samples (3 water samples per farm) were collected and kept in a cooler containing an ice block (Austin and Austin 2016). Five live, symptomatic *C. gariepinus* reared in a monoculture environment, fed commercial fish, and ranging in age from three to eight months were randomly selected using a farm's fishnet. They were caught at around eight in the morning and were kept in a plastic container with pond water to ensure their survival. Within two hours of being collected, the fish and water samples were taken to the Microbiology Laboratory at Ahmadu Bello University in Zaria, where they were processed further (Table 1).

### Clinical and post-mortem examination of the fish samples

Each live diseased fish was examined clinically and a post-mortem examination was performed as described by Austin and Austin, (2016). After which 75 *C. gariepinus* were dissected, 150 tissue specimens (75 skin and gastrointestinal tract respectively) were aseptically extracted and prepared for plating in compliance with ISO 6887-3:201727 (2017) pour plate technique (El-Gendy, et al., 2024). Next, the number of viable microorganisms was measured, computed, and represented as the logarithm of colony-forming units per gram/cm<sup>2</sup>.

Table 1: Distribution of the Fish farms, fish and water samples collected in this study

Culture facility	Number of fish farms	Farm management style	Water source	Water sampled per farm	Total water sampled	Fish sampled per farm	Total number of fish sampled	Average weight (g)	Average total length (cm)
Concrete Ponds	5	Intensive System	Borehole	3	15	5	25	120-1100g	19 -42 cm
Earthen Pond	5	Semi intensive System	Borehole /River, Dam	3	15	5	25	200-1200g	26-47cm
Plastic Tanks	5	Intensive System	Borehole	3	15	5	25	100g-1000g	12-34cm

### Bacterial identification and characterization

Following initial incubation, distinct colonies were picked and streaked onto Nutrient Agar (NA) (Oxoid, UK), MacConkey agar (MCA) (Oxoid, UK), Eosin methylene blue agar (EMB) (Oxoid, UK), and Salmonella Shigella Agar (SS) (Oxoid, UK) and incubated at 37 °C for 18–24 h. The bacteria were identified using Gram staining, morphological characteristics, and conventional biochemical tests, including the oxidase, catalase, triple sugar iron (TSI), indole, urease, citrate utilization, methyl red, oxidative fermentation, Voges Proskauer, nitrate, reduction and gelatine liquefaction tests (**Barrow and Feitham 2003**). After this, a Microbat (24 E) biochemical characterization identification test kit (Oxoid, UK) was also used to confirm the organisms isolated.

### Antibiotic susceptibility test (AST)

Out of the total 220 confirmed isolates, 75 representatives of the bacteria isolates were selected from water (15), skin (30), and GIT (30) of this 50 Gram-negative, with 10 isolates from water, 20 from the skin, and 20 from the GIT. Twenty-five Gram-positive isolates were obtained from the water (5), skin (10), and GIT (10) respectively from diseased *C. gariepinus* and evaluated for antimicrobial susceptibility testing on Mueller-Hinton Agar (MHA) (Oxoid, UK) by using the Kirby-Bauer disc diffusion method with inocula adjusted to an optical density of 0.5 McFarland standard units (**CLSI, 2020**). The antibiotics utilized were amoxicillin (10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), erythromycin (5 µg), gentamycin (10 µg), oxytetracycline (30 µg), streptomycin (10 µg), and tetracycline (30 µg) (Oxoid, UK) embedded disc. The susceptibility tests were conducted according to the guidelines provided by the Clinical Laboratory and Standards Institute (CLSI, 2020), and the MAR indexes for the bacteria isolates were calculated using the results of the disc diffusion method (**Mir et al., 2022**).

