Bacteriological Assessment of some Fish Ponds in Edo State, Nigeria

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Abstract: Fish cultivation in a controlled environment has been found to be influenced by microbial contamination. This study was aimed at assessing the bacteriological qualities of three types of fish ponds in the three Senatorial districts in Edo State, Nigeria. The study was carried out between January, 2017 and December, 2017. The bacteriological analyses were carried out using standard microbiological techniques, antibiotics sensitivity test was carried out using the disk diffusion methods, while the DNA extraction and sequencing were done using standard molecular biology techniques. The total heterotrophic bacterial counts ranged from $34.77 \pm 4.17 \times 10^6$ cfu/ml – $75.22 \pm 5.3 \times 10^6$ cfu/ml. The total coliform counts ranged from $31.88 \pm 3.76 \times 10^6$ cfu/ml -$78.88 \pm 4.29 \times 10^6$ cfu/ml. The isolates were identified to include; *Klebsiella pneumoniae, Streptococcuss pasteurianus, Acinetobacter nosocomialis, Pseudomonas fluorescens, Serratia marcescens, Citrobacter freundii, Staphylococcus aureus*, *Escherichia coli* and *Staphylococcus sciuri*. The bacterial isolates had multiple antibiotic resistance (MAR) index values greater than the permissible limit of 0.2 against cetriazone, augmentin (Amoxicillin clavulonate), ciprofloxacin, gentamicin, cloxacillin, erythromycin, ofloxacin, ranicef and nitrofurantoin. The bacterial contamination of fish ponds intended for human consumption may constitute an impending danger not only in causing disease, but could act as reservoirs of antibiotic resistance organisms leading to treatment failure when improperly cooked fish is consumed. It is therefore, important to understand the microflora associated with fish culture environment and recommended that, adequate public health measures are put in place for the regulatory guidelines for the construction of fish ponds.

Key word: Aquaculture, Antibiotics susceptibility, Bacteriological, Fish Ponds, Water

INTRODUCTION

The fisheries sector contributes roughly 2% of national GDP, 40% of animal protein consumption, and a significant share of employment, particularly in rural areas. Nigeria produces the most aquaculture in Africa, with 15,489 tonnes per year (FAO, 2003). Fish are produced in a variety of culture media or in confined environments such as ponds, and microorganisms have been discovered in these ponds (Biggs *et al.*, 2005; Sheng and Wang, 2020). A fish pond is an artificial reservoir that is made for breeding fishes (Adedayo and Anthony, 2014). The types of fish ponds include the concrete and earthen ponds which are preferred by farmers due to the convenience, cost-effectiveness, easy maintenance and ease of control (Aborisade *et al.*, 2023; Odesiri-Eruteyan and Urhibo (2022). Water of compromised quality and high stocking densities have been blamed for the infectivity (WHO, 2006). The fish diets utilized in these ponds comprise unprocessed materials, which bring a wide range of bacteria into the ponds (Okpokwasili and Ogbulie, 1998). The high cost of feeding is one of the restraints of fish farming, which has led to the use of mammal dung to supplement conventional feed in countries such as China, India, and Nigeria. Unprocessed dung, on the other hand, causes a high concentration of opportunistic and pathogen microorganisms to be released into the pond, posing a public health risk. According to (WHO, 1999), their existence in fish intended for human consumption poses a threat not just in terms of causing sickness, but also in terms of acting as reservoirs for antibiotic resistant organisms, potentially leading to treatment failure if incorrectly prepared fish is consumed. The microbial flora of a cultivated fish is a reflection of its aqueous environment hence, it is critical to understand the microflora associated with the fish culture environment (Erondu and Anyanwu, 2005). Adebami *et al*. (2020) isolated *Staphylococcus* spp., *Enterococcus*

spp., *Streptococcus* spp., *Salmonella* spp., *Micrococcus* spp., *Shigella* spp., *Aeromonas* spp., *Providencia* spp., *Listeria* spp., *Chromobacterium* spp., *Proteus* spp., *Escherichia* spp., *Pseudomonas* spp. and *Klebsiella* spp. from different ponds wastewaters. Coliforms and *Escherichia coli* are bacterial indicators of water quality (Torimiro *et al*., 2014). The presence of microorganisms can affect the stability and quality of the fish ponds. They indicate the level of pollution and play crucial roles in the process of formation and breakdown of organic matter in the water body (Aborisade *et al*., 2023). This study was done to assess the bacteriological qualities of some fish ponds in Edo State, Nigeria.

MATERIALS AND METHODS

Study area: The research was carried out in Edo State's three senatorial districts: Edo South, Edo North and Edo Central. Three sampling stations were chosen for the study in each senatorial district.

