Original Research Article

ISOLATION AND CHARACTERIZATION OF PLASMID-BEARING MULTIPLE ANTIBIOTIC RESISTANCE BACTERIA FROM DIFFERENT AQUATIC SOURCES IN AKURE, NIGERIA.

ABSTRACT

Aims: This study was designed to investigate the plasmid bearing multiple antibiotic resistance bacteria from different aquatic sources.

Place and Duration of Study: This research work was carried out in Akure south area of Ondo state, Nigeria between January, 2018 and June, 2018.

Methodology: The pathogenic bacteria associated with water samples collected from different sources in Akure, Nigeria were isolated and characterized. A total of 521 water samples were collected between January and June, 2018 from sources such as wells, taps, streams, rivers, boreholes and rain. All the samples were subjected to presumptive, confirmed and completed tests to evaluate their microbiological quality. The microbial types in the samples were determined using standard microbiological techniques. All isolates obtained in this study were subjected to antibiotic sensitivity analysis and screened for Beta-lactamase production (ESBL). Plasmid-gene profile analysis of the resistance isolates were carried out using standard method. Furthermore, post curing of the plasmid mediated antibiotic resistance isolates were carried out and data obtained were analyzed and presented using analysis of variance.

Results: Bacterial isolates such as *Acinetobacter baumanni*, *Citrobacter freundii*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurum*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Serratia marcescens*, *Proteus vulgaris* and *Vibrio cholerae* were identified from the water samples. The isolate *E. coli* had the highest percentage distribution of 24.10% in well water and 26.19% in stream water while *Salmonella species* had the highest occurrence of 53.85% in rain water. The Beta-lactamase producing (ESBL) isolates were resistant to multiple antibiotics except Ciprofloxacin, Gentamycin and Pefloxacin that confered antibacterial effect. Plasmid-gene profile analysis of the isolates revealed that *S. typhimurum*, *K. pneumoniae*, *P. aeruginosa* and *P. vulgaris* possess single plasmid each while only *E. coli* contain two plasmid bands. The post plasmid-curing antibiotic sensitivity test of the isolates revealed that the initial antibiotic resistance of the bacterial isolates were plasmid mediated.

Conclusion: Findings from this study suggest the purification of water from these sources before consumption as most microbes found in these samples are potential pathogens that are capable of causing infectious diseases with multiple antibiotic resistant features.

Keywords: Isolation, Characterization, Antibiotic Resistant Bacteria; Aquatic sources, Plasmid profile analysis; Beta-lactamase production.

Comment [F1]: Arrange in alphabetical order

1. INTRODUCTION

Africa faces huge challenges with multiple issues that adversely affect public health, one of which is the ability for both rural and urban Africans to access a clean water supply. According to the WHO [1], only 59% of the world's population had access to adequate sanitation systems, and efforts to achieve the Millennium Development Goal, which is aiming for 75% by the year 2015, has fallen short by nearly half a billion people. The potable water sources most accessible to inhabitants in rural African are largely dams, wells, rivers, streams, ponds, which might harbor pathogen that cause diseases such as diarrhea, cholera, typhoid fever, river blindness, Schistosomiasis among others. Antibiotic resistance is a form of drug resistance whereby some sub-populations of microorganisms, usually bacterial species, are able to survive after exposure to one or more antibiotic [2]. Antibiotic resistance may result from antibiotic resistance genes residing on transmissible plasmids thus facilitating their transferor antibiotic drug misuse by respondents. Therefore, this study aims at isolating and identifying plasmid-bearing multiple antibiotics resistant gram negative bacteria from different water sources in Akure south local government.

2. MATERIALS AND METHODS

2.1 Description of Study Location

This research work was carried out between January, 2018 and June, 2018 in Akure south area of Ondo state, Nigeria. Akure covers an area of 14,798.8 ,993.7 square kilometers and lies at latitude 7°15′0″N, 70 11′ N 5°11′42″E and longitude 5°11′42’E, 5^{o}35’E [3].

