Bacteriological assessment and antibiotics susceptibility profile of bacteria recovered from pond water, fish skin, and gut in Ile-Ife, Osun State, Nigeria

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Abstract

Fish currently provide 6.7% of all protein consumed by humans globally, nevertheless, aquaculture system has been linked to fish and environmental contamination and disease outbreak. This study aims to isolate, identify, and characterise, bacteria in fish and pond water as well as the antibiotic profile of detected Coliforms. The susceptibility of the isolates was tested using the Kirby-Bauer disc diffusion method on Mueller Hinton agar. A total of forty (40) isolates were isolated from the water samples of which (5) species were Gram Positive bacteria and 35 species of Gram Negative bacteria. The temperature for all ponds ranged from 25°C to 28°C. The mean bacteria count for pond C1 to T2 were 4.9×102, 4.9×102, 5.4×102, 2.5×102, 2.2×102, and 1.9×102 CFU/ml respectively. All isolates were 100% resistant to ceftazidime, cefuroxime and augmentin. More resistance to cefixime (80%) and gentamicin (73.3%) and nitrofurantoin (66.7%) was recorded. However, only 16.6% and 8.3% of the isolates were resistant to ciprofloxacin and ofloxacin respectively. The multiple

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antimicrobial resistance index (MARI) ranged from 0.5 to 0.9. The water quality parameters (temperature and pH) and the type of bacteria detected in all pond type did not differ significantly. The Multi-drug resistance bacteria detected could be pathogenic to fish and consumers.

Keywords: Aquaculture; fish; foodborne disease, antimicrobial resistance; pond water; total bacteria count

Background

Fish meat contains high nutritional quality including low-fat content, cholesterol, saturated fat, and high levels of proteins, polyunsaturated fatty acids, and minerals such as calcium, sodium, potassium, phosphorous, and magnesium [1]. Food and agriculture organisation's (FAO) state of world fisheries and aquaculture report estimates that fish now provide 6.7% of all protein consumed by humans globally, passing the 20kg per capita and year mark for the first time [2]. In Nigeria, fish is a staple source of animal protein compared to other animal protein sources such as beef, mutton, pork and poultry. The high consumption of fish also stems from the fact that it is generally accepted without any religious bias [1].

Aquaculture fish production in Nigeria has grown from 0.1% to 0.4% from 1995 to 2016. The FAO estimates an increase in fish production in Nigeria by 2030 to about 18.2%, while export will increase by about 6.6% [3]. These figures show the importance of fish to nutrition and food security. However, recent reports on fish meat safety have heightened consumers fear regarding fish meat and fish products. For instance, cases of fish-borne disease caused by pathogenic microorganisms like *Salmonella*, *Listeria monocytogenes* and *Vibros spp*. has been reported in several parts of the world [4,5].

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Often the primary driver of such food intoxication is the farming environment and feed given to the fish. Since fish lives in water, the quality of water directly impacts fish productivity, fish products, human and environmental health. Water quality is one of the most overlooked aspects of pond management until it adversely affects the quality of fish production. The factors which influence the use of water for fish culture include dissolved oxygen, pH, hardness, turbidity, alkalinity, ammonia and temperature. The level of pollution of a given water body is indicated by other parameters such as biological oxygen demand and chemical oxygen demand [6,7].

Fishes are reared in different water culture media or confinement such as concrete, earthen or plastics ponds. Concrete and earthen ponds have been the widely used culture system for fish. Earthen pond system of fish cultivation has been the most established method of fish culture in Nigeria. Fishes reared in these environments are contaminated by both pathogenic and opportunistic organism's microorganisms. The contamination of these culture systems has been attributed to poor water quality, high stocking densities and the use of animal manure and contaminated feed [6,8].

Due to the high cost of feeding, farmers use animal manure to supplement feeding. The use of organic manure also leads to the release of high concentration of opportunistic and pathogenic microorganisms into the ponds which pose a threat not only to fish health but the environment [9]. Also, these microorganisms in fish and fish ponds portend grave consequences for public health [4,5]. Some of these microorganisms possess resistant determinant which enhances their potential for infecting consumers. For instance, *E. coli* is known to survive well in aquatic environments, and they are highly adept at horizontal gene transfer, a notorious vehicle for antibiotic resistance dissemination. Resistant pathogens are capable of undermining effective health outcomes and prolonging hospitalisation of patients.

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Hence it is essential to document the microflora and antimicrobial resistance associated with the fish environment since the microbial flora of a cultivated fish is an expression of its aqueous environment.

2. Material and methods

2.1 Study area

The study area is Fajuyin and Oke Opa within Ile-Ife, Osun State in Nigeria which lies between latitude 70˚N 50'N and longitude 4˚ 69'E (Figure 1). The climate is tropical, and mix farming system is conventional. Farmers combine fish production, livestock production, and plantain and banana plantation. Hence having a combination of concrete, plastic and earthen fish ponds stocked with Tilapia and Catfish is more profitable. All ponds survey in the study were privately owned. The Earthen ponds and plastic ponds were covered with a net to prevent birds and predatory animal from accessing the pond, while the concrete ponds are in a fenced area and then roofed.