Sample collection: Fish pond water samples were aseptically collected from ponds with the aid of sterile screw capped bottles. Samples were collected in triplicates monthly for 12 months from the concrete, earthen and plastic fish ponds at a depth of 30 cm below the water surface making a total of three hundred and twenty-four (324) fish pond water samples throughout the sampling period. All samples were always collected between 10am-12 noon and immediately taken to the laboratory in an ice-packed container for microbiological analysis within 24 hours.

Media preparation: All agar used were prepared according to the manufacturer's specifications and autoclaved for 15 minutes at 1.5 psi at 121^{o}C.

Water samples preparation, enumeration and isolation of bacteria: This was done in accordance with Cheesbrough's technique (2004). A 1 ml of water was added to 9 ml of sterile distilled water in a test tube (labeled 10^{-1}), 1 ml of the mixture was then transferred into the next tube containing 9 ml distilled water (labeled 10^{-2}) and this was repeated up to 6th dilution. From the tubes, 0.1 ml was then transferred using the pour plate technique into the sterile Petri dish, and the sterilized nutrient agar was introduced under aseptic conditions and allowed to solidify. The plates were incubated at 37^{o}C for 24 hrs. After incubation, the number of discrete colonies was counted and recorded as colony forming units (cfu/ml). Pure colonies were sub cultured on fresh nutrient agar plates; thereafter stocked in slants of nutrient agar and with 1% NaCl for further studies.

The bacteria in the water samples were isolated using a membrane filtration method. The membrane filtration unit's funnel has a capacity of 50 ml, and it was installed on one receptacle attached to the vacuum pump, allowing water to flow over the porous sterile membrane filter (0.45 m). After passing 100 ml of water sample through the membrane filters, sterile forceps were used to deposit them on MacConkey agar plates. Before being inoculated with membrane filters, the media was prepared and autoclaved at 121^{o}C for 15 minutes at 15Ib.

Identification of bacterial isolates: The various morphological and biochemical tests were carried out in accordance with the methods by Cheesebrough (2004). The tests included; Gram staining reaction, catalase, motility, citrate utilization, urease, oxidase, indole and triple sugar ion test.

Molecular identification of the bacterial isolates

DNA extraction from the bacterial isolates: The Zymo bacteria Miniprep kit was used to extract genomic DNA from the bacterial isolates (Zymo Research, Fermentas, USA). The protocol was followed exactly as it was given in the instructions. A NanoDrop Spectrophotometer was used to measure the concentration and purity of DNA (ND-1000, Thermo Fisher Scientific, US) (Shibu *et al.,* 2013). The ABI3500XL analyzers with a 50 cm array were used to perform polymerase chain reaction (PCR) amplification utilizing universal primer sets of 16SrRNA 27F- 5'-AGAGTTTGATCMTGGCTCAG-3' 1492R -5'-CGGTTACCTTGTTACGACTT-3'. The

protocol was followed exactly as it was given in the instructions. Fragments were observed on a 1.5 percent agarose electrophoresis gel stained with Safe view (Mohini and Deshpande, 2011).

Sequencing and identification of the PCR products: The PCR products were purified, and Geneious version 9.0.5 was used to examine the sequenced data. Bio edit Sequence viewer 7.2.1 was used to read the data, which were retrieved as nucleotides in FASTA format (Altschul *et al*., 2007). On the National Centre for Biotechnology and Information website (http://blast.ncbi.nlm.nih.gov), sequences were identified using GenBank's Basic Local Alignment Search Tool (BLAST) algorithm. Using Bio edit software, highly comparable sequences were downloaded from NCBI and subjected to multiple sequence alignment (Hall, 1999; Altschul *et al*., 2007).

Antibiotic susceptibility pattern of the bacterial isolates: Abtek antibiotic sensitivity multi-discs containing nitrofurantoin, amoxicillin, augmentin, gentamycin, cloxacillin, ofloxacin, streptomycin, ceftrazidine, augumentin ciprofloxacin and ranicef were used according to methods of the Clinical and Laboratory Standard Institute (2016).

Plasmid profiling: The characterized bacterial isolates were inoculated into 10 ml of nutrients broth containing 100 ug/ml of the mutagen (acridine orange). The mixture was incubated overnight at 37^{o}C for 24 hrs. After incubation, each mutagen exposed culture was plated on Mueller-Hinton agar medium and incubated at 37^{o}C for 24 hrs.

Analyses of the data obtained from the study: Data obtained from the study were subjected to analysis of variance utilizing the statistical package for social scientist version 21 and Microsoft excel 2016. The *p*-values < 0.05 were considered statistically significant (Ogbeibu, 2005).

RESULTS

The findings of a comparative examination of bacterial load in concrete fish pond water is shown in Table 1. The values range from 34.77 ± 4.17 x 10^6 cfu/ml to 74.55 ± 4.58 x 10^6 cfu/ml. The highest counts were recorded for concrete fish pond water in Edo North in July and the least was recorded for Edo Central and South in August. The total coliform counts ranged from 38.22 ± 4.89 x 10^6 cfu/ml to 78.88 ± 4.29 x 10^6 cfu/ml.