2.2 Sample Collection and Processing

A total of 521 water samples were collected into sterile containers from different areas in Akure metropolis between February 2018 and June 2018. The water samples were collected aseptically from different sources such as tap, borehole, and well, stream, rain and swimming pool. All samples obtained were analyzed microbiologically within 4 hours of collection.

2.3 Test for water quality

The test for quality of the water samples were carried out as described by Cheesbrough [4]. Lactose broth containing Durham tubes were prepared in test tubes. These tubes were inoculated with 1 ml of water sample each and incubated at $37^{O}C$ for 24 hours. Thereafter, lactose broth was examined for change in colour (fermentation) and gas production. Also, plates of Levine Eosine Methylene blue were streaked with the isolates that were able to ferment lactose and subsequently incubated at $37^{O}C$ for 24 hours. Production of Greenish metallic sheen on the plate after the incubation time indicated the presence of *Escherichia coli* while the presence of nucleated colonies (large dark centre) indicated the presence of Gram-negative lactose fermenter (coliform). The isolated microbes were kept and maintained on nutrient agar slants prepared. A Gram-stained slide was made from the slant, and the slide was examined under oil immersion optics. If the organism proves to be a Gram-negative, non-spore-forming rod that ferments lactose, the presence of coliforms was confirmed in the tested water sample.

2.4 Isolation and Identification of bacteria

The pour plate technique was employed in the isolation of bacteria from the water samples as described by Olutiola [5]. 1 mL each of the water samples were pour-plated and incubated at $37^{O}C$ for 24 hours. The media used for the isolation include: Salmonella Shigella Agar, Eosine Methylene Blue Agar and Nutrient Agar. Distinct colonies were then subcultured to obtain pure cultures on which Gram staining and other biochemical tests [Sugar fermentation (glucose, sucrose, lactose, mannitol and triple salt iron), Methyl Red/VogesProskauer, Indole, Nitrate reduction, Oxidase, Coagulase, Citrate, Urease, Motility and Catalase tests] were carried out. The methods described by Willey [6] were adopted for characterization of isolated bacteria. The isolates were further identified with reference to the Bergey's manual of systematic bacteriology [7].

2.5 Standardization of Bacterial inoculum for Sensitivity Test

The McFarland's standard of a one percent (1%v/v) sulphuric acid solution was prepared with one per cent (1%w/v) solution of Barium Chloride ($BaCl.2H_2O$). The turbid solution formed was transferred into a test tube containing 2.0 mL of normal saline until the suspension matches the turbidity of the standard (1% barium sulphate).

2.6 Antibiotic Sensitivity Test of Bacterial Isolates

The Kirby-Bauer test was used to determine the effect of standard antibiotics on bacterial isolates on Mueller Hinton agar. The agar was seeded with 18 hold pure broth cultures of each isolates [8]. Aseptic swabs of the identified bacteria isolates were made on solidified Mueller Hinton Agar. The discs were applied unto the surface of plates and incubated for 24 h at $37^{O}C$ with control as sterile distilled water [9]. The bacterial isolates were tested against a wide range of antibiotics namely; Ofloxacillin (5µg), Amoxicillin (25µg), Ciprofloxacin (10µg), Tetracycline (30µg), Pefloxacin (5µg). Thereafter, a ruler caliberated in millimeter (mm) was used to measure the diameter of the clear zones of inhibition observed on the plates and this was noted as degree of antibiotic resistance as described by [9]. The isolates' zones of inhibition was classified into susceptible (17mm and above), intermediate (13mm-17mm), and resistant (0-12mm) based on the specified standard of mean zone of inhibition for pathogenic gram positive and gram negative bacteria respectively [9].