Table 1: Description of sampling stations

Sampling Stations	Observed Features
C1(Concrete pond 1)	The pond is at Fajuyi area in Ile-Ife. The pond is in an closed, fenced and roofed area; also refuse is being dumped behind the fence and waste water from bathroom flows close to the concrete pond.
C2(Concrete pond 2)	The pond is at Fajuyi area in Ile-Ife. The pond is in an closed, fenced and roofed area; also refuse is being dumped behind the fence and waste water from bathroom flows close to the concrete

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	pond.
E1(Earthen pond 2)	The pond is at Fajuyi area in Ile-Ife. The station is close to a primary school and a farm land, surrounded with plantain and banana plantation with other trees; and the pond was covered with a net to prevent bigger animals from picking up fish.
E2(Earthen pond 1)	The pond is at Fajuyi area in Ile-Ife. The station is in a compound, with a well close to it; surrounded with plantain and banana plantation with other trees, and the pond was covered with a net to prevent bigger animals from picking up fish.
T1(Tank pond 1)	The pond is at Oke Opa area in Ile-Ife. The pond is in a compound where snails and poultry are being reared, having a cashew tree in the compound. The pond is stocked with African cat fish covered with net. It also has a running tap in the compound which is used for the fish farming
T2(Tank pond 2)	The pond is at Oke Opa area in Ile-Ife. The pond is in a compound where snails and poultry are being reared, having a cashew tree in the compound. The pond is stocked with African cat fish covered with a net. It also has a running tap in the compound which is used for the fish farming

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95 2.2 Sample collection, temperature and pH measurement
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97 Water samples were aseptically collected from the ponds using sterile screw-capped kegs.
98 Composites samples were obtained by collecting at different sampling points and depths of
99 30cm below the water surface from two separate concrete ponds, earthen pond, and plastic
100 pond respectively which makes a total of 6 samples. The water samples were transported in a
101 box containing ice packs to the laboratory for microbiological and biophysical analysis. The
102 temperature of water samples was measured at the sites of sampling with a standard
103 laboratory mercury thermometer (Assutech, South Africa). The pH of water samples was
104 determined using pH meter (designer water, South Africa) after its calibration.
105 2.3 Isolation of total heterotrophic bacteria

106 A 1ml each of homogenised samples was seeded into 9ml added of sterilised distilled water
107 in test tubes using a sterile syringe. The first test tube was swirled gently to make 10^{-1}
108 dilution. From 10^{-1} dilution, 1ml of the mixture was transferred to the second tube and also
109 swirled gently to make 10^{-2} dilution. These procedures were taken for the successive dilution
110 in a similar way to give 10^{-3}, 10^{-4}, 10^{-5}, to 10^{-6} dilution with the aid of a sterile syringe. The
111 diluent was inoculated on culture plates using the pour plate method. The plates were
112 incubated in an inverted position for 24-48 hours at 37˚C. Colonies were counted and colony
113 forming unit per ml calculated. Colonies were subcultured to obtain a pure culture and were
114 stored in 20% glycerol for further test.

115 2.4 Isolation of Salmonella/Shigella

116 Salmonella and Shigella identification was done using the *Salmonella/ Shigella* agar (SSA)
117 which was prepared according to the manufacturer's instruction. Briefly, 1ml 10^{-1} and 10^{-2}
118 dilutions were poured into a different petri dish containing SSA. The petri dish was swirled to
119 ensure proper growth. SSA plates were then incubated for 24 to 48h at 37˚ C. After

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incubation; the colonies were subcultured on fresh SSA plates to obtain pure cultures used for further identification.

2.5 Gram Staining Technique

Gram staining was done to differentiate organisms based on the structure of their cell wall [5]. Thin smears from the bacteria colonies were prepared on clean grease free slides; heat fixed slightly and allowed to cool. The smear was covered with crystal violet for 60 seconds and washed off with clean water. Lugol's iodine was added for 60 seconds and rinsed off with clean water. The smear was decolourised rapidly for a few seconds with alcohol and rinsed with clean water. Then the smear was counterstained with safranin for 30 seconds and rinsed with clean water. The back of the slides was cleaned, and the smears were air dried. A drop of oil immersion was placed on the smear, and they were examined microscopically using the 100X oil immersion objective for cells shape, cells arrangement and Gram stain reaction.

2.6 Biochemical Tests

Biochemical characteristics were determined using a conventional biochemical test such as catalase, coagulase, indole, and indole test [10]. Furthermore, bacteria cultures were identified based on their cultural, morphological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology [11,12].

2.6.1 Catalase test

Few drops of 3% hydrogen peroxide were poured into a test tube, and a colony of the test organism was picked and suspended in the test tube [13]. A production of the bubble indicates a positive catalase test. Catalase test differentiates catalase-producing bacteria such as *Staphylococci* from non-catalase producing bacteria such as *Streptococci*.

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143 2.6.2 Coagulase test

144 A drop of distilled water was placed on each end of a clean grease free slide, and a colony of
145 the test organism was emulsified in each of the drops to make two thick suspensions and
146 mixed gently. Plasma is added to one of the suspensions [1]. The clumping of the organism
147 indicates a positive result after 10 seconds. Coagulase test is used to identify *Staphylococcus*
148 *aureus* which produces the enzyme coagulase.