Table 2 illustrates the findings of the comparative study of the bacterial loads of earthen fish ponds water, which range from 34.77 ± 4.17 x10^6 cfu/ml to 75.22 ± 5.4 x 10^6 cfu/ml. The highest counts were recorded for earthen fish pond water in Edo North in July and the least was recorded for Edo South in August. The total coliform counts ranged from 38.22± 4.89 x 10^6 cfu/ml to 75.22 ± 2.63 x 10^6 cfu/ml.

The findings of the comparative analysis of bacterial loads of the plastic fish ponds water are shown in Table 3. The values range from 34.77 ± 4.17 x 10^6 cfu/ml to 74.55 ± 4.58 x 10^6 cfu/ml. The highest counts were recorded for plastic fish pond water in Edo North in July and the least was recorded for Edo South in August. The total coliform counts ranged from 31.44±5.59x10^6 cfu/ml to75.77±2.63x10^6 cfu/ml.

Nine bacterial isolates were phenotypically identified to include; *Serriatia* sp., *Citrobacter* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Escherichia* sp., *Streptococcus* sp, *Staphylococcus* sp., *Klebsiella* sp. and *Staphylococcus aureus*. The following isolates were molecularly identified; *Klebsiella pneumoniae, Streptococcuss pasteurianus, Acinetobacter nosocomialis, Pseudomonas fluorescens, Serratia marcescens, Citrobacter freundii, Staphylococcus aureus, Escherichia coli* and *Staphylococcus sciuri* (Table 4).

Table 5 shows the antibiotic sensitivity pattern of the bacterial isolates from fish ponds. The Gram-positive bacteria isolates, which included *Staphylococcus sciuri, Streptococcus pasteurianus* and *Staphylococcus aureus* were resistant to gentamycin, augumetin, ciprofloxacin, ceptrazidine, erythromycin and augumentin. The Gram-negative bacterial isolates;

Escherichai coli, Klebsiella pneumoniae, Acinetobacter nosocomialis, Serratia marcescens, Citrobacter freundii and *Pseudomonas fluorescens* were resistant to cetriazone, ciprofloxacin, gentamycin, ofloxacin, cloxacilin, rancef augumetin, nitrofurantoin and erythromycin. Multiple antibiotics resistant index revealed that all the tested bacteria were multidrug resistant. Plate 1 represents the agarose gel electrophoresis of the PCR products of nine bacteria samples isolated from the fish pond water samples.

Table 1: Comparative study of bacterial loads from concrete fish pond waters in Edo State, Nigeria

Months	Locations	THBC ($x10^6$ cfu/ml)	Total coliform ($x10^6$ cfu/ml)
J	S	41.22±4.49	38.22±4.89^a
	C	57.00±4.67^b	54.55±4.24^b
	N	68.88±4.23	67.22±4.73^b
	p-value	0.001	0.001
F	S	41.77±4.01^a	42.77±6.51^a
	C	58.44±3.44^b	56.00±6.41^{ab}
	N	70.88±2.82^c	68.00±5.44^b
	p-value	0.000	0.027
M	S	45.33±5.66^a	48.00±6.13^a
	C	61.44±5.39^b	59.88±5.51^{ab}
	N	71.55±5.13^b	70.55±5.33^b
	p-value	0.008	0.003
A	S	38.44±4.81	44.77±7.09^a
	C	54.33±3.38^b	58.11±5.58^{ab}
	N	67.66±3.96	70.33±5.44^b
	p-value	0.000	0.023
M	S	42.11±4.10^a	45.77±5.98^a
	C	60.00±3.21^b	58.77±4.35^{ab}
	N	69.55±4.05^b	69.88±4.87^b
	p-value	0.000	0.010
J	S	49.11±5.70^a	39.22±5.35^a
	C	57.66±8.58^{ab}	55.44±4.68^b
	N	74.55±4.58^b	67.11±4.70^b
	p-value	0.033	0.002
J	S	44.88±5.85^a	44.66±5.46^a
	C	55.44±5.67^{ab}	64.00±5.82^b
	N	67.22±4.90^b	74.88±4.52^b
	p-value	0.029	0.002
A	S	34.77±4.17^a	44.22±5.90
	C	34.77±4.17^a	44.22±5.90
	N	59.33±3.72^b	60.33±5.86
	p-value	0.000	0.104
S	S	44.44±4.09^a	42.88±4.97^a
	C	58.00±5.02^{ab}	56.66±4.56^b
	N	69.55±4.95^b	68.66±4.50^b
	p-value	0.004	0.003
O	S	46.11±5.71^a	42.33±4.39^a
	C	58.33±4.43^{ab}	57.88±4.55^b
	N	70.55±3.52^b	72.22±4.07^c
	p-value	0.031	0.000
N	S	42.33±5.19^a	47.55±6.49^a
	C	54.11±4.40^{ab}	67.88±5.27^b
	N	66.11±4.86^s	78.88±4.29^b
	p-value	0.007	0.002
D	S	40.22±4.35^a	48.66±5.58^a
	C	52.55±4.44^{ab}	61.66±5.71^{ab}
	N	60.44±4.26^b	74.55±5.19^b
	p-value	0.011	0.011