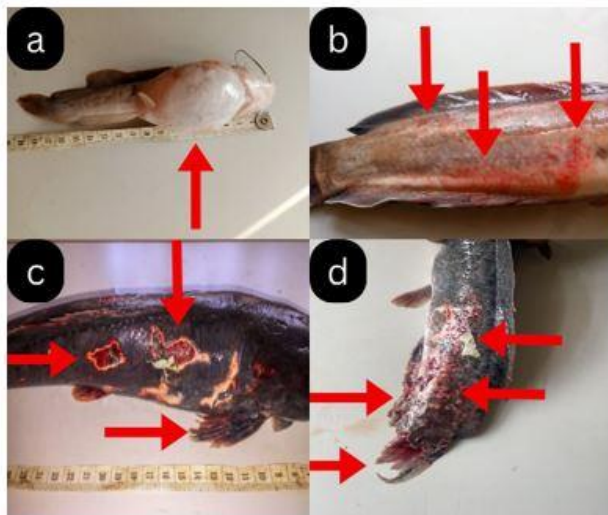
### Data analysis

To calculate absolute frequencies and percentages, data on the isolates were first imported into Microsoft Office Excel version 2016. Using one-way analysis of variance (ANOVA) (SPSS, USA) for Windows version 22.0, the Statistical Package for the Social Sciences, the mean ± standard deviation of the total bacterial count in the water, skin, and GIT from the various types of holding facilities were estimated and compared. Additionally, the percentage of bacteria species resistance was also determined for each antibiotic and the degree of resistance for each antibiotic was compared using the Chi-squared test, and values of  $p < 0.05$  were considered statistically significant.

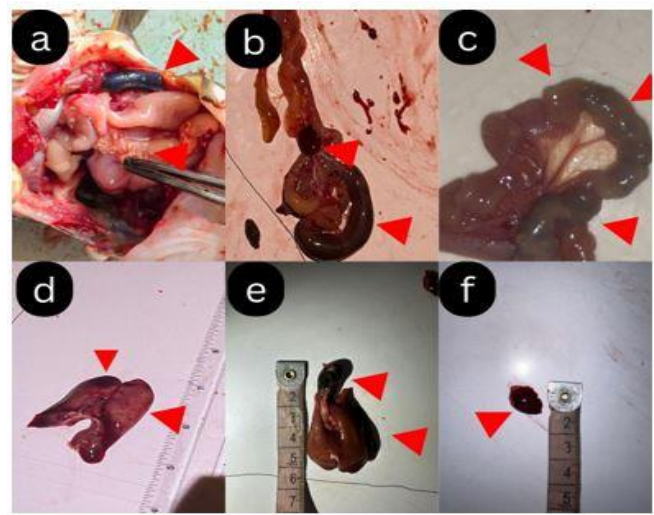
## RESULTS

### Clinical signs and post-mortem findings

On spot examination of moribund *C. gariepinus* samples on the farm, the following were noticed: inappetence, sluggish movements, and dead and moribund fish. Discoloured skin, incomplete barbels, exophthalmia, erosions and severe haemorrhages on the skin, eyes and barbels. Fin rot, white patch, oedema, petechiae haemorrhages and hyperaemia of the abdomen were also observed (**Fig. 1**). The post-mortem examination of the diseased *C. gariepinus* revealed pale gills and whitish necrotic foci; there was enlargement and congestion of the liver, kidney, and spleen; distended gallbladder; and the intestines were haemorrhagic and filled with fluids. The abdomen, in some cases, was enlarged and filled with fluids (ascites) and yellowish-green mucoid in the intestine (**Fig. 2**).



**Fig. 1:** a-d Clinical signs of *Clarias gariepinus* sampled from some selected types of ponds from fish farms in Kaduna State. a) Abdominal drosy in fish from a fish farm in Kaduna state. b) Haemorrhages and pinpoint lesions on the fish from the plastic tank in Kaduna state. c) Ulcers and degeneration of the pectoral fin from a concrete tank in Kaduna state. d) Erosion of the skin and degeneration of the ventral and caudal fin.



**Fig 2:** a-f post-mortem lesion from *Clarias gariepinus* sampled from some selected types of ponds from fish farms in Kaduna State a) distended gall bladder and whitish necrotic foci, b) Enlarged kidney and haemorrhagic intestines filled with fluids c) ballooning of the intestine d) enlarged and congested liver e) distended gall bladder with enlarged liver f) enlarged and congested kidney.

**The total bacterial count in water, skin, and gastrointestinal tract of fish**

The total bacterial count (TBC) in water (log CFU/ml) obtained from the concrete tank ranged from  $4.71 \pm 0.45$  to  $5.80 \pm 0.60$ ; from the skin (log CFU/cm<sup>2</sup>), the values were between  $3.63 \pm 0.01$  and  $5.72 \pm 0.81$ , while the values obtained from the gastrointestinal tract (log CFU/g) ranged from  $4.34 \pm 0.20$  to  $6.41 \pm 0.97$ . The TBC was highest in the GIT, followed by water, and the least observed on the skin. The different farms had a significant difference at  $p < 0.05$  (Table 2).