149 2.6.3 Indole Test

150 It is used to determine the ability of an organism to split indole from the amino acid
151 tryptophan using the enzyme tryptophanase. Tryptophan broth was inoculated with test
152 organism and incubated for 24 h. Drops of Kovac's reagent were added to the broth.
153 Formation of a red ring at the surface of the broth signifies a positive result [14].
154 Enterobacteriaceae produces indole hence the positive result with Kovac's reagent.

155 2.6.4 Citrate utilisation test

156 This test is based on the ability of the organism to use citrate as its source of carbon. Simmon
157 citrate agar medium was prepared as a slant in test tubes. A sterile wire loop was used to
158 inoculate the isolate into the agar slant medium and incubated at 37˚C for 24 h after which it
159 was examined for colour change [15]. A change in colour of the medium to bright blue colour
160 gives a positive citrate utilisation test while green colouration is considered negative.

161 **2.7 Antibiotic Sensitivity Testing**

162 Isolated bacteria from the fish pond in Ile- Ife were tested for antimicrobial susceptibility by
163 the disc diffusion method. The panel of antibiotics (Abtek Biologicals Ltd, UK) was used for
164 testing were ceftazidime (CAZ) 30µg, cefuroxime (CRX) 30µg, gentamicin (GEN) 10µg,
165 cefixime (CXM) 5µg, ofloxacin (OFL) 5µg, augmentin (AUG) 30µg, nitrofurantoin (NIT)

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300µg, ciprofloxacin (CPR) 5µg. The susceptibility of the isolates was tested using the Kirby-Bauer disc diffusion method on Mueller Hinton agar (BIOTEC, Ltd) as described elsewhere [16]. Briefly, 4-5 colonies of pure bacterial isolates were emulsified into a test tube containing 2ml of sterile normal saline (0.85% NaCl) using a sterilised flexible loop and homogenised to give turbidity that is equivalent to 0.5 McFarland standards (equivalent to 1.5 $\times 10^8$ cells). A sterile cotton swab stick was dipped into the suspension, drained to remove excess culture and then streaked on the entire surface of the Mueller Hinton agar (MHA) plate. The inoculated plates were allowed for 3-5 minutes to dry. The antibiotics discs were properly placed aseptically on the surface of the inoculated plates using a sterile forceps and gently to ensure even contact with the medium. After that, the plates were left on the bench for about 5 minutes to allow the antibiotics diffuse into the medium and incubated at 37°C for 18 to 24 hours. The zone of inhibition around the disc was measured, and the result was interpreted as resistance or susceptible based on the interpretative standard according to the Clinical and Laboratory Standards Institute guidelines [17].

3 Results

3.1 Physical parameters and pH

The temperature value of the pond water sample from all sampling stations throughout the study period values ranged from 25°C to 28°C. The water sample from E1 and T1 has the highest value of 28°C respectively while C1 and E1 have the lowest value of 25°C (Table 1). The water sample of T2 recorded the highest pH value of 9.0 while C1 has the lowest pH value of 7.1.

3.2 Total heterotrophic and total coliform bacteria population

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The total bacterial count of the water sample from sampling stations C1 to T2 ranged from 1.9×10^2 CFU/ml to 5.4×10^2 CFU/ml (Table 2). The mean values of the bacterial count for the six sampling stations from C1 to T2 were 4.9×10^2, 4.9×10^2, 5.4×10^2, 2.5×10^2, 2.2×10^2, and 1.9×10^2 CFU/ml respectively. The bacterial count of the water sample from T2 recorded the lowest with the value of 1.9×10^2CFU/ml, while the highest bacterial count was recorded from E1 with the value of 5.4×10^2 CFU/ml. The total coliform count of the water sample of tank pond 2 was lowest with a count of 1.4×10^2 CFU/ml, while concrete pond 1 and earthen pond 1 had the highest coliform count value of 5.4×10^2 CFU/ml (Table 2).

Table 2: Temperature, pH and microbial loads of the water samples from various fish ponds in Ile-Ife, Osun State

Pond type	Temperature (°C)	pH	Total heterotrophic Count (CFU/ml)	Total Coliform Count (CFU/ml)
C1	25	7.1	4.9×10^4	5.4×10^4
C2	27	7.8	4.9×10^4	4.8×10^4
E1	25	8.5	5.4×10^4	5.4×10^4
E2	28	8.8	2.5×10^4	1.5×10^4
T1	28	8.1	2.2×10^4	1.9×10^4
T2	27	9.0	1.9×10^4	1.4×10^4

C1= Concrete pond 1, C2= Concrete pond 2, E1= Earthen pond 1, E2= Earthen pond 2, T2=Tank pond 1, T2=Tank pond 2

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3.3 Bacterial types detected in water samples

The cultural, morphological and biochemical characteristics of dominant bacteria isolated from the various sampling stations during the study are shown in Tables 3-5. A total of forty (40) isolates were recovered from the water samples of which five (5) species were Gram Positive bacteria representing two genera, and thirty-five (35) species of Gram Negative bacteria representing four (4) genera. Isolates C101 to C102 obtained from C1 were identified as *Escherichia coli*, and *Klebsiella* sp. respectively. Isolate C201 was from C2 and was identified as *Salmonella sp*. Isolates E101 and E102 from E1 were identified as *Klebsiella* sp. Isolates E201 to E210, isolates T101 to T114 and isolates T201 to T211 were isolated from E2, T1 and T2 respectively, and the dominant bacteria found in these ponds were identified as *Staphylococcus aureus, Enterococcus* spp, *Enterobacter* spp, *Klebsiella* spp., *Salmonella spp.*, and *Escherichia coli.* These bacteria were similar to those found in the fish gut and skin (Table 6).

