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# Occurrence and antibiogram of chloramphenicol-resistant bacteria in fishponds within Obio-Akpor local government area in Nigeria

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**Abstract**
Use of antibiotics in fishponds may increase occurrence of antibiotic-resistant bacteria thereby posing public health risk. The aim of this study was to determine the occurrence and antibiogram of chloramphenicol-resistant bacteria from fishponds in some communities within Obio/Akpor Local Government Area of Nigeria. Water samples were collected from selected fishponds, and inquiry made into the means of treatment of the sampled fishponds. Samples were analyzed for total heterotrophic bacteria (THB) and Chloramphenicol-resistant bacteria (CRB) using standard methods. Percentage occurrences of CRB in the fishponds were determined, and the CRB were identified through morphological/biochemical means. Identified CRB were subjected to antibiotic susceptibility testing. The results obtained revealed that antibiotics were used in treatment of 64% of the fishponds sampled. THB ranged from $1.33\pm1.87\times10^{10}$ to $3.11\pm0.91\times10^{14}$ CFU/ml; CRB population ranged from $4.88\pm4.92 \times 10^{2}$ to $3.32\pm7.26 \times 10^{6}$ CFU/ml; the occurrence of CRB ranged from $4.83\pm4.80\times10^{-10}$ to $0.12\pm0.29\%$. Correlation between occurrence of CRB and percentage of fishponds treated with antibiotics (FPAB) showed that there was no correlation between them ($r$ = 0.2). The identified CRB included *Vibrio vulnificus*, *Pseudomonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Macrococcus lamae*, *Streptococcus* spp., *Neisseria animaloris*, *Shimwellia pseudoproteus*, *Serratia* spp., and *Proteus hauseri*. Among these, *Shimwellia pseudoproteus* was the most resistant CRB, and most of the CRB were more sensitive to Gentamicin. It is concluded that the presence of antibiotic-resistant bacteria in fishponds may not be due to the use of antibiotics in fishponds, and Gentamicin could be a drug of choice for treatment of infections caused by antibiotic-resistant bacteria.

**Keywords:** Antibiotic-resistant bacteria, chloramphenicol-resistant bacteria, occurrence/incidence of antibiotic-resistance, fishponds, gentamicin

## Introduction
Many antibiotics including enrofloxacin, oxytetracycline, amoxycillin, and sulphadiazine-trimethoprim are frequently used in aquaculture to treat or prevent fish diseases (Chen *et al*., 2022; Miao & Wang, 2020; Pham *et al*., 2015) [4, 16, 23]. Prophylactic antibiotic use to prevent diseases during aquaculture production is on an increasing trend (Miao & Wang, 2020) [16]. Use of antibiotics in fishponds can lead to inducement of antibiotic resistance in bacteria present in the fishponds, and can contribute to increase in occurrence of antibiotic-resistant bacteria. Antibiotic resistance can spread among aquatic microbial communities, and can reach human pathogenic bacteria thereby making vain the use of antibiotics in treatment of infections in humans (Kümmerer, 2009) [15]. Irrational and unrestricted use of antibiotics in aquaculture may contribute to antimicrobial resistance emergence, which may pose a severe threat to human and animal health worldwide.

Pathogenic bacteria in fishponds can be transmitted to humans during handling and processing. An estimated 125 different bacterial species belonging to 34 different bacterial families has been reported to cause various fish diseases as well as infecting humans (Öztürk & Altınok, 2014) [21]. There is growing indication that pathogens among bacteria found in fishponds is widening and becoming more prevalent (Henriksson, 2018) [13]. This implies that fish handlers are becoming more predisposed to fish pathogens. Fish handlers and personnel in fish farming facilities can be exposed to pathogenic bacteria in the fishponds through direct contact with contaminated water, fish, or equipment. Open wounds or cuts on the skin can become entry points for the pathogens, leading to infections. Where the pathogens are antibiotic-resistant bacteria (ARB), then there is eminent danger in the spread of ARB among the human population, thereby posing a significant public health risk.

Use of antibiotics in fishponds may increase the population of ARB in fishponds. Increase in population of ARB in fishponds implies that the fish handlers may become more predisposed to contact with ARB. This poses a public health risk, since infection of the fish handlers with ARB can lead to spread of ARB among the human population. Hence, there is need to compare the occurrence of ARB in fishponds with the use of antibiotics in fishponds, and to determine the antibiotic susceptibility of ARB to different antibiotics. Screening for antibiotics that are still effective against various ARB will be helpful in identifying the antibiotics that can be selected as drug of choice in treating infections caused by ARB.