Table 2: Total bacterial count (TBC) from water, skin, and gastrointestinal tract (GIT) of *Clarias gariepinus* from the different culture facilities

Culture Facilities		Total Bacteria Count (TBC)		
		n (45)	n (75)	n (75)
		Water (Mean ± SD (log CFU ml <sup>-1</sup> ))	Skin (Mean ± SD (log CFU cm <sup>2</sup> -1))	GIT (Mean ± SD (log CFU g-1))
Concrete tanks	Farms			
	1	$4.76 \pm 0.20^a$	$3.63 \pm 0.01^a$	$4.34 \pm 0.20^a$
	2	$5.80 \pm 0.60^b$	$5.72 \pm 0.81^b$	$5.83 \pm 0.20^b$
	3	$5.67 \pm 0.87^c$	$5.63 \pm 0.23^c$	$6.41 \pm 0.97^c$
	4	$4.71 \pm 0.45^d$	$4.69 \pm 0.59^d$	$4.72 \pm 0.15^a$
Earthen ponds	5	$4.80 \pm 0.01^e$	$4.82 \pm 0.15^d$	$5.51 \pm 0.02^d$
	1	$7.88 \pm 0.25^a$	$5.85 \pm 0.77^a$	$5.84 \pm 0.58^a$
	2	$5.66 \pm 0.06^b$	$4.59 \pm 0.62^b$	$4.76 \pm 0.22^a$
	3	$5.79 \pm 0.02^b$	$5.76 \pm 0.52^c$	$5.84 \pm 0.18^a$
	4	$6.68 \pm 0.23^c$	$6.67 \pm 0.08^d$	$7.56 \pm 0.24^b$
Plastic tanks	5	$7.92 \pm 0.24^d$	$6.91 \pm 0.44^e$	$7.97 \pm 0.05^c$
	1	$5.75 \pm 0.79^a$	$5.66 \pm 0.35^a$	$5.16 \pm 0.05^a$
	2	$3.65 \pm 0.10^b$	$3.41 \pm 0.17^b$	$3.52 \pm 0.07^b$
	3	$4.71 \pm 0.33^c$	$4.70 \pm 0.40^c$	$5.51 \pm 0.03^c$
	4	$5.77 \pm 0.32^d$	$4.77 \pm 0.50^d$	$5.68 \pm 0.07^d$
	5	$4.79 \pm 0.48^e$	$4.89 \pm 0.38^d$	$5.51 \pm 0.07^c$

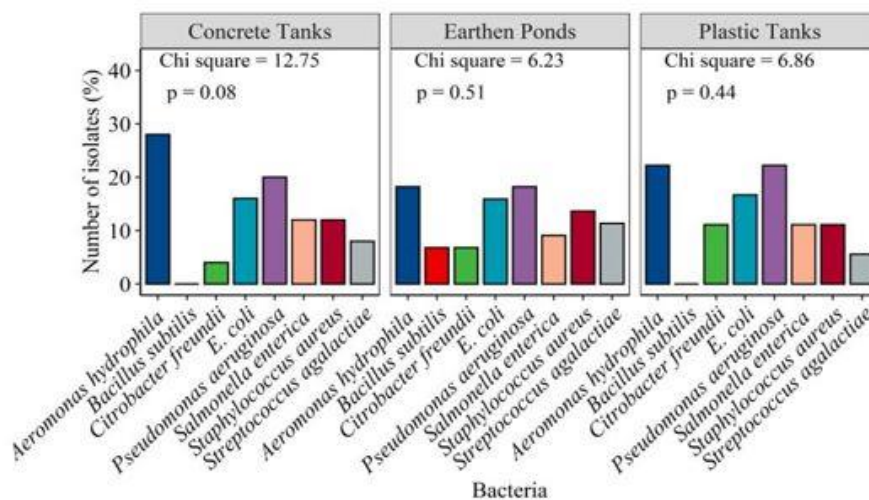
N: Number of samples; Means that are on the same column having different superscript letters (a, b, c, d, and e) are significantly different ( $p < 0.05$ ) (of the same farm category).

The values of TBC obtained from the water samples (log CFU/ml) from the different earthen ponds ranged between  $5.66 \pm 0.06$  and  $7.92 \pm 0.24$ . The values obtained from the skin (log CFU/cm<sup>2</sup>) ranged between  $4.59 \pm 0.62$  and  $6.91 \pm 0.44$ . The values recorded from the GIT (log CFU/g) were  $4.76 \pm 0.22$  and  $7.97 \pm 0.05$ . The TBC was highest in the GIT, followed by water samples, and the least observed on the skin. There was a significant difference at  $p < 0.05$  between the different locations and the different farms (**Table 2**).