Table 3: Biochemical characterisation of bacteria from various fish ponds in Ile-Ife, Osun State

Isolate Code	Cultural characteristics on agar	Cell shape	Gram reaction	Catalase	citrate	Indole	Coagulase	Suspected organism
C101	Pink, round, entire flat on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
C102	Greenish metallic sheen, irregular, entire, flat on EMB agar	R	-	N.A	-	+	-	*E.coli*
C201	Black, round, entire, flat on SSA	R	-	N.A	+	-	-	*Salmonella sp.*

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E101	Pink, round, entire, convex on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
E102	Pink, round, entire, flat on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
E201	Yellow, round, entire, flat on NAG	C	+	+	+	-	+	*S. aureus*
E202	Cream, irregular, entire, raised on NAG	C	-	N.A	+	-	-	*Enterococcus sp.*
E203	Blue. round, entire, flat on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*
E204	Purple, round, entire, flat on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*
E205	Pink, round, entire flat on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
E206	Pink, round, entire, raised on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
E207	Pink, round, entire, flat SSA	R	-	N.A	-	+	-	*E.coli*
E208	Pink, round, entire flat on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
E209	Black, round, entire, flat on SSA	R	-	N.A	+	-	-	*Salmonella sp.*
E210	Purple, round, entire, raised on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*
T101	Cream, round entire flat on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
T102	Cream round entire raised on NAG	C	+	+	+	-	-	*Staphylococcus sp*

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C: Cocci, R: Rod, - : Negative, +: positive, *S. aureus*: *Staphylococcus aureus*, sp: specie, NAG: Nutrient agar, EMB: Eosine methylene blue, SSA: Salmonella and Shigella agar; N.A: Not applicable

Table 4: Biochemical characterisation of bacteria from tank ponds in Ile-Ife, Osun State

Isolate Code	Cultural characteristics on agar	Cell shape	Gram reaction	Catalase	Citrate	Indole	Coagulase	Suspected organism
T103	Pink, irregular, entire, flat on SSA	R	-	N.A	-	+	-	*E.coli*
T104	Black, round, entire, flat on SSA	R	-	N.A	+	-	-	*Salmonella sp.*
T105	Pink, irregular, entire, flat on SSA	R	-	N.A	-	+	-	*E.coli*
T106	Black, round, entire, flat on SSA	R	-	N.A	+	-	-	*Salmonella sp.*
T107	Greenish metallic sheen, irregular, entire, flat on EMB agar	R	-	N.A	-	+	-	*E.coli*

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T108	Blue, round, entire, flat on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*
T109	Purple, irregular, entire, flat on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*
T110	Black, round, entire, flat on SSA	R	-	N.A	+	-	-	*Salmonella sp.*
T111	Pink, round, entire, raised on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
T112	Purple, irregular, entire, flat on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*
T113	Greenish metallic sheen, irregular, entire, flat on EMB agar	R	-	N.A	-	+	-	*E.coli*
T114	Pink, round, entire, raised on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
T201	Yellow, round, entire, flat on NA	C	+	+ve	+	-	+	*S. aureus*
T202	Yellow, round, entire, flat on NA	C	+	+ve	+	-	+	*S. aureus*
T203	Pink, irregular, entire, flat on SSA	R	-	N.A	_	+	-	*E.coli*
T204	Black, round, entire, flat on SSA	R	-	N.A	+	-	-	*Salmonella sp.*
T205	Pink, round, entire, raised on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
T206	Blue, round, entire, flat on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*

234 C: Cocci, R: Rod, - : Negative, +: positive, *S. aureus*: *Staphylococcus aureus*, sp: specie,

235 EMB: Eosine methylene blue, SSA: Salmonella and Shigella agar; N.A: Not applicable

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Table 5: Biochemical characterisation of bacteria from tank ponds in Ile-Ife, Osun State

Isolate Code	Cultural characteristics on agar	Cell shape	Gram	Catalase	Citrate	Indole	Coagulase	
T207	Black, round, entire, flat on SSA	R	-	N.A	+	-	-	*Salmonella sp.*
T208	Pink, round, entire, raised on EMB agar	R	-	N.A	+	-	-	*Klebsiella sp.*
T209	Greenish metallic sheen, irregular, entire, flat on EMB agar	R	-	N.A	-	+	-	*E.coli*
T210	Purple, round, entire, raised on EMB agar	R	-	N.A	+	-	-	*Enterobacter sp.*

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T211	Greenish metallic sheen, irregular, entire, flat on EMB agar	R	-	N.A	-	+	-	*E.coli*

253 C: Cocci, R: Rod, - : Negative, +: positive, *S. aureus*: *Staphylococcus aureus*, sp: specie,
254 EMB: Eosine methylene blue, SSA: Salmonella and Shigella agar; N.A : Not applicable

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263 Table 6: Bacterial Isolates from the Fish (Skin and Gut) Samples from Fish Ponds Tank 1 and
264 Tank 2

