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Chloramphenicol-Resistant Bacteria Proportion in Fishponds and Their Antibiotic Susceptibility

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ABSTRACT

Presence of antibiotic-resistant bacteria in fishponds could pose significant threat to public health worldwide. The aim of this study was to determine the chloramphenicol-resistant bacteria (CRB) proportion among total heterotrophic bacteria (THB) in fishponds and their antibiotic susceptibility. The study area was Rumuolumeni in Obio-Akpor Local Government Area of Rivers State in Nigeria. Water samples were collected from fishponds used for rearing African Catfish, and analyzed for pH, turbidity, Nitrate, Phosphate, and populations of THB and CRB. Populations of THB and CRB were used to calculate CRB proportion in the fishponds. CRB were identified through morphological/biochemical means and were subjected to antibiotic susceptibility testing. Zones of inhibition obtained from the susceptibility testing were compared to standard values. The results obtained showed that the pH of water columns in the fishponds ranged from 6.4 to 7.0, turbidity: 7.5 to 28.0 NTU, Nitrate concentration: 6.1 to 22.4 mg/L, phosphate concentration: 0.06 to 3.82 mg/L, THB population: $7.3\pm0.8 \times 10^{10}$ to $4.7\pm3.2 \times 10^{14}$ CFU/ml, and CRB population: $4.5\pm0.6 \times 10^{1}$ to $7.6\pm0.9 \times 10^{3}$CFU/ml. CRB proportion among the THB in the fishponds was very low (1×10^{-10} to 3×10^{-7} %). Identified CRB included *Vibrio* species, *Pseudomonas fluorescens*, *Staphylococcus* species, *Streptococcus* species, *Bacillus* species, and *Proteus hauseri*. Susceptibility results revealed that Gentamicin was the most effective antibiotic against the CRB. It is concluded that though CRB are present in the fishponds, they are of relatively low population and are susceptible to gentamicin.

Keywords: Chloramphenicol-resistant bacteria, Resistance bacteria proportion, Antibiotic Susceptibility, fishponds, Physicochemistry of fishponds

Introduction

Fishponds are artificial bodies of water designed for rearing fish on a commercial scale to meet the increasing demand for seafood. The operations in fishponds include stocking, feeding, monitoring, and harvesting. During the feeding and monitoring operations, antibiotics are usually added for growth promotion and infection prevention/curative purposes (Depaola *et al.,* 2015). This practice can lead to the development and spread of antibiotic-resistant bacteria from the aquatic environment to terrestrial environment, thereby posing a public health risk. Antibiotic resistance is a growing concern globally, with significant implications for human health, agriculture, and the environment.

Bacterial resistance to antibiotics has been attributed to the misuse and overuse of antibiotics, selective pressure, and possession of antibiotic-resistant plasmids (Bradford, 2020). In aquaculture, antimicrobials are administered either through feeds or directly into the pond water. Both methods may result in heavy use of antimicrobial agents thereby leading to a selective pressure in the aquatic microorganisms (Landers *et al.,* 2012), and emergence of antibiotic-resistant bacteria. Antibiotic resistance has been observed in several bacterial species found in fishponds including *Azotobacter chroococcum*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. (Aina and Olaleye, 2023). Transmission of antibiotic-resistant bacteria (ARB) from fish aquaculture to fish handlers and consumers is likely to occur (Ferri *et al*., 2022;

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Krahulcová *et al*., 2023), thereby establishing ARB within the human population. Although fish borne zoonosis are prevailing worldwide, the problem is more of concern in middle- and low-income countries (Chai *et al.,* 2005). However, the geographical limits and populations at risk are expanding because of growing international markets, improved transportation systems, and demographic changes such as population movements. Fish-associated zoonotic pathogens are mainly bacteria. Often, fish unsusceptible to these infections are capable to cause serious sickness in humans (Rahman *et al*., 2020). However, opportunistic fish-borne bacterial infections are limited. Zoonotic diseases associated with fish contact that are caused by bacteria include those caused by infections with *Mycobacterium*, *Erysipelothrix*, *Campylobacter*, *Aeromonas*, *Vibrio*, *Edwardsiella*, *Escherichia*, *Salmonella*, *Klebsiella* and *Streptococcusiniae* (Ziarati *et al*., 2022). *Aeromonas* is more common is freshwater species and *Vibrio* is more likely in saltwater species (Gauthier, 2015; Komar *et al.,* 2006). Contact may result in wound infections and ingestion can result in gastroenteritis with vomiting and diarrhea. More severe and potentially life-threatening diseases and septicemia may occur in immuno-compromised people. Often bacterial infection of fish does not make fish appear ill but can cause serious illness in humans.