The values obtained from water (log CFU/ml) from plastic tanks, on the other hand, varied and ranged from  $3.65 \pm 0.10$  and  $5.77 \pm 0.32$ . The values from the skin (log CFU/cm<sup>2</sup>) ranged between  $3.41 \pm 0.17$  and  $5.77 \pm 0.32$ , while the GIT (log CFU/g) values ranged between  $3.52 \pm 0.07$  and  $5.68 \pm 0.07$ . A similar pattern was observed in the TBC from plastic tanks, with the highest values observed in the GIT, followed by the water, and the lowest values obtained from the skin. Between the various locations and the different farms, there was a significant difference ( $p < 0.05$ ) (**Table 1**). The TBC from the different types of culture facilities and samples (GIT, skin, and water) was highest in earthen ponds, followed by concrete tanks, and the lowest values were obtained from plastic tanks. There was a significant difference between the concrete and plastic tanks and earthen tanks ( $p < 0.05$ ) (**Table 2**).

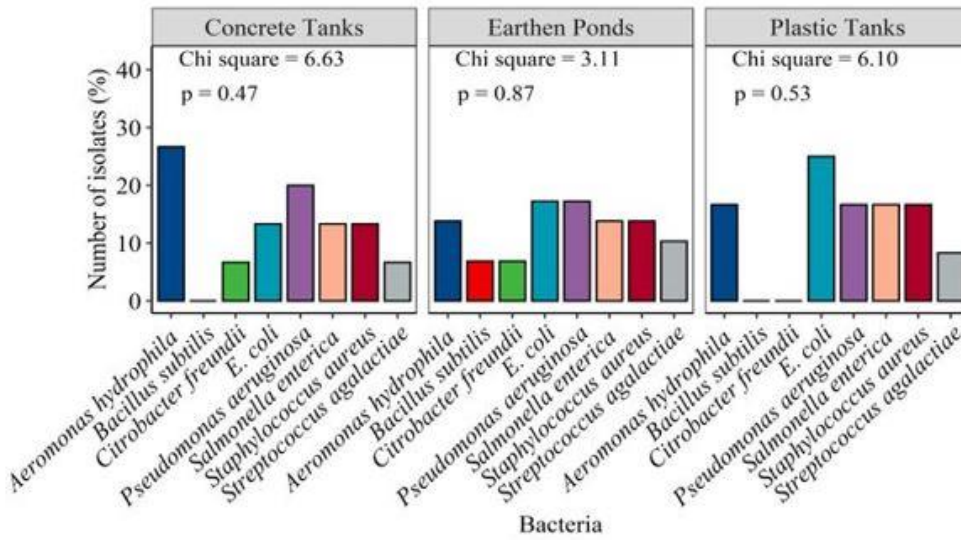
### Bacteria identification and distribution

In total, 220 bacteria isolates were obtained from this study, of which 87 bacteria isolates were obtained from water samples, 56 isolates from the skin, and 77 isolates from GIT of *C. gariepinus* belonging to 8 genera were isolated from the different types of culture facilities. The isolates comprised three genera of Gram-positive bacteria (*B. subtilis*, *S. agalactiae*, and *S. aureus*) and five genera of Gram-negative bacteria (*A. hydrophila*, *C. freundii*, *E. coli*, *P. aeruginosa*, and *S. enterica*). Water samples from earthen ponds had the most bacteria isolates, 44/87 (50.6%), followed by water from concrete tanks, 25/87 (28.7%), and water from plastic tanks, 18/87 (20.7%). Seven common bacteria were isolated from the different types of culture facilities, and *A. hydrophila* was the most prevalent species isolated from concrete tank 7 (28.0%), while *P. aeruginosa* and *A. hydrophila* were the most prevalent species from earthen ponds 8 (18.18%) and plastic tanks 4 (22.22%). The bacteria recovered from the various types of culture environments did not significantly differ from one another (**Fig 3**).