Keys: J–January, F- February, M – March, A- April, M –May, J-June, J-July, A-August, S –September, O- October, N-November, D-December. S - South, C - Central, N – North, THBC- Total heterotrophic bacterial counts. Values with the similar superscripts indicates no statistically significant difference ($p>0.05$).

Table 2: Comparative study of bacterial loads from earthen fish pond waters in Edo State, Nigeria

Months	Locations	THBC ($x10^6$ cfu/ml)	Total coliform ($x10^6$ cfu/ml)
J	S	41.22±4.49	38.22±4.89[a]
	C	60.00±3.21	52.55±4.44[ab]
	N	69.55±1.23	60.44±4.26[b]
	p-value	0.000	0.011
F	S	41.77±4.01[a]	42.77±6.51[a]
	C	54.44±4.41[a]	54.55±4.24[b]
	N	68.44±4.00[b]	67.22±4.73[b]
	p-value	0.001	0.001
M	S	45.33±5.66[a]	48.00±6.13[a]
	C	60.00±5.68[ab]	65.00±4.16[b]
	N	69.66±5.46[b]	75.77±2.63[b]
	p-value	0.025	0.001
A	S	38.44±4.81[a]	44.77±7.09[a]
	C	62.00±5.70[ab]	58.11±5.76[ab]
	N	75.00±4.97[b]	66.77±5.56[b]
	p-value	0.014	0.098
M	S	42.11±4.10[a]	45.77±5.98[a]
	C	54.11±4.40[ab]	58.22±4.77[ab]
	N	66.11±4.86[b]	68.22±5.15[b]
	p-value	0.007	0.013
J	S	49.11±5.70[a]	39.22±5.35[a]
	C	60.22±3.77[b]	58.00±6.20[ab]
	N	71.22±4.17[b]	74.33±5.14[b]
	p-value	0.000	0.006
J	S	44.88±5.85[a]	44.66±5.46[a]
	C	61.88±5.79[ab]	59.22±5.77[ab]
	N	75.22±5.43[b]	71.66±6.02[b]
	p-value	0.007	0.008
A	S	34.77±4.17[a]	44.22±5.90[a]
	C	59.33±3.72[b]	55.55±4.23[a]
	N	71.88±4.18[c]	68.22±4.02[b]
	p-value	0.000	0.002
S	S	44.44±4.09	42.88±4.97[a]
	C	58.11±5.76	53.88±4.62[ab]
	N	66.77±5.56	64.11±5.17[b]
	p-value	0.098	0.013
O	S	46.11±5.71[a]	42.33±4.39[a]
	C	59.00±4.28[b]	59.00±3.86[ab]
	N	73.22±4.15[b]	71.00±4.93[b]
	p-value	0.002	0.005
N	S	42.33±5.10[a]	47.55±6.49[a]
	C	62.55±5.64[ab]	56.66±4.13[b]
	N	73.44±5.02[b]	66.33±4.64[b]
	p-value	0.048	0.007
D	S	40.22±4.35[a]	48.66±5.58[a]
	C	56.33±5.66[ab]	56.77±5.97[ab]
	N	69.44±5.89[b]	66.55±5.58[b]
	p-value	0.068	0.109

Keys: J–January, F- February, M – March, A- April, M –May, J-June, J-July, A-August, S –September, O- October, N-November, D-December. S - South, C - Central, N - North, THBC-Total heterotrophic bacterial counts. Values with the similar superscripts indicates no statistically significant difference ($p>0.05$).

Table 3: Comparative study of bacterial loads from plastic fish pond waters in Edo State, Nigeria