## Materials and Methods

### Assessment of antibiotic use in fishponds in the Study Area

The study area was within Obio-Akpor LGA of Rivers State in Nigeria, and included the following communities: Rukpokwu, Rumuagholu, Nkpolu-Rumuigbo, Atali, Agbada, and Iwofe. The location and proximity of these communities as geo-referenced with the aid of a calibrated compass and map plotting tools available at https://www.mapcustomizer.com/ is presented in Figure 1. On getting to a/some fishpond(s) location, an introduction and purpose of the research was explained to the fish-fishpond owners/managers, and questionnaires given so as to obtain information on the use of antibiotics in the fishponds.

### Sample Collection

Sampling was carried out in a stratified random sampling manner, and the number of fishponds sampled in each location was dependent on the total number of fishponds available in the location. In total, water samples were collected from 50 fishponds out of 298 fishponds encountered; leading to a sample size of 16.8% of the total number of fishponds encountered (Table 1). Samples were collected using disinfected plastic water bottles (200 ml capacity), and transported in an ice box to the Microbiology Laboratory in Rivers State University, Nigeria, for analyses.

### Enumeration of bacteria in the fishponds

The populations of total heterotrophic b**acteria** (THB) and chloramphenicol-resistant bacteria (CRB) in the water column of the fishponds were determined using Nutrient Agar (NA) and NA supplemented with Chloramphenicol (30 µg/ml). Turbid water samples were serially diluted to $10^{-12}$ dilution through 10-fold serial dilution, and then 0.1 ml of $10^{-10}$ to $10^{-12}$ dilutions were inoculated on NA plates using the spread plate technique; lightly turbid samples were serially diluted to $10^{-6}$, and then 0.1 ml of $10^{-4}$ to $10^{-6}$ dilutions were inoculated on NA plates; clear samples were serially diluted to $10^{-4}$, and then 0.1 ml of $10^{-2}$ to $10^{-4}$ dilutions were inoculated on NA plates; 0.1 ml of all the samples (undiluted and $10^{-1}$ dilution) was inoculated on NA supplemented with Chloramphenicol (NAC). Inoculated NA plates were incubated at 35 °C for 24 hours, while inoculated NAC plates were incubated at ambient temperatures (27 – 32 °C) for 48 hours. After incubation, ensuing colonies on NA and NAC plates were counted and used to calculate the population of THB and CRB respectively.

### Isolation and Identification of Chloramphenicol-Resistant Bacteria

Colonies on NAC plates were isolated and sub-cultured onto sterile NA plates and coded. The coded isolates were subjected to morphological, physiological, and biochemical tests used in identification of bacteria. The tests included Gram staining & microscopic examination, Catalase, Oxidase, motility, citrate utilization, Indole production, Methyl red, Vogues-Proskauer, 7% salt (NaCl) tolerance, casein hydrolysis, starch hydrolysis, haemolysis, Lecithinase production, lipase production, and fermentation tests using glucose, lactose, maltose, Mannitol, sucrose, Xylose, & glycerol. The tests were carried out as described by Peekate (2022) [22].

### Antibiotic susceptibility testing of Chloramphenicol-Resistant Bacteria

Identified Chloramphenicol-Resistant Bacteria (CRB) were subjected to antibiotic susceptibility testing using the well in agar method (Balouiri *et al*., 2016) [3]. The antibiotics and their quantity used as prescribed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were Ampicilin – 10 µg, Ampiclox – 30 µg, Chloramphenicol – 30 µg, Ciprofloxacin – 5 µg, Erythromycin – 15 µg, Gentamicin – 10 µg, Ofloxacin – 5 µg, Streptomycin – 10 µg, and Tetracycline – 30 µg (EUCAST, 2023) [9]. Solutions of the antibiotics were prepared such that their respective quantity will be available in a delivery volume of 40 µl. Broth cultures of the CRB were prepared by inoculating their colonies into 7 ml sterile nutrient broth. The inoculated broths were incubated at 35 °C for about 24 hours. After incubation, the absorbance of the broth cultures was measured and compared with the absorbance of a freshly prepared 0.5 McFarland standard (prepared as outlined in the Clinical and Laboratory Standards Institute manual; CLSI, 2012). Sterile normal saline was added to the broth cultures so as to adjust their absorbance to the absorbance of the McFarland standard. Absorbance measurements were achieved with the aid of a spectrophotometer (721 VIS Spectrophotometer, Huanghua Faithful Instrument Co. Ltd; China) set at 600 nm. The standardized broth cultures were spread inoculated, onto sterile Mueller-Hinton agar (MHA) plates with the aid of sterile swab sticks. The MHA plates were prepared such that the agar thickness was about 7 – 8 mm. Equidistant wells of about 6 mm in diameter were bored into inoculated MHA with the aid of sterile cork borer. Aliquots of 40 µl of the different antibiotic solutions were placed separately into the wells with the aid of an automatic micro-pipette. The plates were allowed in the upright position for 30 minutes for adsorption of the antibiotic solutions into the agar. Then the plates were incubated in inverted position at 35 °C for 24 hours. After incubation, zones of inhibitions around the wells were measured and recorded.