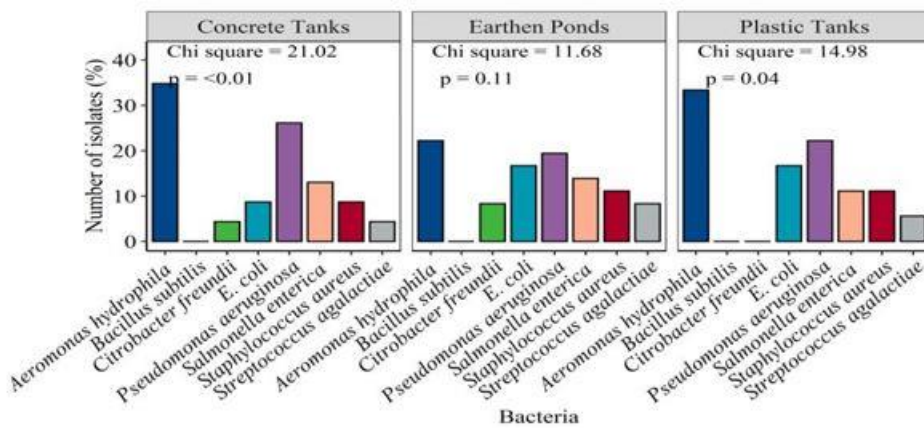
**Fig. 3:** Distribution of the different species of bacteria isolated from water from the different culture facilities.

Skin sampled from the earthen pond had the highest isolates at 29/56 (51.8 %), followed by skin sampled from concrete tanks at 15/56 (26.8 %), and the least was isolated from skin sampled from plastic tanks at 12/56 (21.4 %). The most prevalent species isolated from concrete tanks were *A. hydrophila* 4 (26.71 %), while the most prevalent bacteria from the different earthen ponds were *E. coli* and *P. aeruginosa* 5 (17.24%) and *E. coli* 3 (25 %) from plastic tanks. The bacteria that were isolated from the various holding culture facilities showed no significant difference ( $p < 0.05$ ) (**Fig. 4**). In addition, a total of 36/77 (46.8 %) of the bacteria were isolated from the GIT of fish obtained from the earthen ponds; 23/77 (29.9%) were isolated from the GIT obtained from concrete tanks and the least bacteria isolates of 18/77 (23.4 %) were obtained from the GIT of fish obtained from a plastic tank. The most prevalent organism was *A. hydrophila* from the different culture facilities.

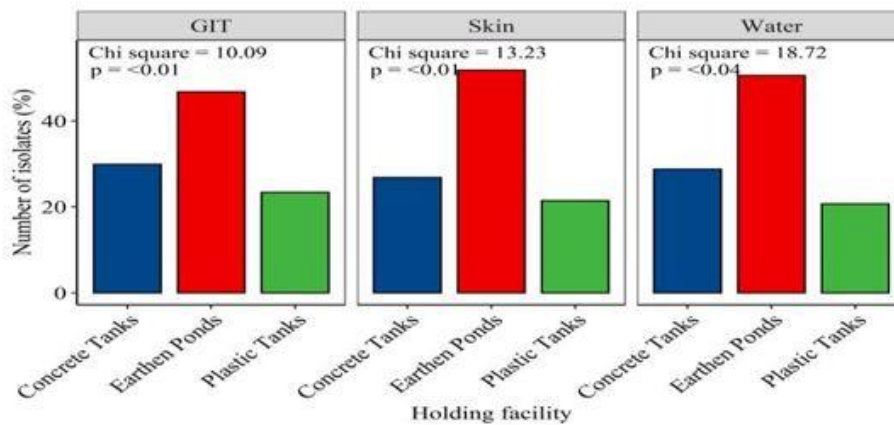


**Fig.4:** Distribution of the bacteria isolates on the skin of the *Clarias gariepinus* from different culture facilities

The bacteria were isolated from both concrete and plastic tanks (**Fig. 5**) and between the different locations (water, skin, and GIT), and from the different culture facilities differed significantly ( $p < 0.05$ ) (**Fig. 6**).



**Fig. 5:** Distribution of the different Bacteria isolates on the gastrointestinal tract of the fish from the different culture facilities