2.7 Molecular Characterization of Multiple Antibiotic Resistant Bacteria via Plasmid Profile

Plasmid profile analysis of the multiple antibiotic resistant bacteria isolates were carried out using protocols described by Chan [10] and Matsui [11]. Thereafter, a 1% SDS-PAGE gel was prepared and loaded into electrophoresis chamber containing between 4 wells; this was buffered with 20 mM sodium acetate, 2mM EDTA and then adjusted to pH 7.8 with acetic acid. The sample buffer contained 25% sucrose, 5mM sodium acetate, 0.05% bromophenol blue and 0.1% SDS. Electrophoresis was allowed to proceed at room temperature. After electrophoresis, gels were stained with ethidium bromide (1µl/ml) and observed with UV transillumination. The molecular marker used was the bacteriophage *Hind III* digest. The multiple antibiotic resistant isolates were cured of their plasmid afterwards by exposing overnight grown bacterial cultures at $37\ ^{O}C$ with 10mg/mL of ethidium bromide by adopting the methods described in Birnboim and Dolly [12] as well as Brown [13].

2.8 Antibiotic Sensitivity Test after Plasmid Curing

The characterized multiple antibiotic resistant bacterial isolates were subjected to antibiotic sensitivity test post plasmid curing using broad spectrum antibiotics by adopting the method described in Matsui [11].

2.9 Data Analysis

Analyzed sample treatments were in triplicates and data means obtained were subjected to a 2-way analysis of variance. The treatment means were separated using Duncan's New Multiple Range test at $P\leq 0.05$ levels of significance.

3. RESULTS AND DISCUSSION

3.1 Water Samples Quality and Percentage Frequency Distribution of Bacteria Isolates

The quality of water and the frequency of distribution of bacteria isolated from the different water sources are presented in Table 1.

3.2 Microscopic and Biochemical Characteristics of Bacterial Isolates from Water Samples

The characterization of the bacterial isolates obtained from the water samples across the different locations are presented in Table 2. The Gram negative bacteria isolates include: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Salmonella typhi*, *Salmonella typhimurum*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Serratia marcescens*, *Proteus vulgaris* and *Vibrio cholerae*.

Table 1: water quality and percentage distribution of the bacteria isolated from different water sources

T.I	Gram st	Glu	Suc	Lac	Ma	TSI	O/C	Mt	Ci	Ur	Ni	In	Mr	VP	COT	NA	EMB

Sources	Water Quality				No of Isolates / Frequency of Distribution (%)											
	PT	CFT	CT	(NO/F.D)	AB	CF	EA	EC	KP	PV	PA	SS	SM	SD	VC	TOTAL
RAIN	+ve	GMT	CC	NO	-	-	-	36	12	-	24	84	-	-	-	156
				F.D	-	-	-	23.07	7.69	-	15.38	53.85	-	-	-	100
STREAM	+ve	GMT	CC	NO	12	18	60	132	60	24	48	72	36	36	-	504
				F.D	2.38	4.76	11.90	26.10	11.90	4.76	9.52	14.28	7.14	7.14	-	100
S.P	-ve	-ve	-ve	NO	-	-	-	-	-	-	24	-	-	12	-	36
				F.D	-	-	-	-	-	-	66.67	-	-	33.3	-	100
TAP	+ve	GMT	CC	NO	12	-	36	84	48	-	156	216	-	84	-	636
				F.D	1.89	-	5.67	13.21	7.55	-	24.52	33.16	-	13.20	-	100
WELL	+ve	GMT	CC	NO	24	36	108	564	108	60	540	444	48	408	-	2340
				F.D	1.02	1.54	4.62	24.10	4.62	2.56	23.00	18.97	2.05	17.44	-	100
B.H	+ve	GMT	CC	NO	6	-	18	40	20	10	22	50	19	12	-	97
				F.D	3.04	-	9.13	20.30	10.15	5.07	11.06	25.38	9.64	6.09	-	100

Keys: AB= *Acinetobacter baumanni*, CF= *Citrobacter freundii*, EA= *Enterobacter aerogenes*, EC= *Escherichia coli*, KP= *Klebsiella pneumonia*, PA= *Pseudomonas aeruginosa*, PV= *Proteus vulgaris*, SP= *Salmonella paratyphi*, ST= *Salmonella typhi*, STY= *Salmonella typhimurum*, SM= *Serratia marcescens*, SD= *Shigella dysenteriae*, VC= *Vibrio cholerae,* PT= Presumptive Test, CFT= Confirmed test, CT= Completed Test, NO= Number of Isolates, F.D= Frequency of Distribution(%), GMT= Green metallic Sheen , CC=Coliform Confirmed.