Tank/Tissue	Media	Colour on media	Gram	Reaction	Cell Shape	Catalase	Citrate	Indole	Coagulase	Suspected Organism
T1/GUT	SSA	Black	-		R	N.A	+	-	-	*Salmonella sp.*
	EMB	Blue	-		R	N.A	+	-	-	*Enterobacter sp*
T2/GUT	EMB	Greenish metallic	-		R	N.A	-	+	-	*E. coli*

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		sheen							
TI/SKIN	EMB	Pink	-	R	N.A	+	-	-	*Klebsiella sp*
T2/SKIN	EMB	Greenish metallic sheen	-	R	N.A	-	+	-	*E. coli*
	SSA	Black	-	R	N.A	+	-	-	*Salmonella sp*

EMB: Eosin methylene blue agar, SSA: Salmonella Shigella agar, R: Rod, N.A: Not applicable

3.4 Antibiotics susceptibility testing

All isolates were 100% resistant to ceftazidime, cefuroxime and augmentin. Resistance was also recorded to cefixime (80%) and gentamicin (73.3%) and nitrofurantoin (66.7%). However, only 16.6% and 8.3% of the isolates were resistant to ciprofloxacin and ofloxacin respectively. The multiple antimicrobial resistance index (MARI) ranged from 0.5 to 0.9 (Table 7).

Table 7: Antibiotics Susceptibility Pattern of Isolates from the Pond Water Samples

Isolate (code)	Antibiotic susceptibility								Susceptibility %			MARI
	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	R	I	S	
E.coli (T107)	R	R	R	R	I	R	S	I	62.5	25	12.5	0.6
E.coli (C102)	R	R	R	R	R	R	S	R	87.5	0	12.5	0.9
E.coli (T105)	R	R	S	R	S	R	I	I	50	25	25	0.5
Kleb (E205)	R	R	R	R	S	R	S	I	62.5	12.5	25	0.6
Kleb (T114)	R	R	R	I	S	R	R	R	75	12.5	12.5	0.8

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Kleb (E206)	R	R	R	R	S	R	R	I	75	12.5	12.5	0.8
Ent (E202)	R	R	I	R	S	R	R	I	62.5	25	12.5	0.6
Ent (T109)	R	R	R	R	S	R	R	I	75	12.5	12.5	0.8
Ent (E203)	R	R	R	R	S	R	R	I	75	12.5	12.5	0.8
Sal (C201)	R	R	R	R	S	R	R	I	75	12.5	12.5	0.8
Sal (TI04)	R	R	R	R	S	R	R	I	75	12.5	12.5	0.8
Sal (T105)	R	R	S	R	S	R	R	I	62.5	12.5	25	0.6
Resistance %	100	100	75	91.6	8.3	100	66.6	16.6				

CAZ: Ceftazidime (30 µg), CRX: Cefuroxime (30 µg), GEN: Gentamicin (10 µg), CXM: Cefixime (5 µg), OFL: Ofloxacin (5 µg), AUG: Augmentin (30 µg), NIT: Nitrofurantoin (300 µg) and CPR: Ciprofloxacin (5 µg), R: Resistance, I: Intermediate, S: Susceptible, *Kleb*: *Klebsiella* sp., *Ent*: *Enterobacter* sp., *Sal*: *Salmonella* sp., *Staph*: *Staphylococcus aureus*

4 Discussions

The pH (7.1-9.0) and temperature (25-28˚C) recorded in all the ponds were within the optimum range necessary for aquaculture. Optimum pH and temperature directly stabilise the physicochemical parameters of pond water enhancing fish health, and productivity and the maintenance of a proper balance of the microbial ecology in pond water. A study conducted in Kenya, reported a temperature range from 20.04˚C to 32.63˚C [6], elsewhere, the temperature was lower than in the present study [9]. Rapid and fluctuating changes in pH and temperature have been reported to cause extreme stress in the fish.

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In the present study, more Gram-negative bacteria were isolated than the Gram-positive from the various fish pond water. The bacteria isolated were *E. coli*, *Salmonella* spp, *Klebsiella* spp., *Enterobacter* spp., *Staphylococcus* spp., and *Enterococcus spp.* Some of these bacteria belong to the Enterobacteriaceae family that is well-known to cause sepsis, pneumonia, urinary tract infections, wound, bacteremia, cystitis, and meningitis [18]. Coliform bacteria in the water samples of the fish ponds were similar to the bacteria isolated from different fish parts such as the skin and guts. The bacterial load observed in the skin and guts of fish could be due to the bacteria already present in the water. This viewpoint was shared in similar research on the bacteriological and elemental quality of *Clarias gariepinus* from river Lavun, Bida Niger state, Nigeria [1]. Also, the bacterial load in water could be as a result of the effect of anthropogenic activities in the location where the ponds are sited. The coliforms isolated are an indication of the contamination of the pond water with faecal materials. Faecal contamination of pond water may result in the introduction of pathogenic organisms into the pond. Such pathogens have been shown to lead to fish diseases or foodborne disease [4,5,19].