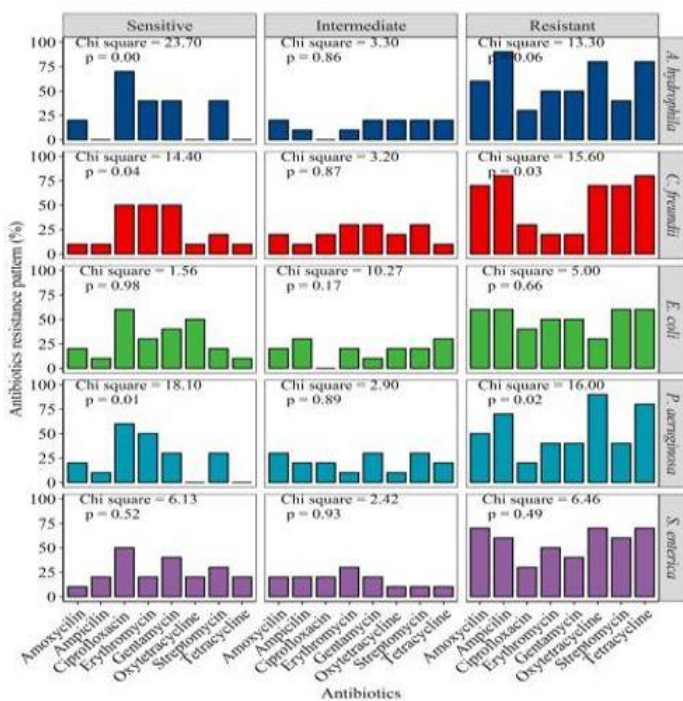


**Fig.6:** Distribution of the diversity of bacteria isolates from *Clarias gariepinus* gastrointestinal tract, skin, and water from the different culture facilities.

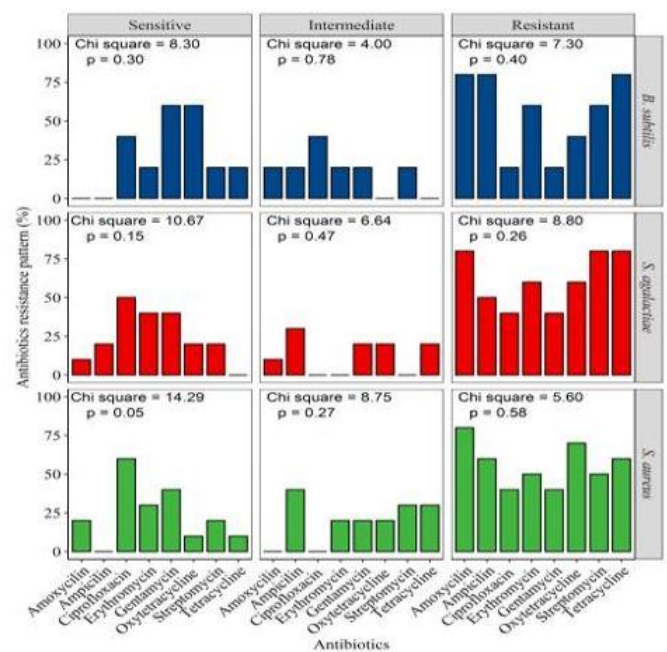
**Antibiotic susceptibility of the bacteria isolates**

The antibiotic-resistant pattern of the 50 Gram-negative bacteria 10 per identified isolate to the eight antibiotics used is shown in **Fig. 7**. Antibiotic resistance in *A. hydrophila* ranged between 30 and 90 %, with the least resistance recorded for ciprofloxacin and the highest resistance observed for ampicillin. The least resistant pattern for *C. freundii* was observed for gentamycin and amoxicillin (20 %), and the highest resistant activity was observed for tetracycline and ampicillin (80 %). But 50 % of the *C. freundii* were susceptible to gentamycin, erythromycin, and ciprofloxacin. The resistance pattern for *E. coli* was between 30 and 60 %, but it was sensitive to ciprofloxacin (60 %) and oxytetracycline (50 %). The *P. aeruginosa* resistant pattern was between 20 and 90 %, with the highest resistance observed for oxytetracycline and the least for ciprofloxacin. However, these *P. aeruginosa* were sensitive to ciprofloxacin (60 %) and erythromycin (50%). Furthermore, these *S. enterica* antibiotic-resistant patterns ranged between 30 and 70 %, with most of the isolates resistant to more than 5 antibiotics tested. The antibiotic resistance patterns for *A. hydrophila*, *C. freundii*, and *P. aeruginosa* differed significantly ( $p < 0.05$ ).

The antibiotic-resistant pattern of the 25 Gram-positive bacteria to the eight antibiotics used is shown in **Fig. 8**: The resistant pattern for *B. subtilis* ranged between 20 and 80 %. However, tetracycline, ampicillin, and amoxicillin showed the highest resistance (80%), whereas gentamycin and ciprofloxacin showed the least resistance (20%). For *S. agalactiae*, the resistance pattern ranged between 40 and 80 %. Amoxicillin, streptomycin, and tetracycline showed the highest resistance, at 80%, while ciprofloxacin and Gentamycin showed the least, at 40%. Additionally, *S. agalactiae* showed resistance to five of the administered drugs. The resistance pattern in *S. aureus* ranged from 40 to 80%. Amoxicillin showed the most resistance at 80%, while ciprofloxacin and Gentamycin showed the least resistance at 40%. Nonetheless, it exhibited 60% ciprofloxacin sensitivity. The antibiotic-resistant pattern for *S. aureus* sampled differed significantly ( $p < 0.05$ ).