Table 2: Biochemical Characteristics of Bacterial Isolates from Water Samples

A.B	-ve	+ve		-ve	+ve	+ve	-ve/+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	Blue/R	+ve(Bluish)
C.F	-ve	+ve	+ve	+ve	+ve	+ve	-ve/+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	Grey/F	+ve
E.A	-ve	+ve	+ve	+ve	-ve	-ve	-ve/+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	Cream/R	+ve(BDC)
E.C	-ve	+ve	+ve	+ve	+ve	-ve	-ve/+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	Cream/R	+ve(GMS)
K.P	-ve	+ve	+ve	+ve	+ve	-ve	-ve/+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	Cream/R	+ve(BDC)
P.A	-ve	-ve	-ve	-ve	-ve	-ve	-ve/+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	Cream/R	+ve(Pink)
P.V	-ve	+ve	+ve	-ve	-ve	+ve	-ve/+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	Blue/F	+ve(Pink)
S.P	-ve	+ve	-ve	-ve	+ve	+ve	-ve/+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	Cream/F	+ve
S.T	-ve	+ve	+ve	+ve	+ve	+ve	-ve/+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	Black/F	+ve
S.TY	-ve	+ve	-ve	-ve	+ve	+ve	-ve/+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	Cream/F	+ve
S.M	-ve	+ve	-ve	-ve	-ve	-ve	-ve/+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	Red/F	+ve(Red)
S.D	-ve	+ve	-ve	-ve	+ve	-ve	-ve/+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	Pink/R	+ve
V.C	-ve	+ve	+ve	-ve	+ve	-ve	+ve/+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	Yellow/R	+ve(Yellow)

Keys: AB-*Acinetobacter baumanni*, CF-*Citrobacter freundii*, EA-*Enterobacter aerogenes*, EC-*Escherichia coli*, KP-*Klebsiella pneumonia*, PA-*Pseudomonas aeruginosa*, PV-*Proteus vulgaris*, SP-*Salmonella paratyphi*, ST- *Salmonella typhi*, STY-*Salmonella typhimurum*, SM-*Serratia marcescens*, SD-*Shigella dysenteriae*, VC-*Vibrio cholerae*. +ve-positive, -ve - negative, Gl-Glucose, Su-Sucrose, La-Lactose, Ma-Mannitol, TSI-Triple Sugar Iron test, O/C-Oxidase/catalase, Mt-Motility test, Ci- Citrate, Ur- Urease, Ni-Nitrate test, IN- Indole, MR- Methyl Red, VP- Voges-proskaurs, COT- Coagulase test, NA-Nutrient Agar, EMB-Eosine Methylene Blue agar, BDC-Black Dark centre, GMS- Greenish Metallic Sheen, T.I.- Tentative Identity.

3.3 Antibiotics Sensitivity Pattern of the Bacterial Isolates

The results of the antibiotics sensitivity pattern of the bacterial isolates before plasmid curing based on their zones of inhibitions subjected to statistical analysis at $p \leq 0.05$ levels of significance are presented in table 3, while their deduced antibiotic resistance patterns of the multiple antibiotic resistant bacteria are presented in table 4. The resistance patterns were denoted by comparison of analyzed data with accepted standards for Gram negative bacteria. The zones of inhibition ranges from 10.00±0.577 mm to 24.67±0.577 mm with septrin being the least effective on *Acinetobacter baumanni* and Ciprofloxacin being the highest on *Escherichia coli*

The antibiotic resistance patterns in Tables 4 were all denoted as either Susceptible (S) at ≤ 16.00 mm and above, Intermediate (I) at ≤ 12.00 - 15.00 mm or Resistant (R) at ≤ 11.00.

3.4 Plasmid Profiles of Multiple Antibiotic Resistant Bacterial Isolates

The Gram negative bacterial isolates which were resistant to more than two antibiotics were termed multiple antibiotic resistant isolates (MDRIs) and were subsequently profiled for plasmid analysis as represented in the electropherogram (Plate 1).