Furthermore, the fertilisation of the ponds with animal manure poses the risk of introduction of potentially pathogenic and resistant bacteria into the pond. The resistant bacteria could infect fishes, humans and contaminate the environment. Animal faeces have been shown to harbour a plethora of potentially dangerous bacteria, some of which are resistant to antibiotics of choice used in the treatment of human and animal diseases [16,20]. Resistant bacteria are capable of transferring and conferring resistance to commensal bacteria through horizontal gene transfer using mobile genetic components such as conjugative plasmids transposons, and phages [21].

The different groups of bacteria isolated from these fish ponds are in concordance with studies conducted elsewhere [14,22–24]. Contaminated feed added to the ponds introduces allochthonous bacteria which subsequently become the principal source of bacterial

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313 contaminant of pond water and affecting fish health. The presence of pathogenic
314 microorganisms especially *Enterobacter spp*. *E.coli, Salmonella,* and *Staphylococcus spp.*
315 can lead to the transmission of water-borne diseases such as Typhoid fever, Cholera, food
316 poisoning, gastroenteritis and skin infections in humans. Often the main route of disease
317 transmission is the consumption of improperly cooked fish cultivated in these ponds [19,22].
318 The antibiotics susceptibility test (AST) of the study shows that 100% of the isolates were
319 100% resistant to ceftazidime, cefuroxime and augmentin. Up to 73.3% were resistant to
320 gentamicin. About 80% of the isolates were resistant to cefixime, 6.7% were resistant to
321 ofloxacin. Also, 66.7% were resistant to nitrofurantoin. The AST result corresponds to the
322 findings in Singapore where 100.0%, 97.0%, and 97.0% of isolates were resistant to
323 cefazolin, ceftazidime and cefepime respectively [25]. A similar result was obtained with *V.*
324 *parahaemolyticus* isolates in Malaysia, where high resistance to ampicillin, amikacin,
325 kanamycin, cefotaxime, and ceftazidime was reported [26]. On the other hand, a relatively
326 low prevalence of antibiotic resistance was detected in a marine fish cage-culture area of
327 Guangdong, China [27].

328 The 100% resistance to augmentin found in this study, did not come as a surprise given that
329 augmentin is listed among the top 20 antibiotics that contribute 61% towards the total
330 systemic antibacterial market [28]. Nonetheless, a report on an outbreak of food-borne
331 disease among school teachers at Rob Ferreira High School in White River, Mpumalanga,
332 South Africa, found no resistance among *S. enterica* serotype Virchow [29]. However, a 14%
333 resistance to augmentin was reported in *Salmonella typhi* antibiotic sensitivity pattern in
334 Dubai, United Arab Emirates [30]. Even though AMR is a global public health problem,
335 Nigeria already faces a precarious situation of poor antibiotic prescription monitoring and
336 prescription only medicines (POM) including antimicrobials that are routinely sold Over-
337 The-Counter (OTC) in pharmacies and by patent proprietary medicines vendors (PPMVs).

Easy access to critical antibiotics of last resort by patients increases its imprudent use, further worsening the problem of AMR. There are, currently no available studies outlining the full burden of AMR and its health and economic impact on Nigerians [31]. Hence the implication of the finding of this study could be far-reaching and could be used a baseline study on AMR in the environment.

In this study, the multiple antibiotic resistance indexes for all isolated bacteria ranged from 0.5 to 0.9 indicating a high-risk environment. A study with a similar finding was conducted in southeastern Nigeria on the prevalence of antibiotic-resistant diarrhoeagenic bacterial species in Surface Waters. The result of this study shows that *Salmonella spp*. had the highest MARI value of 0.75 whereas the least MARI value of 0.44 was obtained for *Staphylococcus spp.* [32]. In Benin City Nigeria, multiple antibiotic resistance index mean value of 0.365 for Vibrio isolates was obtained from four different fish pond facilities [33].

Antimicrobial resistance is a global public health problem sustained by multifactorial antibiotic usage. The spread of antibiotic resistance in the marine environment is poorly understood [34]. However, antibiotic resistance genes (ARGs) in environmental reservoirs and a transferability of these genes through horizontal gene transfer (HGT) would likely account for the growing prevalence of ARGS in the aquaculture settings. Furthermore, global usage of antimicrobial agent to control infections in humans and animals is a common practice. In these circumstances, antimicrobial agents used in marine and aquaculture system not only escalates the pathogenic bacterial resistance to antibiotics but also increases the likelihood of HGT transfer to common commensal bacteria thus promoting resistance to multiple antibiotics [13].

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Although the present study did not characterise the antimicrobial resistant genes (ARGs) conferring resistance against the antimicrobial agent tested, this limited nevertheless did not diminished the criticality of the multi-drug resistant phenotype found in the study.

5. Conclusion

The concrete pond (C1 and C2), and earthen (E1) pond had the highest load of heterotrophic and coliform bacteria. The water quality parameters (temperature and pH) and the type of bacteria detected in all pond type did not differ significantly. The study also revealed that all the ponds were contaminated with potentially pathogenic bacteria that could affect fish health and fish product. These organisms could lower fish yield, cause diseases and economic loss and equally endanger public health, particularly if the fish harvested from the ponds are not properly cooked before consumption. Discharging of contaminated pond water into the environment could further enhance the transfer of resistant bacteria to the environment. Hence the regular monitoring of pond water for microbial contamination is necessary to maintain aquaculture and public health. Good agricultural practices; such as the use of good quality water, regular draining of pond water after a specific period, closure of ponds to the public will aid in preventing pond water contamination. The use of modern techniques such as polymerase chain reaction and matrix-assisted laser desorption/ionisation (MALDI) used with time-of-flight mass spectrometer (TOF) to quantify and characterise bacteria from pond water is necessary for accurate and timely identification.