### Data Analysis

The *t* test statistics and Analysis of Variance (ANOVA) were used to determine significant difference in the biological data obtained from the various analyses.

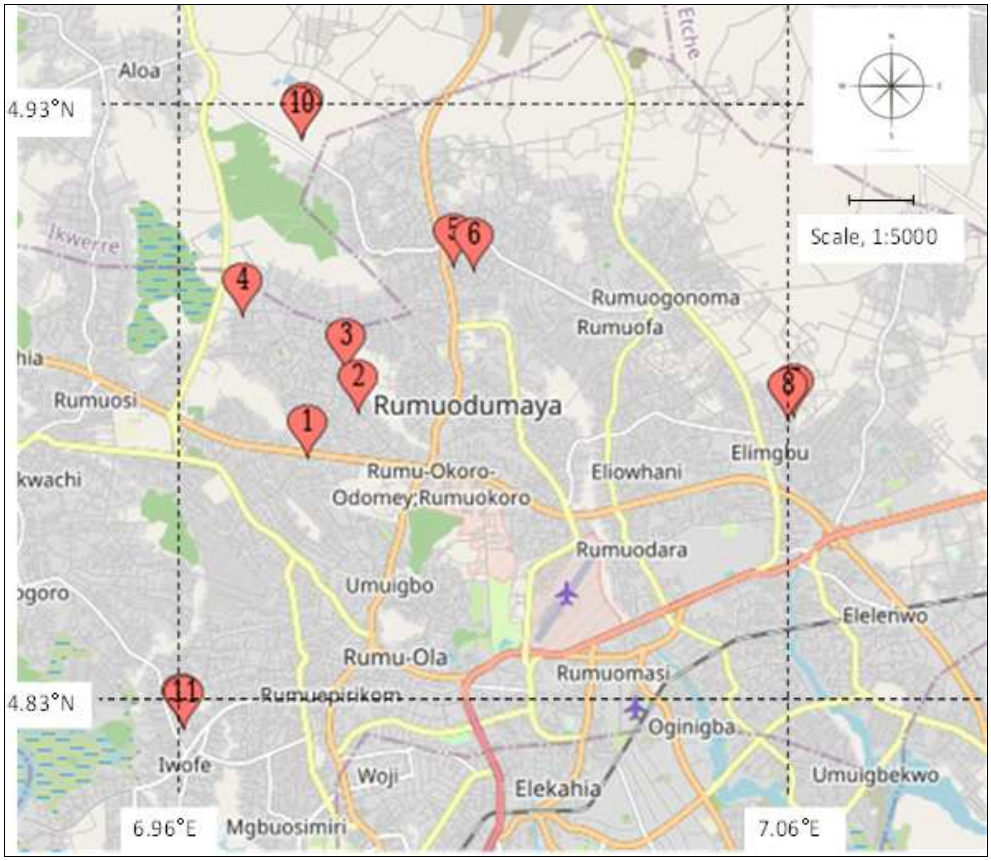


**Fig 1:** Location and proximity of the sampled fishponds in the study area.

**Table 1:** Sampling location and Number of Fishponds Encountered

| N | Community | LWSC (code) | NFE | NFS | PPES (%) |
|---|---|---|---|---|---|
| 1 | Rukpokwu | SARS road (1.1) | 11 | 5 | 45.5 |
| | | Rukpokwu1 (1.2) | 10 | 5 | 50.0 |
| | | Rukpokwu 2 (1.3) | 4 | 2 | 50.0 |
| 2 | Rumuagholu | Rumuagholu (2.0) | 14 | 6 | 42.9 |
| 3 | Rumuigbo | Nkpolu (3.0) | 5 | 3 | 60.0 |
| 4 | Atali | Atali (4.1) | 200 | 10 | 5.0 |
| | | Palace road (4.2) | 15 | 5 | 33.3 |
| 5 | Agbada | Agbada (5.0) | 25 | 6 | 24.0 |
| 6 | Iwofe | Iwofe (6.0) | 14 | 8 | 57.1 |
| Total | | | 298 | 50 | 16.8 |

LWSC: Location within sampled community, NFE: Number of fish-fishponds encountered, NFS: Number of fish-fishponds sampled, PPES: proportion of total number of fishponds encountered that was sampled.