Intensive use of antimicrobial agents in fishponds can culminate in the pond fishes becoming reservoirs of antimicrobial-resistant bacteria. From these reservoirs resistant genes may reach human pathogens through horizontal gene transfer, or the resistant pathogens may reach humans directly from the aquatic environment. The consequences of antimicrobial resistance in bacteria that infect humans are increased number of infections, increased frequency of treatment failures and increased severity of infection (Salam *et al*., 2023). Apart from human health risk due to antimicrobial resistant bacteria, some antibiotics can accumulate in the flesh of fishes, and consumption of such flesh can lead to health problems such as allergy (Arsène *et al*., 2022).

Research (Afolabi*et al*., 2020; Amponsah *et al*., 2021; Oké and Goosen, 2019) indicate that fishponds for rearing African Catfish (*Clarias gariepinus*) are abundant in some African countries. And there are indications that broad-spectrum antibiotics are mostly used by the fishpond operators to control disease and/or foster growth of the fishes (Depaola *et al.,* 2015; Lulijwa *et al*., 2020).

The use of broad-spectrum antibiotics in fishponds could invariably lead to emergence of bacteria resistant to broad-spectrum antibiotics such as chloramphenicol. Therefore, the aim of this study was to determine the chloramphenicol-resistant bacteria (CRB) proportion among total heterotrophic bacterial population in fishponds and their antibiotic susceptibility. Elucidation of the susceptibility of the CRB will help in development of effective treatment strategies in cases of outbreak of infections resulting from CRB emanating from fish aquaculture.

Materials and Methods

Study area

The study area was Rumuolumeni in Obio-Akpor Local Government Area of Rivers State in Nigeria. The location of the encountered fishponds used for rearing African Catfish (*Clarias gariepinus*), as geo-referenced with the aid of a calibrated compass were within latitude 4°49'37" to 4°49'44" N, and longitude 6°57'14" to 6°57'25" E.

Sample collection

Water samples were collected from 8 concrete fishponds from within the study area. Samples were collected from the upper water column (0 – about 30 cm depth) of the ponds; disinfected 200 ml plastic bottles were submerged within the depth and water flowed in through natural action. Samples were placed in ice packed box after collection and transported within 24 hours to the Microbiology laboratory of the Department of Microbiology, Rivers State University, Nigeria for analysis.

Physicochemical analyses

The water samples were analyzed for pH, turbidity, nitrate concentration, and phosphate concentration.

Determination of pH: The pH of the water samples was determined using a handheld pH-meter (Hanna Instruments, USA). The meter was first calibrated using acetate buffer of pH 4.0 and phosphate buffer of pH 6.8. After calibration, its probes were dipped into the samples and upon equilibration, the readings were recorded.

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Determination of turbidity: The turbidity of the water samples was determined using Nephelometric method as described in United States Environmental Protection Agency (EPA) 2015 document (https://www.epa.gov/sites/default/files/2015-08/documents/method_180-1_1993.pdf). Turbidity standards of 2, 4, 6, 8, and 10 NTU formazin solutions were prepared. Absorbance of the solutions at 650 nm was measured using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). Values of the turbidity standards were then plotted against the absorbance to obtain a standard turbidity graph. The absorbance of the samples at 650 nm was then measured using the Spectrophotometer, and the turbidity determined by extrapolation from the standard turbidity graph.