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385 **Conflict of interest**

386 None to declare

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388 **References**

389 1. Aliyu, A.; Ibrahim, Y. K.; Oyi, R. A. Bacteriological and Elemental Quality of Clarias
390 gariepinus (cat fish) Samples from River Lavun, Bida Niger state, Nigeria. *Niger. J. Pharm.*
391 *Res.* **2018**, *12*, 139–147.

392 2. FAO *The State of World Fisheries and Aquaculture 2016*; Food and Agriculture
393 Organization of the United Nations: Rome, 2016.

394 3. FAO *The state of world fisheries and aquaculture-meeting the sustainable development*
395 *goals*; Food and Agriculture Organization of the United Nations: Rome, 2018.

396 4. Li, Y.; Pei, X.; Yan, J.; Liu, D.; Zhang, H.; Yu, B.; Li, N.; Yang, D. Prevalence of
397 foodborne pathogens isolated from retail freshwater fish and shellfish in China. *Food Control*
398 **2019**, *99*, 131–136.

399 5. Yanestria, S. M.; Puspita Rahmaniar, R.; Wibisono, F. J.; Effendi, M. H. Detection of invA
400 gene of Salmonella from milkfish (Chanos chanos) at Sidoarjo wet fish market, Indonesia,
401 using polymerase chain reaction technique. *Vet. World* **2019**, *12*, 170–175.

402 6. Mukwabi, D.; Okemo, K. P. Temperature and ph influence on bacterial pathogens
403 infecting farmed Nile tilapia in aquaculture systems in Bungoma county, Kenya. *Int. J. Fish.*
404 *Aquat. Stud.* **2019**, *7*, 190–197.

405 7. Yang, P.; Lai, D. Y. F.; Huang, J. F.; Tong, C. Effect of drainage on CO2, CH4, and N2O
406 fluxes from aquaculture ponds during winter in a subtropical estuary of China. *J. Environ.*
407 *Sci.* **2018**, *65*, 72–82.

408 8. Abdel-Wahed, R. K.; Shaker, I. M.; Elnady, M. A.; Soliman, M. A. M. Impact of Fish-
409 farming Management on Water Quality, Plankton Abundance and Growth Performance of

410 Fish in Earthen Ponds. *Egypt. J. Aquat. Biol. Fish.* **2018**, *22*, 49–63.

411 9. Wang, W.; Gu, X.; Zhou, L.; Chen, H.; Zeng, Q.; Mao, Z.; Wang, W.; Gu, X.; Zhou, L.;

412 Chen, H.; Zeng, Q.; Mao, Z. Antibiotics in Crab Ponds of Lake Guchenghu Basin, China:

413 Occurrence, Temporal Variations, and Ecological Risks. *Int. J. Environ. Res. Public Health*

414 **2018**, *15*, 548.

415 10. Umana, S.; Uko, M. P.; Umana, S. I.; Okon, U. C.; Bassey, M. P. Occurrence of Bacterial

416 Contaminants in Fin-fishes (Clarias gariepinus and Coptodon guineensis) from Humic

417 Freshwater Ecosystem of Eniong River, Itu, Akwa Ibom State, Nigeria. *J. Adv. Microbiol.*

418 **2017**, *6*, 1–12.

419 11. Ogbonna, D. N.; Inana, M. E.; Castro Escarpulli, G. Characterization and Multiple

420 Antibiotic Resistance of Bacterial Isolates Associated with Fish Aquaculture in Ponds and

421 Rivers in Port Harcourt, Nigeria. *J. Adv. Microbiol.* **2018**, *10*, 1–14.

422 12. Noel, K. R.; John, H. G. *Bergey's Manual of Systematic Bacteriology*; Barbara Tansill,

423 Ed.; Volume 1.; Wiliam & Wilkins: Baltimore, USA, 1984.

424 13. Ramesh, D.; Souissi, S. Antibiotic resistance and virulence traits of bacterial pathogens

425 from infected freshwater fish, Labeo rohita. *Microb. Pathog.* **2018**, *116*, 113–119.

426 14. Kumar Kanth, M.; Rupam Kumari, S. Bacteriological investigation the patna urban. *Int.*

427 *J. Curr. Res.* **2018**, *10*, 72816–72818.

428 15. Alfiansah, Y. R.; Hassenrück, C.; Kunzmann, A.; Taslihan, A.; Harder, J.; Gärdes, A.