**Fig. 7:** Antibiogram of Gram-Negative bacteria isolates from diseased farmed *Clarias gariepinus*.



**Fig. 8:** Antibiotic resistance patterns of Gram-positive bacteria isolated from diseased farmed *Clarias gariepinus*

The multiple antibiotic resistance index value of the bacteria isolated from the different types of culture environments ranged between 0.63 and 0.75, with the highest MAR value recorded for *E. coli*, *A. hydrophila*, *S. enterica*, *B. subtilis*, and *S. aureus* isolated in this study (**Table 3**).

Table 3: Multiple antibiotic resistance (MAR) index for bacteria isolated from diseased *Clarias gariepinus*.

Bacteria species	Number of ineffective antibiotics	Multiple antibiotic resistance (MAR) Index
<i>Escherichia coli</i>	6	0.75
<i>Citrobacter freundii</i>	5	0.63
<i>Pseudomonas aeruginosa</i>	5	0.63
<i>Aeromonas hydrophila</i>	6	0.75
<i>Salmonella enterica</i>	6	0.75
<i>Bacillus subtilis</i>	6	0.75
<i>Streptococcus agalactiae</i>	5	0.63
<i>Staphylococcus aureus</i>	6	0.75

## DISCUSSION

Several clinical signs have been associated with diseases of cultured *C. gariepinus* worldwide, and this study has recorded Gram-positive and Gram-negative bacteria responsible for these infections. These findings have also been recently reported by **Kozlov et al., (2021)** in catfish, **Nadia et al., (2019)** in *Oreochromis niloticus*, and **Mekasha and Linke, (2021)** in *Danio rerio*. The presence of these clinical signs and post-mortem lesions recorded in the fish samples are indications of bacterial disease infection (**Kozlov, et al., 2021; Okon et al., 2023**). The total bacterial load is a broad indicator of bacterial contamination, which is affected by hygienic conditions and management practices on fish farms (**Ogbukagu et al., 2021**). This study showed that the bacterial load varied from the water, skin, and GIT from the various types of culture facilities and the GIT was higher than those observed in the water and on the skin. Similarly, **Ajani et al., (2016)** and **Nwankwo and Akani (2017)** reported that heterotrophic bacteria populate the GIT of *C. gariepinus* in higher numbers than those found in other parts of the fish and pond water. The bacteria that enter the GIT with the fish's feed during ingestion may have adapted and created a symbiotic relationship within the GIT, suggesting that these organisms can thrive in the GIT.

The values of total bacteria count in water, skin, and GIT observed are consistent with the findings of **Amuneke et al., (2020)**, but a higher result was obtained by **Ajani et al., (2016)** and **Efiong and Isaac (2019)**. Nevertheless, the result obtained from this study was also lower than the values reported by **Nwankwo and Akani (2017)** and **Jesumirhewe and Ibeh (2020)**. This may be due to different management practices on the farms.

The TBC in the water, skin, and GIT of *C. gariepinus* cultured in earthen ponds were higher than that of concrete and plastic tanks. The difference in value may be attributed to the water sources used in earthen ponds, which are untreated surface water derived from dams, streams, rivers, lakes, and runoff water, whereas most concrete and plastic tanks use subsurface water (**Adah et al., 2021**). Furthermore, the occasional application of organic manure in earthen ponds has been linked with the buildup of organic matter, thereby leading to an increase in bacteria load (**Omorogiuwa, 2019**).

The various types of bacterial isolates recovered from the diseased *C. gariepinus* and water from the various holding facilities in the study are similar to the reports of **Dirisu et al., (2017)** and **Wise et al., (2021)**. However, Gram-negative bacteria were observed to be the most isolated bacteria species from *C. gariepinus*, showing clinical signs and post-mortem lesions; this was similar to the findings of **Pękala-Safińska (2018); Nwankwo and Akani (2017)** and **Dirisu et al., (2017)**. However, this result differed from the finding of **Al-Taee et al., (2024)** who found 50% of both Gram positive and Gram-negative bacteria was obtained in caged carp fish. This finding may be attributed to differences in the culture facility, species of fish and location of sampling.