Determination of nitrate concentration: The nitrate concentration of the water samples was determined using the Brucine-sulfanilic acid method (Sa'id and Mahmud, 2013). Equal volume of 13N sulphuric acid was added to 10 ml samples in 50 ml capacity volumetric flasks. The resulting hot solutions were allowed to cool down to ambient temperature, and then 0.5 ml brucine-sulfanilic acid mixture was added followed by addition of deionized water to the 50 ml mark. The flasks and their contents were placed in a hot water bath at 100 ºC for 25 minutes for colour development to occur. After this, the flasks were allowed to cool, and the absorbance of the solutions was measured at 410 nm using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). The absorbance readings were used to extrapolate nitrate concentrations with the aid of a nitrate-absorbance graph previously obtained.

Determination of phosphate concentration: The phosphate concentration of the water samples was determined using the Ascorbic acid method (APHA, 1992). The water samples were filtered through a Whatman No. 1 filter paper, and then 40 ml of the filtrates transferred into 50 ml capacity volumetric flasks. Phosphate analyzing reagent was then prepared according to the following composition: 373.2 ml distilled water, 0.06 g Potassium antimony tartrate, 2.4 g Ammonium molybdate, 2.12 g Ascorbic acid, and 26.8 ml conc. sulphuric acid.

The phosphate analyzing reagent (6.5 ml) was added to the filtrate, and the content of the flasks made up to the 50 ml mark using distilled water. The flasks were gently shaken and allowed for blue colour development. The absorbance of the resulting blue colour solutions were then measured at 880 nm using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). The absorbance readings were then used to extrapolate the concentration of phosphate from a previously obtained standard phosphate-absorbance graph.

Determination of chloramphenicol-resistant bacteria proportion

Population of total heterotrophic bacteria (THB) was determined using Nutrient Agar (NA), while population of chloramphenicol-resistant bacteria (CRB) was determined using NA supplemented with 30 µg/ml Chloramphenicol (NAC) as described by Peekate and Abu (2017). Serial dilution was carried out on water samples from the fishponds, to between 10^{-8} to 10^{-12} dilutions. Inoculum volumes of 0.1 ml of the different dilutions were inoculated on NA plates in duplicates, whereas undiluted and 10^{-1} dilutions were inoculated on NAC. Inoculated NA and NAC plates were incubated at ambient temperatures (24 – 33 °C) for 24 and 48 hours respectively. After incubation, colonies on NA and NAC plates were counted and used to calculate the population of THB and CRB respectively. From the values obtained, the chloramphenicol-resistant bacteria proportion was determined using the formula:

$$\text{CRB proportion} = \frac{\text{CRB population}}{\text{THB population}} \times 100\ \%.$$

Isolation and identification of chloramphenicol-resistant bacteria

Enumerated colonies on the NAC plates were isolated and sub-cultured onto sterile NA plates and coded. The coded isolates were subjected to Gram staining and microscopic examination, and the following physiological and biochemical tests: 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, and glycerol.

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The tests were carried out as described by Peekate (2022). The results of the tests were submitted to the search dialogue of the Advanced Bacteria Identification Software (ABIS), bio-database software available at https://www.tgw1916.net/bacteria_abis.html, so as to reveal the possible identity of the isolates.

Antibiotic susceptibility testing

Identified CRB were subjected to antibiotic susceptibility testing using well in agar method (Balouiri *et al*., 2016). 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 (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.1_Breakpoint_Tables.pdf). Solutions of the antibiotics were prepared such that their respective quantity was available in a delivery volume of 40 µl. The absorbance of prepared broth cultures of the CRB was measured and adjusted to the absorbance of 0.5 McFarland standard (CLSI, 2012) through the addition of sterile normal saline. 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 5 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 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. Zones of inhibition were compared to standard values in EUCAST so as to classify tested CRB as resistant, intermediate, or susceptible to the antibiotics used.

Results

Physicochemical characteristics of the fishponds

The physicochemical characteristics of water from the fishponds are presented in Table 1. The pH ranged from 6.4 to 7.0 with a mean of 6.9±0.2 generally indicating slight acidity. The turbidity ranged from 7.5 to 28.0 NTU with a mean of 20.1±7.5 NTU indicating high turbidity, nitrate concentration ranged from 6.1 to 22.4 mg/L with a mean of 12.3±6.5 mg/L indicating moderate nitrate concentration, and phosphate concentration ranged from 0.06 to 3.82 mg/L with a mean of 2.36±1.49 mg/L indicating low phosphate concentration.