## Results

### Antibiotic use in fishponds in the Study Area

Collation of the responses obtained from the fishpond owners/managers showed that antibiotics were used in treatment of 64% of the fishponds sampled (Table 2). The minimum frequency of antibiotic treatment was once in a month, while the maximum was once a week; once in a month was carried out in 27 fishponds (54%), while once a week was carried out in 5 fishponds (10%). The antibiotics used included Ampiclox, Chloramphenicol, and Tetracycline.

### Bacterial populations in the fishponds

The population of total heterotrophic bacteria (THB) in the fishponds across the sampled locations ranged from $1.33\pm1.87 \times 10^{10}$ to $3.11\pm0.91 \times 10^{14}$ CFU/ml; with the mean lowest population in fishponds in Rukpokwu 2, and the mean highest population in fishponds in Nkpolu-Rumuigbo (Figure 2). On the other hand, the population of chloramphenicol-resistant bacteria (CRB) ranged from $4.88\pm4.92 \times 10^{2}$ to $3.32\pm7.26 \times 10^{6}$ CFU/ml; with the mean lowest in fishponds around SARS road, and the mean highest in fishponds in Rukpokwu 1.

The percentage of THB that were CRB in the fishponds across the sampled locations ranged from $4.83\pm4.80 \times 10^{-10}$ to $0.12\pm0.29\%$ (Figure 3); with the mean lowest in fishponds in Nkpolu-Rumuigbo, and the mean highest in fishponds in Rumuagholu.

### Correlation of percentages of CRB and fishponds treated with antibiotics

Calculation of the correlation between percentage of THB that were CRB (PCRB) and percentage of fishponds treated with antibiotics (FPAB) in the different sampled locations showed that there was no correlation between PCRB and FPAB ($r = 0.2$).

### Identity of the Chloramphenicol-resistant bacteria

The morphological and physiological characteristics of the chloramphenicol-resistant bacteria (CRB) isolated from the fishponds across the sampled locations are presented in Table 3a, while the results of the biochemical tests carried out on them are presented in Tables 3b and 3c. Use of combination of morphological & physiological characteristics and results from the biochemical tests in searching the database of ABIS (https://www.tgw1916.net/bacteria_abis.html) revealed that the CRB possibly included the following bacteria: *Vibrio vulnificus*, *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Staphylococcus saprophyticus*, *Staphylococcus xylosus*,

*Bacillus coagulans*, *Bacillus subtilis*, *Micrococcus lamae*, *Streptococcus sobrinus*, *Streptococcus porcinus*, *Streptococcus iniae*, *Neisseria animaloris*, *Shimwellia pseudoproteus*, *Serratia odorifera*, *Serratia ficaria*, and *Proteus hauseri*.

**Antibiotic Susceptibility Pattern of the Chloramphenicol-Resistant Bacteria**

Antibiotic susceptibility results of the Chloramphenicol-resistant bacteria (Table 4) revealed that they were all resistant to Chloramphenicol. Also, the results revealed that out of the 18 Chloramphenicol-resistant bacteria (CRB), Tetracycline had no effect on 12 of them; Ciprofloxacin had no effect on 11 of them; Ofloxacin had no effect on 10 of them; Ampicilin, Ampiclox, and Erythromycin had no effect on 7 of them; Streptomycin had no effect on 5 of them; and Gentamicin had no effect on 3 of them. This means that Gentamicin is the most effective antibiotic against the chloramphenicol resistant bacteria. On the other hand, the most resistant CRB was *Shimwellia pseudoproteus*; it showed resistance to all the antibiotics.

**Table 2:** Means of fishpond treatment

| Location | N | FPAB (%) | FABU |
|---|---|---|---|
| SARS road | 5 | 5 (100) | Once in a month |
| Rukpokwu 1 | 5 | 5 (100) | Weekly |
| Rukpokwu 2 | 2 | 0 (0) | 0 |
| Rumuagholu | 6 | 5 (83.3) | Once in a month |
| Nkpolu, Rumuigbo | 3 | 0 (0) | 0 |
| Atali | 10 | 10 (100) | Once in a month |
| Atali, palace road | 5 | 0 (0) | 0 |
| Agbada | 6 | 6 (100) | Once in a month |
| Iwofe | 8 | 1 (12.5) | Once in a month |
| Total | 50 (100%) | 32 (64%) | |

N: Number of fishponds, FPAB: number of fishponds in which antibiotics is used, FABU: Frequency of antibiotic use.