From this plate, isolate in lane 2, 6, 7, 8 and 11, all have plasmid band with isolate in lane 11 showing double bands. All of these isolates have plasmid band ranging from 1567bp to 2027bp

The electropherogram depicts the different plasmid sizes of the profiled Gram negative producing MDRIs and their magnitudes.

Table 3: Antibiotics Sensitivity pattern of the Isolated bacteria

1	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
AB	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	15.67±0.577^{d}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}
CF	13.67±0.577^{c}	15.67±0.577^{c}	14.67±0.577^{d}	14.67±0.577^{d}	11.67±0.577^{b}	10.00±0.577^{a}	13.67±0.577^{c}	15.67±0.577^{c}	18.67±0.577^{e}	13.67±0.577^{c}
EA	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{E}	10.00±0.577^{a}	10.00±0.577^{a}	15.67±0.577^{d}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}
EC	17.67±O.577^{d}	10.00±0.577^{a}	16.67±0.577^{c}	24.67±0.577^{f}	10.00±0.577^{a}	10.00±0.577^{a}	24.67±0.577^{f}	24.67±0.577^{f}	23.67±0.577^{e}	11.67±0.577^{b}
KP	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	14.67±0.577^{d}	10.00±0.577^{a}	10.00±0.577^{a}	14.67±0.577^{d}	10.00±0.577^{a}	10.00±0.577^{E}	10.00±0.577^{a}
PA	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	23.67±0.577^{d}	10.00±0.577^{a}	10.00±0.577^{a}	12.67±0.577^{b}	10.00±0.577^{a}	10.00±0.577^{E}	13.67±0.577^{c}
PV	10.00±0.577	12.67±0.577^{c}	19.67±0.577	19.67±0.577	14.67±0.577^{d}	15.67±0.577^{d}	19.67±0.577^{e}	22.67±0.577	14.67±0.577^{c}	10.00±0.577^{a}
SP	11.67±0.577^{b}	13.67±0.577^{c}	12.67±0.577^{c}	15.67±0.577^{d}	11.67±0.577^{b}	10.00±0.577^{a}	16.67±0.577^{d}	17.67±0.577^{d}	16.67±0.577^{f}	13.67±0.577^{c}
ST	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	12.33±0.577^{c}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	12.33±0.577^{b}	10.00±0.577^{a}
STY	10.00±0.577^{a}	10.00±0.577^{a}	10.00±0.577^{a}	23.67±0.577^{e}	10.00±0.577^{a}	10.00±0.577^{a}	20.46±0.57^{b}	24.67±0.57	22.67±0.577^{c}	20.00±0.000^{a}
SM	17.67±0.577^{d}	15.67±0.577^{d}	21.67±0.577^{b}	19.67±0.577^{e}	10.00±0.577^{a}	10.00±0.577^{a}	17.67±0.577^{d}	21.67±0.577	20.67±0.577^{g}	14.67±0.577^{d}
SD	10.00±0.577^{a}	10.00±0.577^{a}	12.33±4.041^{b}	14.33±0.577^{d}	10.00±0.577^{a}	10.00±0.577^{a}	22.67±0.577^{c}	14.67±0.577^{d}	19.67±0.577^{e}	10.00±0.577^{a}
VC	10.00±0.577^{E}	10.00±0.577^{E}	10.00±0.577^{E}	17.67±0.577^{d}	10.00±0.577^{E}	10.00±0.577^{E}	17.67±0.577^{d}	15.67±0.577^{d}	13.67±0.577^{c}	10.00±0.577^{E}

Means followed by the same letter(s) within the group along the same column are not significantly different at $p \leq 0.05$ levels of significance using Duncan's new multiple range test.