Table 1: Physicochemical characteristics of water from the fishponds

N	Sample code	pH	Turbidity (NTU)	Nitrate (mg/L)	Phosphate (mg/L)
1	IWA	6.4	7.5	6.4	0.24
2	IWB1	6.8	10.5	14.1	3.82
3	IWB2	7.0	23.0	22.4	3.33
4	IWB3	7.0	28.0	7.7	1.90
5	IWB4	7.0	20.5	6.4	0.06
6	IWB5	7.0	23.5	16.0	2.99
7	IWB6	6.8	28.0	19.2	3.68
8	IWB7	7.0	19.5	6.1	2.87
	Mean ± SD	6.9±0.2	20.1±7.5	12.3±6.5	2.36±1.49

Chloramphenicol-resistant bacteria proportion in the fishponds

The populations of total heterotrophic bacteria (THB) and chloramphenicol-resistant bacteria (CRB) in the fishponds across the sampled locations are presented in Table 2. THB ranged from numbers in magnitudes of 10^{10} to 10^{14}CFU/ml ($7.3\pm0.8 \times 10^{10}$ to $4.7\pm3.2 \times 10^{14}$ CFU/ml) with a mean of $1.1\pm0.8 \times 10^{14}$ CFU/ml. On the other hand, CRB ranged from numbers in magnitudes of 10^{1} to 10^{3} CFU/ml ($4.5\pm0.6 \times 10^{1}$ to $7.6\pm0.9 \times 10^{3}$ CFU/ml) with a mean of $2.6\pm1.8 \times 10^{3}$ and CFU/ml. From these values, the chloramphenicol-resistant bacteria proportion in the fishponds across the sampled locations were calculated to range from 1.0×10^{-10} to 3.0×10^{-7} % with a mean of $5.4\pm1.8 \times 10^{-8}$ %. These values indicate very low chloramphenicol-resistant bacteria proportion.

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Table 2: Populations of total heterotrophic bacteria (THB) and chloramphenicol-resistant bacteria (CRB) in the fishponds

N	Sample code	THB (CFU/ml)	CRB (CFU/ml)	POC (%)
1	IWA	$7.3\pm0.8 \times 10^{10}$	$4.5\pm0.6 \times 10^{1}$	6×10^{-8}
2	IWB1	$2.9\pm0.4 \times 10^{12}$	$1.7\pm0.3 \times 10^{2}$	6×10^{-9}
3	IWB2	$3.1\pm0.5 \times 10^{14}$	$7.4\pm1.7 \times 10^{3}$	2×10^{-9}
4	IWB3	$5.7\pm2.3 \times 10^{13}$	$7.5\pm2.1 \times 10^{1}$	1×10^{-10}
5	IWB4	$5.7\pm0.8 \times 10^{11}$	$1.7\pm0.2 \times 10^{3}$	3×10^{-7}
6	IWB5	$5.9\pm1.5 \times 10^{12}$	$3.7\pm1.3 \times 10^{3}$	6×10^{-8}
7	IWB6	$1.5\pm0.5 \times 10^{13}$	$1.4\pm0.3 \times 10^{2}$	1×10^{-9}
8	IWB7	$4.7\pm3.2 \times 10^{14}$	$7.6\pm0.9 \times 10^{3}$	2×10^{-9}
	Mean ± SD	$1.1\pm1.8 \times 10^{14}$	$2.6\pm1.8 \times 10^{3}$	$5.4\pm1.8 \times 10^{-8}$

POC: Proportion of Chloramphenicol-resistant bacteria $= \frac{\text{CRB}}{\text{THB}} \times 100$.