**Table 3a:** Morphological and Physiological characteristics of the CRB isolates

| IC | COFIF | ACN | GS/MP | MT | ST |
|---|---|---|---|---|---|
| 1K1 | 1.1, 1.3, 2.0, 4.2, 5.0, 6.0 | 1 | - rods | + | - |
| H3 | 1.1, 1.2, 3.0, 4.1, 4.2, 5.0, 6.0 | 2 | - rods | + | - |
| J2 | 1.1, 1.2, 3.0, 4.1, 4.2, 6.0 | 3 | + cocci | - | + |
| 1L2 | 1.1, 1.2, 2.0, 3.0, 4.2, 5.0, 6.0 | 4 | + rods | + | - |
| 1P1 | 1.1, 1.2, 2.0, 3.0, 4.2, 5.0, 6.0 | 5 | +cocci | + | + |
| 1G2 | 1.1, 1.2, 2.0, 3.0, 4.2, 5.0, 6.0 | 5 | +cocci | + | - |
| R2 | 1.1, 1.2, 4.1, 4.2, 6.0 | 6 | + cocci | - | + |
| C2 | 1.2, 3.0, 5.0, 6.0 | 7 | + rods | + | + |
| K1 | 1.2 | 8 | - rods | + | - |
| K2 | 1.2, 2.0 | 9 | - cocci | - | + |
| 1R2 | 1.3, 2.0 | 10 | - rods | + | - |
| 1V4 | 1.3, 5.0 | 11 | + rods | + | + |
| M1 | 2.0 | 12 | + cocci | - | - |
| C1 | 3.0, 4.1, 5.0, 6.0 | 13 | + cocci | - | - |
| P4 | 4.1 | 14 | - rods | + | - |
| J1 | 4.1, 4.2, 6.0 | 15 | + rods | + | - |
| 1E3 | 4.1, 4.2, 6.0 | 15 | - rods | + | - |
| Y2 | 5.0, 6.0 | 16 | + cocci | - | + |

IC: Isolate-code, COFIF: Code of fishponds where isolate was found, ACN: Assigned Cluster-number, GS/MP: Gram stain reaction/Morphology, MT: Motility, ST: Salt tolerance

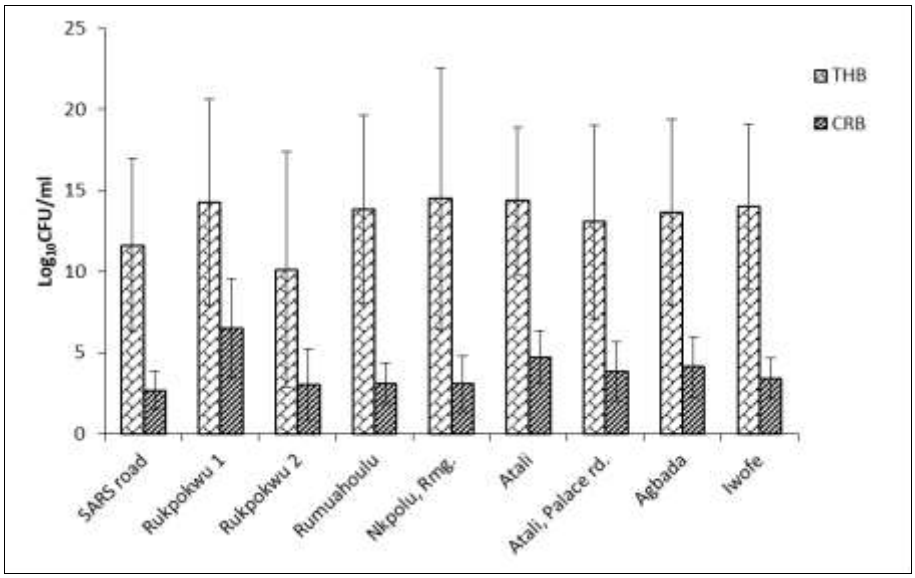


**Fig 2:** Total heterotrophic bacteria (THB) and chloramphenicol resistant bacteria populations in fishponds from the different locations