Keys: 1-Isolates, SXT-Septrin, CH-Chloramphenicol, SP-Sparfloxacin, CPX-Ciprofloxacin, AM-Amoxacillin, AU-Augmentin, CN-Gentamycin, PEF-Pefloxacin, OFX-Ofloxacin, S-Streptomycin, AB-*Acinetobacter baumanni*, CF-*Citrobacter freundii*, EA-*Enterobacter aerogenes*, EC-*Escherichia coli*, KP-*Klebsiella pneumoniae*, PA-*Pseudomonas aeruginosa*, PV-*Proteus vulgaris*, SP-*Salmonella paratyphi*, ST- *Salmonella typhi*, STY-*Salmonella typhimurum*, SM-*Serratia marcescens*, SD-*Shigella dysenteriae*, VC-*Vibrio cholerae*

Table 4: Deduced Antibiotics Sensitivity pattern of Multiple Antibiotic Resistant Bacteria

AT	AB	CF	EA	EC	KP	PA	PV	SP	ST	STY	SM	SD	VC
SXT	R	I	R	S	R	R	R	R	R	R	R	R	R
CH	R	I	R	R	R	R	R	R	R	R	R	R	R
SP	R	I	R	I	R	R	R	R	R	R	R	R	R
CPX	R	I	R	S	I	S	R	R	R	R	R	R	R
AM	R	R	R	R	R	R	R	R	R	R	R	R	R
AU	R	R	R	R	R	R	R	R	R	R	R	R	R
CN	I	I	S	S	I	I	R	R	R	R	R	I	R
PEF	R	I	R	S	R	R	S	R	R	I	I	R	R
OFX	R	S	R	S	R	R	I	S	I	S	S	S	I
S	R	I	R	R	R	I	R	S	R	S	S	R	R

Keys: R-Resistance, S-Susceptible, I-Intermediate, AT- Antibiotic, SXT-Septrin, CH-Chloramphenicol, SP-Sparfloxacin, CPX-Ciprofloxacin, AM-Amoxacillin, AU-Augmentin, CN-Gentamycin, PEF-Pefloxacin, OFX-Ofloxacin, S-Streptomycin, AB-*Acinetobacter baumanni*, CF-*Citrobacter freundii*, EA-*Enterobacter aerogenes*, EC-*Escherichia coli*, KP-*Klebsiella pneumoniae*, PA-*Pseudomonas aeruginosa*, PV-*Proteus vulgaris*, SP-*Salmonella paratyphi*, ST- *Salmonella typhi*, STY-*Salmonella typhimurum*, SM-*Serratia marcescens*, SD-*Shigella dysenteriae*, VC-*Vibrio cholerae*

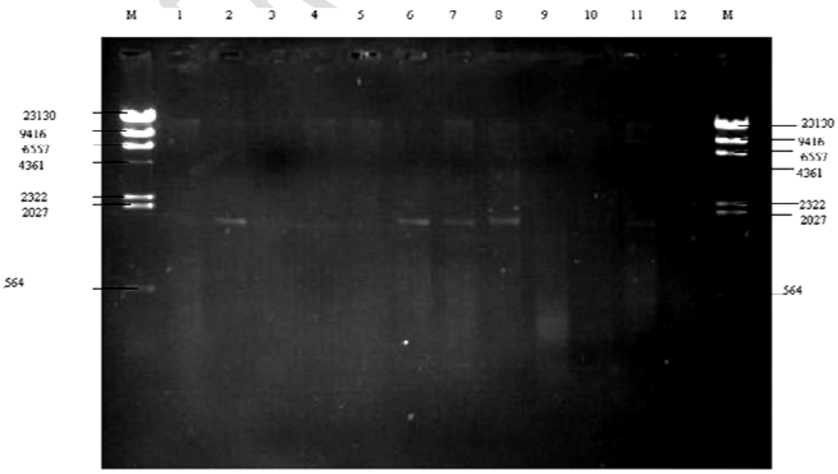

Plate 1: Electropherogram of multiple antibiotic resistant bacteria plasmids of screened isolates