Identity of the chloramphenicol resistant bacteria

The morphological and biochemical/physiological characteristics of the chloramphenicol resistant bacteria (CRB) isolated from the fishponds across the sampled locations are presented in Table 3. Bacteria with the same characteristics were placed in the same cluster. Based on their characteristics, ABIS revealed their identity as follows: *Vibrio vulnificus*, *V. alginolyticus*, *Pseudomonas fluorescens*, *Staphylococcus saprophyticus*, *Staphylococcus xylosus*, *Streptococcus porcinus*, *Streptococcus iniae*, *Bacillus subtilis*, *B. coagulans*, and *Proteus hauseri*.

Antibiotic susceptibility of the chloramphenicol resistant bacteria

Antibiotic susceptibility of the chloramphenicol resistant bacteria (CRB) is presented in Table 4a-4c. In the Tables, all the CRB were resistant to at least two antibiotics, chloramphenicol inclusive. However, eight out of the ten CRB were 100 % susceptible to Gentamicin (Table 4b).

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Table 3: Morphology and Biochemical/Physiological characteristics of the CRB isolates

CL	IC	MP	GS	MT	ST	CT	OX	IP	CU	MR	VP	HM	SH	CH	LC	LP	GF	LF	MlF	MnF	SF	XF	GyF	PB (% SM)
1	1D1,1F1, 1L1, 1J1, 1O2,1K1	R	-	+	-	-	+	+	-	+	-	.γ	+	-	+	+	A	-	A	-	-	-	-	*Vibrio vulnificus* (92)
2	O2, H3, N2,	R	-	+	-	+	+	-	+	-	-	.β	-	-	+	+	-	-	-	-	-	-	A	*Pseudomonas fluorescens* (89)
3	N3, D1, O3, 1H1, J2, 1E2	C	+	-	+	+	-	-	-	-	-	.γ	-	-	-	-	A	-	A	A	A	-	A	*Staphylococcus saprophyticus* (93)
4	T4,1W1, B1, 1S1, 1V1,1V2, 1O1, X1, 1L2	R	-	+	-	+	-	-	-	-	+	.γ	+	-	+	-	A	-	A	A	A	-	-	*Vibrio alginolyticus* (87)
5	T3, 1X1, U2, R2, S2, 1B4, 1C3, X2, Y1, 1A4, 1D2	C	+	-	+	-	+	+	-	-	-	.β	-	-	+	+	A	-	A	A	-	-	-	*Streptococcus porcinus* (76)
6	Y3, C2, H1	R	+	+	+	+	+	-	+	-	-	.β	+	-	+	+	A	-	A	A	-	-	-	*Bacillus subtilis* (84)
7	T1, C1, H2	C	+	-	-	-	-	-	+	+	-	.γ	+	-	+	-	A	-	-	A	A	-	-	*Streptococcus iniae* (83)
8	1A1, 1B3, 1C1	R	+	+	-	+	-	-	-	-	-	.γ	+	-	-	-	A	-	A	-	-	-	-	*Bacillus coagulans*(84)
9	1A3, 1C2, 1E3	R	-	+	-	+	-	+	-	+	-	.γ	+	-	-	-	A	-	A	-	A	A	-	*Proteus hauseri* (93)
10	Y2, 1V3, 1B2	C	+	-	+	+	-	+	+	-	-	.γ	-	-	+	+	A	A	A	A	A	A	-	*Staphylococcus xylosus* (85)

CL: Cluster code, IC: Isolate code, MP: Morphology, GS: Gram stain reaction, R: Rods, C: Cocci, MT: Motility, ST: Salt tolerance, 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, GF, LF, MlF, MnF, SF, XF, and GyF: Glucose, Lactose, Maltose, Mannitol, Sucrose, Xylose, and Glycerol fermentation respectively, A: Acid production, PB: Probably bacteria, % SM: Percent Similarity.