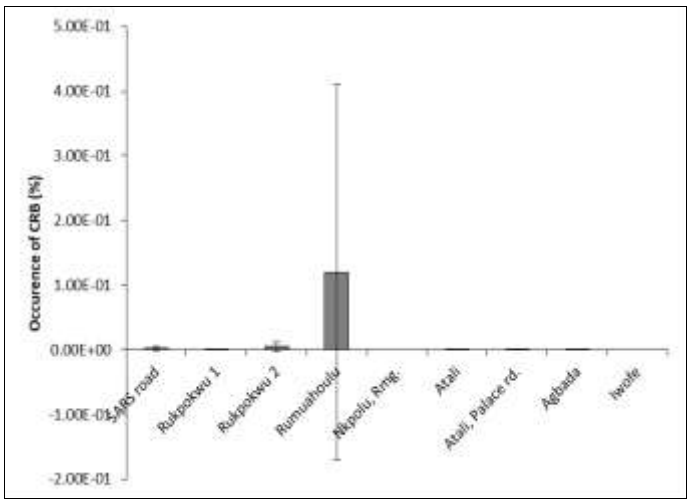


**Fig 3:** Proportion of THB that were CRB in the fishponds in the different locations.

**Table 3b:** Biochemical characteristics of the CRB isolates

| ACN/IC | CT | OX | IP | CU | MR | VP | HM | SH | CH | LC | LP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1/1K1 | - | + | + | - | + | - | .γ | + | - | + | + |
| 2/H3 | + | + | - | + | - | - | .β | - | - | + | + |
| 3/J2 | + | - | - | - | - | - | .γ | - | - | - | - |
| 4/1L2 | + | - | - | - | - | + | .γ | + | - | + | - |
| 5/1P1 | + | - | + | - | + | - | .γ | + | - | - | - |
| 5/1G2 | + | - | + | - | - | + | .γ | + | - | + | - |
| 6/R2 | - | + | + | - | - | - | .β | - | - | + | + |
| 7/C2 | + | + | - | + | - | - | .β | + | - | + | + |
| 8/K1 | + | + | - | - | - | + | .γ | - | - | - | - |
| 9/K2 | + | + | - | - | + | - | .γ | + | - | + | + |
| 10/1R2 | + | - | - | - | - | - | .γ | - | - | + | - |
| 11/1V4 | + | - | + | + | - | - | .γ | - | - | + | + |
| 12/M1 | - | - | + | - | - | - | .β | + | - | - | - |
| 13/C1 | - | - | - | + | + | - | .γ | + | - | + | - |
| 14/P4 | + | - | - | + | - | - | .γ | + | - | + | + |
| 15/J1 | + | - | - | - | - | - | .γ | + | - | - | - |
| 15/1E3 | + | - | + | - | + | - | .γ | + | - | - | - |
| 16/Y2 | + | - | + | + | - | - | .γ | - | - | + | + |

ACN/IC: Cluster-number/Isolate-code, CT: Catalase, OX: Oxidase, IP: Indole production, CU: Citrate utilization, MR: Methyl red, VP: Voges-Proskauer, HM: Haemolysis, SH: Starch hydrolysis, CH: Casein hydrolysis, LC: Lecithinase production, LP: Lipase production

**Table 3c:** Biochemical characteristics (sugar fermentation) of the CRB isolates

| ACN/IC | GF | LF | MlF | MnF | SF | XF | GyF | SB.ABIS (% similarity) |
|---|---|---|---|---|---|---|---|---|
| 1/1K1 | A | - | A | - | - | - | - | *Vibrio vulnificus* (92) |
| 2/H3 | - | - | - | - | - | - | A | *Pseudomonas fluorescens* (89) |
| 3/J2 | A | - | A | A | A | - | A | *Staphylococcus saprophyticus* (93) |
| 4/1L2 | A | - | A | A | A | - | - | *Bacillus coagulans* (73.2) |
| 5/1P1 | A | - | A | A | A | - | - | *Macrococcus lamae* (92) |
| 5/1G2 | A | - | A | A | A | - | - | *Streptococcus sobrinus* (86) |
| 6/R2 | A | - | A | A | - | - | - | *Streptococcus porcinus* (67) |
| 7/C2 | A | - | A | A | - | - | - | *Bacillus subtilis* (84) |
| 8/K1 | A | - | - | - | A | - | - | *Pseudomonas fragi* (85) |
| 9/K2 | A | - | - | - | - | - | - | *Neisseria animaloris* (98) |
| 10/1R2 | A | - | A | - | - | A | - | *Shimwellia pseudoproteus* (90) |
| 11/1V4 | A | - | A | A | A | A | - | *Serratia odorifera* (85) |
| 12/M1 | A | - | A | A | A | - | - | *Streptococcus iniae* (99) |
| 13/C1 | A | - | - | A | A | - | - | *Streptococcus iniae* (83) |
| 14/P4 | A | - | A | A | A | A | - | *Serratia ficaria* (88) |
| 15/J1 | A | - | A | - | - | - | - | *Bacillus coagulans*(84) |
| 15/1E3 | A | - | A | - | A | A | - | *Proteus hauseri* (93) |
| 16/Y2 | A | A | A | A | A | A | - | *Staphylococcus xylosus* (85) |