Months	Locations	THBC (x10^6 cfu/ml)	Total coliform (x10^6 cfu/ml)
J	S	43.33±5.94^a	40.22±4.35^a
	C	53.33±4.88^{ab}	52.55±4.44^{ab}
	N	67.00±3.85^b	60.44±4.26^b
	p-value	0.009	0.011
F	S	39.88±4.24^a	41.44±4.38^a
	C	57.22±3.89^b	56.44±3.80^b
	N	71.00±4.10^c	65.77±2.76^b
	p-value	0.000	0.000
M	S	46.66±5.56^a	52.77±4.46^a
	C	60.00±5.68^{ab}	65.00±4.16^b
	N	69.66±5.46^b	75.77±2.63^b
	p-value	0.025	0.001
A	S	48.11±6.15^a	40.22±5.86^a
	C	58.11±5.76^{ab}	53.88±4.62^{ab}
	N	66.77±5.56^b	64.11±5.17^b
	p-value	0.098	0.013
M	S	45.44±5.03^a	39.77±4.50^a
	C	58.22±4.77^{ab}	55.55±3.09^b
	N	68.22±5.15^n	69.11±4.31^c
	p-value	0.013	0.000
J	S	49.11±5.70^a	42.77±5.81^a
	C	57.66±8.58^{ab}	58.77±4.58^b
	N	74.55±4.58^b	69.55±4.19^b
	p-value	0.033	0.003
J	S	44.88±5.85^a	48.00±4.98^a
	C	52.11±5.35^{ab}	60.88±4.36^{ab}
	N	67.22±4.90^b	70.44±4.08^b
	p-value	0.022	0.006
A	S	34.77±4.17^a	43.66±4.38
	C	59.33±3.72^b	55.55±4.23^b
	N	71.88±4.18^c	68.22±4.02^{ab}
	p-value	0.000	0.002
S	S	45.22±7.31^a	38.33±4.90^a
	C	61.33±5.45^{ab}	54.33±3.65^b
	N	70.22±5.17^b	67.44±3.56^c
	p-value	0.024	0.000
O	S	43.44±5.65^a	46.11±5.71^a
	C	59.22±5.77^{ab}	58.33±4.43^{ab}
	N	71.66±6.02^b	70.55±3.52^b
	p-value	0.008	0.004
N	S	44.77±6.15^a	44.22±4.99^a
	C	58.00±6.20^{ab}	57.77±4.53^{ab}
	N	74.33±5.14^b	71.44±4.74^b
	p-value	0.006	0.002
D	S	49.44±4.18^a	31.88±3.76^a
	C	64.44±6.00^{ab}	31.44±5.59^a
	N	73.00±5.30^b	54.66±2.93^b
	p-value	0.013	0.007

Keys: J–January, F- February, M – March, A- April, M –May, J-June, J-July, A-August, S –September, O- October, N-November, D-December. S - South, C - Central, N – North, THBC - Total heterotrophic bacterial counts. Values with the similar superscripts indicates no statistically significant difference ($p>0.05$).

Table 4: Molecular identification of bacterial isolates from fish pond water samples

Isolates	Closest Match	Percentage Identity	Sequence ID
1	*Klebsiella pneumoniae*	94.9	NR-1176831
2	*Streptococcus pasteurianus*	98.7	NR-04360.1
3	*Acinetobacter nosocomialis*	99.3	NR-117931.1
4	*Pseudomonas fluorescens*	98.5	NR-115715.1
5	*Serratia marcescens*	98.4	NR-036886.1
6	*Citrobacter freundii*	99.2	NR-113340.1
7	*Staphylococcus aureus*	99.0	NR-037007.2
8	*Esherichia coli*	99.3	NR-025520.1
9	*Staphylococcus sciuri*	96.1	NR-114419.1

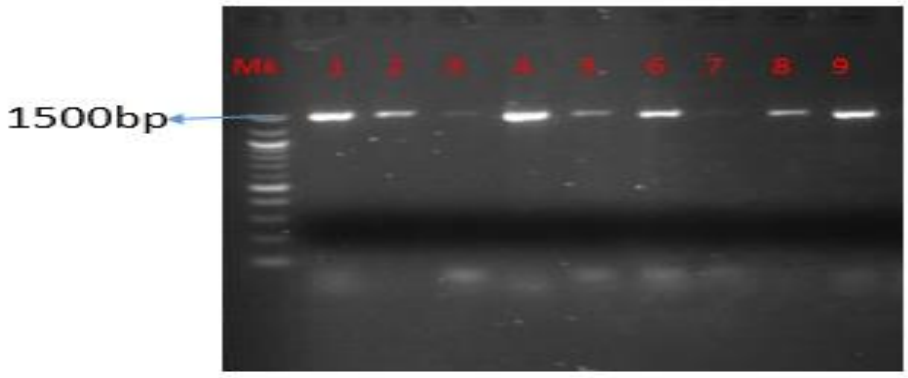

Plate 1: Agarose gel electrophoresis of the PCR products of nine bacteria samples isolated from fish pond water samples. Band size approximately 1500 bp confirms positive amplification. Identified isolates from 1-9 are *Klebsiella pneumonia, Streptococcus pasteurianus, Acinetobacter nosocomialis, Pseudomonas fluorescens, Serratia marcescens, Citrobacter freundii, Staphylococcus aureus, Staphylococcus sciuri* and *Escherichai coli* respectively.