429 Bacterial Abundance and Community Composition in Pond Water From Shrimp Aquaculture

430 Systems With Different Stocking Densities. *Front. Microbiol.* **2018**, *9*, 2457.

431 16. Iwu, C. J.; Jaja, I. F.; Iweriebor, B. C.; Obi, L. C.; Okoh1, A. I. Antibiotic resistance

432 profiles of Escherichia coli O26, O145, and O157:H7 isolated from swine in the Eastern Cape

433 Province, South Africa. *Asian Pacific J. Trop. Dis.* **2017**, *7*, 553–559.

434 17. CLSI *Performance Standards for Antimicrobial Susceptibility Testing*; 27th Editi.;

435 Clinical and Laboratory Standards Institute: Wayne, USA, 2017.

436 18. AlTamimi, M.; AlSalamah, A.; AlKhulaifi, M.; AlAjlan, H. Comparison of phenotypic
437 and PCR methods for detection of carbapenemases production by Enterobacteriaceae. *Saudi*
438 *J. Biol. Sci.* **2017**, *24*, 155–161.

439 19. Elbashir, S.; Parveen, S.; Schwarz, J.; Rippen, T.; Jahncke, M.; DePaola, A. Seafood
440 pathogens and information on antimicrobial resistance: A review. *Food Microbiol.* **2018**, *70*,
441 85–93.

442 20. Jaja, I. F.; Bhembe, N. L.; Green, E.; Oguttu, J.; Muchenje, V. Molecular characterisation
443 of antibiotic-resistant Salmonella enterica isolates recovered from meat in South Africa. *Acta*
444 *Trop.* **2019**, *190*, 129–136.

445 21. Al-tawfiq, J. A.; Laxminarayan, R.; Mendelson, M. How should we respond to the
446 emergence of plasmid-mediated colistin resistance in humans and animals ? *Int. J. Infect. Dis.*
447 **2017**, *54*, 77–84.

448 22. dos Santos, R. R.; Xavier, R. G. C.; de Oliveira, T. F.; Leite, R. C.; Figueiredo, H. C. P.;
449 Leal, C. A. G. Occurrence, genetic diversity, and control of Salmonella enterica in native
450 Brazilian farmed fish. *Aquaculture* **2019**, *501*, 304–312.

451 23. Akeju, A. V.; Simpson, V. B.; Akeju, A. V; Simpson, V. B. Microbiological Study of
452 Common Iced Fish Consumed in Akure, Ondo State, Nigeria. *J. Adv. Microbiol.* **2019**, *14*,
453 44269.

454 24. Anyiam, A.; Albert, A. C.; Felix, E. C.; Cynthia, E. C.; Eromosele, O. S. Effect of Habitat
455 Locations on the Bacterial and Water Quality Changes in Freshwater Tilapia (Oreochromis
456 niloticus) Using Small Scale Depuration Food Quality Control and Food Safety View project
457 Effect of Habitat Locations on the Bacterial and Water Qual. *J. Adv. Microbiol.* **2016**, *1*, 1–
458 10.

459 25. Ng, C.; Chen, H.; Goh, S. G.; Haller, L.; Wu, Z.; Charles, F. R.; Trottet, A.; Gin, K.

460 Microbial water quality and the detection of multidrug resistant E. coli and antibiotic
461 resistance genes in aquaculture sites of Singapore. *Mar. Pollut. Bull.* **2018**, *135*, 475–480.
462 26. Letchumanan, V.; Pusparajah, P.; Tan, L. T.-H.; Yin, W.-F.; Lee, L.-H.; Chan, K.-G.
463 Occurrence and Antibiotic Resistance of Vibrio parahaemolyticus from Shellfish in Selangor,
464 Malaysia. *Front. Microbiol.* **2015**, *6*, 1417.
465 27. Wu, J.; Su, Y.; Deng, Y.; Guo, Z.; Cheng, C.; Ma, H.; Liu, G.; Xu, L.; Feng, J. Spatial
466 and temporal variation of antibiotic resistance in marine fish cage-culture area of Guangdong,
467 China. *Environ. Pollut.* **2019**, *246*, 463–471.
468 28. Global Antibiotic Resistance Partnership Situation analysis: Antibiotic use and resistance
469 in South Africa. *South African Med. J.* **2011**, *101*, 549–596.
470 29. Smith, A. M.; Gouws, A. M.; Hoyland, G.; Sooka, A.; Keddy, K. H. Outbreaks of food-
471 borne disease - A common occurrence but rarely reported. *South African Med. J.* **2007**, *97*,
472 1272.
473 30. Abro, A. H.; Al Deesi, Z. O.; Abdou, A. M. S. Salmonella typhi: Antibiotic sensitivity
474 pattern in Dubai, United Arab Emirates. *Pakistan J. Med. Sci.* **2011**, *27*, 295–299.
475 31. Federal ministries of Agriculture, health and E. *Antimicrobial Use and Resistance in*
476 *Nigeria - situation analysis and recommendations*; Abuja, Nigeria, 2017.
477 32. Stanley, C. O.; Onuoha, S. C. Diarrhogenic Bacterial Species in Surface Waters, South
478 Eastern Nigeria. *J Heal. Sci* **2017**, *27*, 319.
479 33. Igbinosa, E. O. Detection and Antimicrobial Resistance of *Vibrio* Isolates in Aquaculture
480 Environments: Implications for Public Health. *Microb. Drug Resist.* **2016**, *22*, 238–245.
481 34. Stange, C.; Yin, D.; Xu, T.; Guo, X.; Schäfer, C.; Tiehm, A. Distribution of clinically
482 relevant antibiotic resistance genes in Lake Tai, China. *Sci. Total Environ.* **2019**, *655*, 337–
483 346.