ACN/IC: Assigned Cluster-number/Isolate-code, GF, LF, MlF, MnF, SF, XF, & GyF: Glucose, Lactose, Maltose, Mannitol, Sucrose, Xylose, and Glycerol fermentation respectively, SB.ABIS: Suspected bacteria as determined using ABIS online tools.

**Table 4:** Growth inhibition of the Chloramphenicol-resistant Bacteria by Antibiotics

| CRB (IC) | APN | APX | CHL | CIP | ERY | GEN | OFL | STR | TET |
|---|---|---|---|---|---|---|---|---|---|
| | Zone of inhibition (mm) | | | | | | | | |
| *Vibrio vulnificus* (1K1) | 20 | 16 | 0 | 0 | 0 | 0 | 0 | 18 | 0 |
| *Pseudomonas fluorescens* (H3) | 15 | 12 | 0 | 34 | 32 | 36 | 35 | 35 | 10 |
| *Staphylococcus saprophyticus* (J2) | 22 | 24 | 0 | 13 | 32 | 26 | 25 | 22 | 30 |
| *Bacillus coagulans* (1L2) | 0 | 0 | 0 | 0 | 10 | 24 | 0 | 17 | 0 |
| *Macrococcus lamae* (1P1) | 27 | 25 | 0 | 0 | 28 | 22 | 0 | 12 | 0 |
| *Streptococcus sobrinus* (1G2) | 30 | 30 | 0 | 0 | 40 | 9 | 18 | 25 | 0 |
| *Streptococcus porcinus* (R2) | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 15 | 0 |
| *Bacillus subtilis* (C2) | 10 | 0 | 0 | 0 | 10 | 24 | 0 | 14 | 0 |
| *Pseudomonas fragi* (K1) | 0 | 10 | 0 | 0 | 0 | 22 | 0 | 14 | 0 |
| *Neisseria animaloris* (K2) | 28 | 12 | 0 | 20 | 30 | 30 | 28 | 28 | 16 |
| *Shimwellia pseudoproteus* (1R2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Serratia odorifera* (1V4) | 30 | 34 | 0 | 30 | 0 | 34 | 36 | 26 | 40 |
| *Streptococcus iniae* (M1) | 36 | 34 | 0 | 18 | 24 | 35 | 32 | 27 | 0 |
| *Streptococcus iniae* (C1) | 44 | 40 | 0 | 28 | 30 | 30 | 32 | 0 | 24 |
| *Serratia ficaria* (P4) | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 26 |
| *Bacillus coagulans* (J1) | 25 | 25 | 0 | 20 | 20 | 35 | 35 | 0 | 0 |
| *Proteus hauseri* (1E3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Staphylococcus xylosus* (Y2) | 0 | 0 | 0 | 0 | 10 | 25 | 0 | 19 | 0 |

CRB (IC): Chloramphenicol resistant bacteria (Isolate code), APN: Ampicilin, APX: Ampiclox, CHL: Chloramphenicol, CIP: Ciprofloxacin, ERY: Erythromycin, GEN: Gentamicin, OFL: Ofloxacin, STR: Streptomycin, TET: Tetracycline

## Discussion

Means available in the treatment of fishponds include the use of antibiotics and changing of water (Ibemere & Ezeano, 2014; Niyi-David *et al*., 2021; Yanong, 2006; Zhong *et al*., 2018) [14, 18, 27, 28]. These reduce the population of bacteria that could cause disease to the fishes. In this study, it is revealed that 64% of fishpond owners/managers that were interviewed use antibiotics in treatment of their fishponds, and the frequency of antibiotic use was mostly once in a month. In a related study (Agoba *et al*., 2017) [1], it was revealed that 7.8% of fishpond owners within a certain region outside Nigeria use antibiotics in fish farming. Higher percentages have been reported in other related works carried out in Nigeria (Dandi *et al*., 2024; Oka *et al*., 2023) [8, 19]. Dandi *et al*. (2024) [8] reported a percentage of 52.75% among medium and large-scale fish farmers, and stated that the antibiotic use was once a month. Oka *et al* (2023) [19] reported a percentage of 91% among fish farmers with also a frequency of antibiotic use of once a month. It is evident from these observations that more than 50% of fishpond owners/managers in Nigeria prefer to use antibiotics in their fishponds. This point to the possibility that many fishpond owners/managers in Nigeria may have experience loss of fishes in the past, and have resulted to the use of antibiotics to prevent further loss.