The bacteria isolated in this study are the common pathogenic bacteria associated with the clinical manifestation observed in the fish and are capable of causing disease leading to mortality and great economic loss (**Junior et al., 2018; Adah et al., 2021; Duman et al., 2021; Santos et al., 2021**). **Del Rio-Rodriguez et al., 1997** and **El-Gendy et al., 2024**, showed the survival ability of *E. coli* in fish intestines frequently utilized as a pollution indicator in water. Recently, **Ava et al., (2020)** also reported the presence of *E. coli* in fish and water in Bangladesh.

*Aeromonas* and *Pseudomonas* species are considered to be the most important pathogenic bacteria responsible for ulcerative syndrome, hemorrhagic septicaemia, tail and fin rot, bacterial gill rot, and dropsy, as observed in this study (**Junior et al., 2018**). There has been an increased incidence of diseases caused by *C. freundii* in fish farms, and antibiotic-resistant *C. freundii* has been found in aquatic environments that were heavily impacted by human activities and hence cause a risk to public health and constitute a reservoir for antibiotic resistance (**Guma et al., 2022**). *Streptococcus agalactiae* and *Staphylococcus aureus* have also been associated with anorexia, display disoriented whirling motion, exophthalmia, and haemorrhages on the eyes, operculum, around the



mouth and barbels leading to mortality (Duman *et al.*, 2021). Santos *et al.*, 2021; Abd El Tawab *et al.*, 2022). There was co-infection of the bacteria isolated from the diseased fish; thus, it was difficult to link a specific or single bacteria isolate to the disease observed in this study.

Furthermore, regardless of the different culture facilities, there was an association between the bacteria species isolated from the water and the different fish parts sampled. This suggests that the various types of culture environments may impact the health of aquatic species and the makeup of the bacteria in fish (Adah *et al.*, 2021).

Antibiotic resistance in fish culture has been widely documented, particularly in farms with a history of antibiotic usage (Jama *et al.*, 2020). The antibiogram in this study showed that the pathogens are multi-resistant to the antibiotics used and varied significantly. The Gram-negative bacteria were susceptible to ciprofloxacin. Gram-positive bacteria were susceptible to ciprofloxacin and gentamycin. The findings of the present study regarding resistance to antibiotics are in agreement with the reports of Agoba *et al.*, (2017); Gufe *et al.*, (2019); Omorogiuwa (2019) and Jama *et al.*, (2020). The bacteria's susceptibility to ciprofloxacin and gentamycin may be due to their less frequent use in fish farming.

However, both Gram-negative and Gram-positive bacteria were resistant to amoxicillin, ampicillin, oxytetracycline, and tetracycline, as reported by Apenteng *et al.*, (2017); Agoba *et al.*, (2017), and Adah *et al.*, (2020). The widespread and irrational use of over-the-counter antibiotics, ampicillin, oxytetracycline, and tetracyclines, which have been the gold standard of antimicrobial therapy, may have been the cause of the high resistance of the bacterial species (Prestinaci, *et al.*, 2015; Manyi-Loh *et al.*, 2018).

As a result, only a few antibiotics may be effectively used to treat bacterial disease outbreaks on fish farms (Pepi and Focardi, 2021). More specifically, the increase in multidrug-resistant bacteria is a serious public health concern and requires effective policies and implementation plans to address these issues. Antibiotic resistance spread quickly among aquatic microbial communities and then to human pathogenic bacteria, rendering antibiotic usage for human health ineffective (Manyi-Loh *et al.*, 2018).

## CONCLUSION

This study reported the varying clinical signs of bacterial disease, TBC, and the presence of 8 pathogenic bacteria genera comprising of *B. subtilis*, *S.*

*agalactiae*, *S. aureus*, *A. hydrophila*, *C. freundii*, *E. coli*, *P. aeruginosa*, and *S. enterica* from the water, skin and gastrointestinal tract of diseased farmed *Clarias gariepinus*. Also, antibiotic resistance was widespread in this study, with potential consequences on the environment, public health, and the global community. This study has therefore established the need for the identification and monitoring of pathogens, AMR, and usage of antibiotics in fish farming and the adoption of proper biosecurity measures in aquaculture facilities. This will boost productivity and contribute significantly to the production of wholesome, healthy fish.

## Conflict of Interests

The authors declare that there is no conflict of interest

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## Authorship contributions

All authors contributed to the idea and design of the study. Material preparation and investigation were performed by Adah Arimie Deborah and Adah Adakole Sylvanus. The writing/editing was carried out by Adah Arimie Deborah and all authors have read and approved the article

## Data availability

All relevant data is in the article.

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