Table 5: Antibiotic susceptibility pattern of bacterial Isolates from fish ponds water

GRAM POSITIVE	No. of Isolates	GEN	AUG	CRX	RAN	ERY	OFL	CPT	CXC	MARI
Staphylococcus sciuri	22	4(18.2)	2(9.1)	5(22.7)	5(22.7)	2(9.1)	2(9.1)	6(27.3)	3(27.3)	0.75
Streptococcus pasteurianus	53	2(3.8)	2(3.8)	2(3.8)	2(3.8)	6(11.3)	12(22.6)	14(26.4)	12(22.6)	1.00
S. aureus	61	34(55.7)	26(42.6)	30(49.2)	37(60.7)	18(29.5)	18(29.5)	26(42.6)	30(49.2)	1.00
GRAM NEGATIVE	No. of Isolates	RAN	CRX	GEN	CTR	OFL	AUG	NIT	CXC	MARI
Escherichai coli	40	20(50.0)	23(57.5)	12(10.0)	15(37.5)	21(52.5)	9(22.5)	12(30.0)	11(27.5)	1.00
K. pneumonia	47	13(27.7)	5(10.6)	5(10.6)	17(36.2)	19(40.4)	22(46.8)	27(57.4)	13(27.6)	1.00
A. comialis	18	3(16.7)	2(11.1)	6(33.3)	9(50.0)	5(27.8)	10(55.6)	10(55.6)	5(27.8)	1.00
S. marcescens	35	17(48.6)	15(42.8)	15(42.8)	22(62.9)	20(57.1)	20(57.1)	12(34.3)	16(45.7)	1.00
C. freundii	67	35(52.5)	26(38.8)	20(29.9)	10(14.9)	10(14.9)	10(14.9)	23(34.3)	22(32.8)	1.00
P. fluorescens	55	12(21.8)	10(18.2)	15(27.3)	15(27.3)	25(45.5)	25(45.5)	25(45.5)	30(54.5)	1.00

Keys: R – Resistant, S – Sensitivity, OFL- ofloxacin, CRX – cetriazone, NIT-nitrofurantoin, CXC-cloxacillin, CRX-cefuroxime, ERY – erythromycin, AUG – augmentin, GEN – gentamicin, CPT- ciprofloxacin, CAZ-ceptrazidine, CTR –Ceftriazone, RAN–rancef. MARI – Multiple Antibiotic Resistant Index, R = Resistance, S = Sensitivity, I = Intermediate, Sensitivity standard for disc : 0-11 resistant, 12-14 intermediate, 14-30 susceptible, MARI- Multiple antibiotics resistant index
MARI ≥ 0.2 (public health significance).

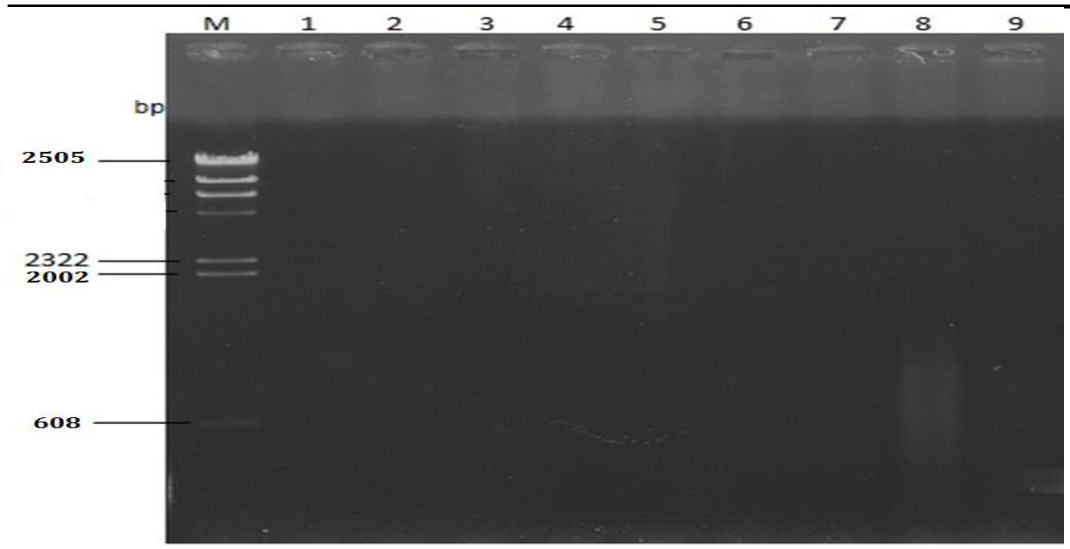

Plate 2: Plasmid profile of the fish pond water samples resistant bacterial isolates
Lane 1-9 are; *Klebsiella pneumoniae, Streptococcus pasteurianus, Pseudomonas fluorescens, Serratia marcescens, Acinetobacter nosocomialis, Escherichai coli, Citrobacter freundii,, Staphylococcus aureus* and *Staphylococcus sciuri* isolated from fish pond water were found to carry antibiotic resistant genes that are not encoded in the plasmids.