Bacterial population in fishponds has been shown to range from values in magnitude of $10^3$ to $10^7$ CFU/ml (Danba *et al*., 2014; Torimiro *et al*., 2014; Aina & Olaleye, 2023) [7, 2, 26]. In this study the total heterotrophic bacteria varied significantly ($p< 0.05$) between the various fishponds. The lowest population of total heterotrophic bacteria was in magnitude of $10^{10}$ CFU/ml, while the highest was in magnitude of $10^{14}$ CFU/ml. This population size is higher than what has been observed in previous studies. High magnitude of bacterial populations in fishponds can be attributed to the number of fishes in the ponds and/or frequency of water change. High number of fishes per pond means increased fish droppings and therefore higher microbial load. Also, higher microbial load is expected when the duration of water change is quite long.

Presence of antibiotic-resistant bacteria in fishponds has been revealed in some studies (Aina & Olaleye, 2023; Danba *et al*., 2015; Torimiro *et al*., 2014) [6, 2, 26]. The occurrence/incidence of antibiotic-resistant bacteria in fishponds assessed in these studies range from 0.72 to 80%. The occurrence of chloramphenicol-resistant bacteria in the fishponds assessed in this study ranged from $10^{-10}$ to 0.12%. This is less than what has been observed in other studies. The comparatively low occurrence of antibiotic-resistant bacteria could be due to the use of low amount of antibiotics and/or frequency of antibiotic use in fishponds in the study area.

Antibiotic-resistant bacteria that have been identified in fishponds include *Staphylococcus aureus*, *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Proteus* spp., *Aeromonas* spp., *Bacillus* spp., *Streptococcus* spp., *Shigella* spp., *Vibrio cholerae*, *Enterococcus faecalis* and *Enterobacter aerogenes* (Hafsat *et al*., 2015; Olukunle & Oyewumi, 2017; Danba *et al*., 2015) [12, 20, 6]. The Chloramphenicol-resistant bacteria in the fishponds assessed in this study included *Vibrio vulnificus*, *Pseudomonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Macrococcus lamae*, *Streptococcus* spp., *Neisseria animaloris*, *Shimwellia pseudoproteus*, *Serratia* spp., and *Proteus hauseri*. Most of the Chloramphenicol-resistant bacteria found in this study including *Vibrio*, *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Streptococcus*, and *Proteus* are among the antibiotic-resistant bacteria that have been found in the other studies. This is an indication that antibiotic-resistant bacteria are wide spread globally.

Antibiotics shown to be still effective against some antibiotic-resistant bacteria include Ofloxacin, Gentamicin, Ofloxacin, and Ciprofloxacin (Farzana *et al*., 2015; Gurdabassi *et al*. 2000; Neela *et al*. 2012; Rahman *et al*., 2008) [10, 17, 24]. In this study, Gentamicin was shown to be effective against 15 out of the 18 identified chloramphenicol-resistant bacteria, followed by Streptomycin which was effective against 13 out of the 18 of them. Gentamicin, the most effective antibiotic against the chloramphenicol-resistant bacteria in this study, has been shown in the other related works to be among the few antibiotics that are effective against antibiotic-resistant

bacteria. The susceptibility of antibiotic-resistant bacteria to Gentamicin could be attributed to its broad-spectrum and bactericidal nature; Gentamicin inhibits protein synthesis by binding to both large and small subunits of ribosomes in bacteria (Tangy *et al*. 1985) [25]. Also, Gentamicin was not reported in the literatures assessed for this study as one of the antibiotics used in fishponds; reasonably so due to its relatively high cost. Since Gentamicin is shown in this study and other related study to be active against antibiotic-resistant bacteria, it follows that Gentamicin can be an antibiotic of choice in the treatment of infections caused by antibiotic resistant-bacteria.

## Conclusion

In this study, it was revealed that even though most fishpond owners/managers use antibiotics in treatment of their fishponds, the occurrence of chloramphenicol resistant bacteria in their fishponds were quite low. The chloramphenicol resistant bacteria isolated in this study were more sensitive to Gentamicin than the other antibiotics used. However, *Shimwellia pseudoproteus* was resistant to Gentamicin, and all the antibiotics used.

## Conflict of Interest

There was no conflict of interest.

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