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Table 4a: Susceptibility of the chloramphenicol-resistant bacteria (CRB) to ampicilin, ampiclox, and chloramphenicol

CRB	N	APN			APX			CHL		
		% R	% I	% S	% R	% I	% S	% R	% I	% S
Vibrio vulnificus	6	0	16.7	83.3	0	100	0	100	0	0
Pseudomonas fluorescens	3	0	100	0	0	100	0	100	0	0
Staphylococcus saprophyticus	6	0	33.3	66.7	0	16.7	83.3	100	0	0
Vibrio alginolyticus	9	100	0	0	100	0	0	100	0	0
Streptococcus porcinus	11	93.3	6.7	0	86.7	13.3	0	100	0	0
Bacillus subtilis	3	33.3	66.7	0	100	0	0	100	0	0
Streptococcus iniae	3	0	33.3	66.7	0	0	100	100	0	0
Bacillus coagulans	3	0	33.3	66.7	0	0	100	100	0	0
Proteus hauseri	3	100	0	0	100	0	0	100	0	0
Staphylococcus xylosus	3	100	0	0	66.7	33.3	0	100	0	0

CRB: chloramphenicol resistant bacteria, N = number of isolates, APN: Ampicilin (10 µg), APX: Ampiclox (30 µg), CHL: Chloramphenicol (30 µg), R: resistant, I: intermediate, S: sensitive.

Table 4b: Susceptibility of the CRB to ciprofloxacin, erythromycin, and gentamicin

CRB	N	CIP			ERY			GEN		
		% R	% I	% S	% R	% I	% S	% R	% I	% S
Vibrio vulnificus	6	66.7	33.3	0	100	0	0	83.3	16.7	0
Pseudomonas fluorescens	3	0	0	100	0	33.3	66.7	0	0	100
Staphylococcus saprophyticus	6	16.7	83.3	0	0	0	100	0	0	100
Vibrio alginolyticus	9	77.8	22.2	0	0	100	0	0	0	100
Streptococcus porcinus	11	73.3	26.7	0	93.3	6.7	0	0	0	100
Bacillus subtilis	3	66.7	33.3	0	0	100	0	0	0	100
Streptococcus iniae	3	0	0	100	0	0	100	0	0	100
Bacillus coagulans	3	0	0	100	0	0	100	0	0	100
Proteus hauseri	3	66.7	33.3	0	100	0	0	66.7	33.3	0
Staphylococcus xylosus	3	66.7	33.3	0	0	100	0	0	0	100

CIP: Ciprofloxacin (5 µg), ERY: Erythromycin (15 µg), GEN: Gentamicin (10 µg).

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Table 4c: Susceptibility of the CRB to ofloxacin, streptomycin, and tetracycline

CRB	N	OFL			STR			TET		
		% R	% I	% S	% R	% I	% S	% R	% I	% S
Vibrio vulnificus	6	83.3	16.7	0	0	33.3	66.7	100	0	0
Pseudomonas fluorescens	3	0	0	100	0	0	100	66.7	33.3	0
Staphylococcus saprophyticus	6	0	0	100	0	0	100	0	0	100
Vibrio alginolyticus	9	100	0	0	0	22.2	77.8	88.9	11.1	0
Streptococcus porcinus	11	93.3	6.7	0	20	80	0	100	0	0
Bacillus subtilis	3	66.7	33.3	0	33.3	66.7	0	100	0	0
Streptococcus iniae	3	0	0	100	100	0	0	0	33.3	66.7
Bacillus coagulans	3	0	0	100	66.7	33.3	0	100	0	0
Proteus hauseri	3	100	0	0	100	0	0	66.7	33.3	0
Staphylococcus xylosus	3	100	0	0	0	33.3	66.7	100	0	0

OFL: Ofloxacin (5 µg), STR: Streptomycin (10 µg), TET: Tetracycline (30 µg)

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Discussion

Review of physicochemical characteristics of fish ponds (Ehiagbonare and Ogunrinde, 2010; Kiran, 2010; Olukunle and Oyewumi, 2017) indicates that pH of waters in fish ponds vary from slightly acidic through neutral to slightly alkaline; nitrate concentration vary from as low as 2.21 mg/L to as high as 80 mg/L; whereas phosphate concentration always remain low from values of 0.51 to 4.51 mg/L. Also, turbidity ranges from very low values of 0.6 NTU to very high values of 170 NTU. The range of values of the physicochemical parameters of the fishponds assessed in this study falls within the ranges of values that have been observed in the other related studies (Ehiagbonare and Ogunrinde, 2010; Kiran, 2010; Olukunle and Oyewumi, 2017). Variation within these ranges could be attributed to differences in geographical locations of the fishponds, especially with regards to pH, and the rate of influx of organic material including feeds.