DISCUSSION

Physicochemical properties of the water used to cultivate fish must be optimal for fish and other aquatic creatures if not, the water will not yield maximally. The initiation of fish (*Clarias garipinus*) farming in this study is influenced by water temperature, which is an important parameter. Setting baseline conditions and criteria for water quality is crucial.

Gram negative bacteria were found to be the most commonly isolated bacteria from the ponds, according to the findings of the bacteriological study. The Gram negative bacteria isolated were *Escherichai coli*, *Klebsiella pneumoniae*, *Acinetobacter nosocomialis*, *Serratia marcescens*, *Citrobacter freundii* and *Pseudomonas fluorescens*, while Gram positive bacteria isolated included *Staphylococcus sciuri*, *Streptococcus pasteurianus* and *Staphylococcus aureus*. Coliform bacteria found in the fish pond water was an indicator of faecal contamination, which could lead to the presence of pathogenic organisms in fish if their concentration is higher than (10^4 -10^6) in the skin and (10^4 - 10^7 cfu/g). The faeces may have entered the ponds as a consequence of pond fertilization with animal manure that was dumped directly into the fish ponds, or as a result of fish excretion into the ponds (Kay *et al*., 2008). The presence of pathogenic bacteria such as *Klebsiella pneumoniae* in fish ponds could be explained by the fact that *Klebsiella* spp. are considered indigenous bacteria in aquatic environments and are naturally present in fish up to 10^2-10^3 cfu/g, multiplying under favourable temperature conditions above 15 °C. As a result, the pond's temperature promoted *Klebsiella pneumoniae* development and multiplication. Although, *Klebsiella pnuemoniae* is prevalent in the natural flora of the mouth, skin, and intestines, Ryan and Ray (2004) stated that if aspirated (inhaled), it can induce damaging alterations to human and animal lungs, notably to the alveoli (in the lungs), resulting in bloody sputum.

Citrobacter freundii, which was isolated in this investigation, has been found in fish pond water and has been linked to gastrointestinal tract infections, according to Ampofo and Clerk (2010). The *C. freundii* is a soil organism, according to Amand *et al.* (2010), but it can also be found in water,

sewage, food, and the intestinal systems of animals and humans. In immunocompromised patients, *C. freundii* has been linked to opportunistic nosocomial infections of the respiratory tract, urinary tract, blood, and a variety of other typically sterile locations (Rezaei *et al.,* 2016). *Staphylococcus sciuri* can colonize the nasopharynx, skin and the urogenital tract, with low carrier rates in the nasopharynx, skin, and urogenital tract. Peritonitis, endocarditis, septic shock, urinary tract infection, and wound infections are all caused by *Staphylococcus sciuri* which is a zoonotic agent.

Staphylococcus aureus is a persistent and pervasive colonizer of the skin. Although, it is not usually harmful, patients with weakened immune systems are at risk of infection. *Staphylococcus aureus* is the organism that causes impetigo, folliculitis, septic arthritis, osteomyelitis, septicaemia, pneumonia, and meningitis, among other disorders (Kozitskaya *et al.,* 2005). *Staphylococcus aureus* could have gotten into the fish pond water by fish faeces or contact with contaminated soil surfaces. Consumption of undercooked fish raised in these ponds, or contact with infected fish and water, can cause urinary tract infections and osteomyelitis. The different families of bacteria identified from these ponds are consistent with Okpokwasili and Ogbulie (1998) findings on pond water, which suggested that bacteria from pond feed constitute the primary source of bacteria of health concern. In a microbiological examination of an Elquanter fish pond, Daboor (2008) found similar organisms. *Serratia marcescens* is a Gram negative bacillus that lives in the soil and releases a red pigment when exposed to light. Bacteria can be found in a variety of areas in our environment, including human and animal wastes, dust, soil, and surface waterways. *Serratia marcescens* was found in all of the ponds, particularly the earthen pond, due to the fact that it is a naturally occurring soil bacteria in this study, which contradicts a previous finding by another researcher (Ikpi and Offem 2011). *Pseudomonas fluorescens* is a Gram negative, nonpathogenic saprophyte that colonizes soil, water, and plant surfaces. It secretes a soluble greenish fluorescent pigment called fluorescein, which is especially useful when there is a lack of iron. Except for few strains that can use NO_3 as an electron acceptor instead of O_2, it is an obligate aerobe. Multiple polar flagella help it to move around. *Pseudomonas fluorescens* has minimal nutritional needs and thrives on mineral salts media supplemented with a variety of carbon sources. It was documented in all of the fish ponds in this investigation because they are well acclimated to soil. In both concrete and earthen ponds, *Klebsiella pneumoniae* was the most common organism (Ampofo and Clerk, 2010). Food poisoning has been linked to *Pseudomonas* sp., *Proteus* sp. and *Staphylococcus* sp. (Oni *et al*., 2013). In both ponds, *Acinetobacter nosocomialis* was found. This bacterium is one of the most opportunistic pathogens for freshwater fish, and it is one of the principal etiological agents in illness outbreaks in India, where multiple deaths have been reported (Das and Mukheyce, 1999).