Heterotrophic bacterial populations in water column of fishponds have been shown to occur in magnitudes of 10^5 to 10^9 CFU/ml (Aborisade *et al*., 2023; Njoku *et al*., 2015; Obire and Vincent, 2017). This range of value is lesser than what was recorded in this study. This could be attributed to the difference in turbidity of the water in the fishponds, for in this study the mean turbidity was higher than the mean turbidity recorded in the other studies. It is well known that more turbid waters have higher bacterial population than less turbid waters (Agedah *et al*., 2015; Azhdarpoor *et al*., 2019). In another related study (Hafiz and Rahman, 2024), chloramphenicol-resistant bacteria (CRB) population in magnitudes of $10^2$to 10^3 CFU/ml were observed in several fishponds in Bangladesh, India. This is in agreement with what was observed in this study and could imply that irrespective of the geographical location of fishponds, CRB will always be present at relatively low population range. It can therefore be hypothesized that CRB and other broad-spectrum antibiotic-resistant bacteria naturally occur in fishponds, though at low population.

CRB isolated from fishponds in other related studies (Aina and Olaleye, 2023; Hafiz and Rahman, 2024; Lin *et al*., 2023; Petersen *et al*., 2002) include *Acinetobacter* spp., *Aeromonas* spp., *Azotobacter chroococcum*, *Bacillus* spp., *Enterococcus* spp., *Enterobacter* spp., *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas* spp., and *Shigella* spp. Some CRB isolated in these studies, including *Pseudomonas*, *Bacillus*, and *Proteus*, were isolate in this study. The other CRB (*Vibrio*, *Staphylococcus*, and *Streptococcus* spp.) which were isolated in this study but were not isolated in the other related studies could be due to peculiar practices in operation of the investigated fishponds. The practices may have resulted in inclusion of marine species (*Vibrio* spp.) through objects used in marine environment, and accidental contamination with human fluid in the case of *Staphylococcus* and *Streptococcus* spp.

Gentamicin has been shown to be effective against multidrug-resistant bacteria (Bala *et al*., 2016; Sitohy *et al*., 2024). Multidrug-resistant bacteria (MDRB) resist the actions of antibiotics through removal of the antibiotic molecules from their cells using efflux pumps located on their cell wall or membrane, modification of sites targeted by antibiotic molecules, and enzymatic inactivation of antibiotic molecules (Gaurav *et al*., 2023; Helmy *et al*., 2023; Rozwadowski and Gawel, 2022). These mechanisms mostly depend on the actions of enzymes whose synthesis are controlled by special resistant genes which are acquired through vertical or horizontal gene transfers. Gentamicin binds with 30S subunit of bacterial ribosome in such a way that it induces errors in protein and enzyme synthesis (Rivera *et al*., 2021). It also causes substantial slowdown in the elongation rate of peptide chains in bacterial cells. In an additional mechanism, gentamicin causes the two subunits of the ribosome to stay together even after translation therefore creating a pool of inactive ribosomes that can no longer re-initiate and translate more polypeptides (Borovinskaya *et al*., 2007). It is likely that the mechanisms of action exhibited by gentamicin have not been overwhelmed by the mechanisms developed by MDRB against other antibiotics, probably due to the special molecular structure of gentamicin.

Conclusion

Chloramphenicol-resistance proportions in the fishponds within the study location were relatively quite low. However, many of the chloramphenicol-resistant bacteria of these low proportions were resistant to at least two antibiotics.

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On the other hand, most of the chloramphenicol-resistant bacteria were highly susceptible to Gentamicin. This indicates that Gentamicin could be a drug of choice in the treatment of infections caused by antibiotic-resistant bacteria.

Conflict of Interest

The authors of the manuscript declare that they have no competing interest in having this work published.

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