Antibiotic resistance of the bacterial isolates from fish pond water revealed that all Gram negative bacteria such as *Escherichai coli, Klebsiella pneumoniae, Acinetobacter nosocomialis, Serratia marcescens, Citrobacter freundii* and *Pseudomonas fluorescens* were resistant to all tested antibiotics such as gentamycin, ciprofloxacin, ofloxacin, augumetin, nitrofurantoin and cloxacilin. *Streptococcus pasteurianus* and *Staphylococcus aureus*, both Gram positive bacteria, were likewise resistant to all of the antibiotics tested. This could be due to the overuse of antibiotics in clinical practice, particularly in asymptomatic patients (Dick *et al*., 2015). This is contrary to the report of Abu and Wondikom (2018) who reported *Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa,* and *Klebsiella* spp. were susceptible to augmetin, cetriazone, gentamycin, cloxacillin,

erythromycin, rancef, tarivid and ciprofloxacin. The resistance of *Citrobacter freundii* to all the antibiotics is contrary with the study of Fish *et al*. (2015). Most antibiotic resistance in bacterial isolates is likely attributable to chromosomally or plasmid-mediated resistant genes in the bacteria's genetic make-up. This is associated with indiscriminate usage of antibiotics. This finding concurs with Nain *et al*. (2015) who found that *Klebsiella* sp. and *Citrobacter* sp. were resistant to ceftazidime, ciprofloxacin, amoxicillin, gentamicin, tobramycin, ofloxacin, clindamycin, oxacillin, erythromycin and cefotazime.

Most of the tested isolates from cat fish showed multiple antibiotic resistances to the tested antibiotics especially, *Citrobacter freundii, Streptococcus pasteurianus* and *Pseudomonas fluorescens* which showed resistance to gentamycin, augumentin and ciproflxacin. This is similar with the studies of Poonia *et al*. (2014) who reported *Citrobacter freundii, Pseudomonas* spp. and *Klebsiella* spp. to be multidrug resistant to augmetin, gentamycin, ampiclox, pefloxacin, zinnacef and ciprofloxacin. Also, *Staphylococcus aureus, Escherichai coli, Klebsiella pneumoniae, Acinetobacter nosocomialis* and *Serratia marcescens* isolated from cat fish in this study were found to be resistant to five or more antibacterial agents tested. Lupo *et al.* (2012) previously reported that antibiotic resistance in bacteria has developed considerably over the last decade, which supports this finding.

The variations in the multiple antibiotic resistance bacterial isolates from fish pond water and cat fish observed in this study may be due to multi drug resistance to strains of the bacterial isolates (Diwan *et al*., 2010; Subramani and Vignesh, 2012). Antibiotics are employed in the treatment of bacterial infections in both humans and animals like fishes. Abuse of antibiotics induces resistance by bacterial isolates in and around the fish farms or and transfer of resistance along the food chains (Diwan *et al*., 2010). Antibiotic resistance genes were found in bacteria isolated from fish and fish pond water in this investigation. Bacteria on fish farms and in the surrounding environment may develop antibiotic resistance as a result of antibiotics released into the environment from nearby households and/or hospitals, as well as other fish farms and animal farms. Pathogenic fish bacteria may have a higher multiple antibiotic resistance (MAR) index due to their exposure to a wide spectrum of antibiotics, in addition to antibiotics particularly administered for disease control on fish farms. Antibiotics resistant genes were found in multiple-antibiotic-resistant *Klebsiella pneumoniae, Streptococcus pasturianus, Pseudomonas fluorescens, Serratia marcescens, Citrobacter freundii, Staphylococcus aureus* and *Staphylococcus sciuri* isolated from fish pond water, which agrees with Cernat *et al*. (2007). Previous researchers studied the incidence of antibiotic resistant genes in Gram negative bacteria from fish ponds (Nawaz *et al.,* 2006; Balta *et al.,* 2010; Boran *et al.,* 2013).

CONCLUSION

The study revealed that the ponds contaminated with pathogenic bacteria that could affect fish cultivated, since the microbial quality of any fish pond water is a reflection of the microbial flora of the fish itself. These organisms could lower fish yield, causes disease and economic loss and equally endanger the ultimate consumers (humans) particularly if the fish harvested from the ponds are under processed.

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