3.5 Post-Curing Antibiotics Sensitivity Analysis of Cured MDRIs Bacterial Isolates

The five multiple antibiotic resistant bacterial isolates that possess plasmid bands were cured of their plasmids. They were then re-subjected to antibiotics sensitivity test to elucidate their resistance pattern. The antibiotic sensitivity pattern of the MDRIs bacterial isolates after plasmid curing are presented in Table 5 while their deduced antibiotic resistant patterns are contained in Table 6. The multiple antibiotic resistant isolates (MDRIs) were screened out of the Gram negative bacterial isolates using sensitivity discs containing Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxacillin, Augmentin, Gentamycin, Pefloxacin, Ofloxacin, and Streptomycin. The zones of inhibition ranges from From 10.67±0.577 mm to 27.667±0.577 mm with chloramphenicol being the least effective on P. vulgaris and Ciprofloxacin being the highest on *Escherichia coli*. From the table, it can be deduced that *E. coli* was resistant to Amoxicillin, Augmentin, Chloramphenicol, while it was susceptible to Ciprofloxacin, Gentamycin, Septrin and Pefloxacin. *Enterobacter aerogenes* was resistant to Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxacillin, Augmentin, Pefloxacin, Ofloxacin, and Streptomycin and they are all significantly different from Gentamycin which had the effect of inhibiting it. Also, *Klebsiella pneumoniae* was resistant to Septrin, Chloramphenicol, Amoxacillin, Augmentin, Pefloxacin, Tarivid, Streptomycin but it was susceptible to Ciprofloxacin and Gentamycin.

Salmonella paratyphi was resistant to Augmentin, Amoxacillin and Septrin while it was susceptible to Pefloxacin, Gentamycin and Ofloxacin, Pefloxacin, *Salmonella typhi* was resistant to Septrin, Chloramphenicol, Sparfloxacin, Amoxacillin, Augmentin, Gentamycin, Pefloxacin, and Streptomycin but was inhibited and by Ciprofloxacin and Ofloxacin. Also, *Pseudomonas aeruginosa* was resistant to Septrin, Chloramphenicol, Sparfloxacin, Amoxacillin, Augmentin, Pefloxacin, Ofloxacin while it was susceptible to Ciprofloxacin, Gentamycin and Streptomycin. Augmentin had no effect on *C. freundii* while Septrin, Gentamycin, Streptomycin were all having the same effect on *C. freundii* and they were significantly different from others which was capable of inhibiting *C. freundii.*

Salmonella typhimurum was resistant to Amoxacillin, Augmentin, Sparfloxacin, Chloramphenicol, Septrin and Streptomycin while susceptible to Gentamycin, Ofloxacin, Ciprofloxacin and Pefloxacin. Only Gentamycin was capable of inhibiting *Acinetobacter baumanni* while it was resistant to the other antibiotics.

Table 5: Post-Curing Antibiotics sensitivity analysis of cured Bacteria Isolates

1	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
E.C	21.667±0.577^{d}	19.667±0.577^{d}	18.667±0.577^{c}	27.667±0.577^{f}	21.667±0.577^{d}	20.667±0.577^{d}	25.667±0.577^{f}	25.667±0.577^{f}	23.667±0.577^{e}	19.667±0.577^{d}
K.P	15.667±0.57^{b}	15.667±0.57^{b}	19.667±0.57^{d}	25.667±0.57^{f}	14.667±0.57^{b}	17.667±0.577^{c}	24.667±0.57^{e}	19.667±0.57^{d}	16.667±0.577^{c}	16.667±0.577^{c}
P.A	17.667±0.577^{c}	15.667±0.577^{c}	19.667±0.577^{d}	20.667±0.57^{d}	13.667±0.57^{b}	11.667±0.577^{a}	22.667±0.57^{e}	18.667±0.57^{c}	23.667±0.577^{e}	17.67±0.577^{c}
P.V	11.667±0.577^{a}	10.67±0.577^{a}	19.667±0.577^{d}	19.667±0.57^{d}	14.667±0.577^{b}	15.667±0.577^{b}	19.667±0.57^{d}	22.667±0.57^{e}	14.667±0.57^{b}	10.67±0.57^{a}
S.T	21.667±0.577^{d}	19.667±0.577^{d}	18.667±0.577^{c}	27.667±0.577^{f}	21.667±0.577^{d}	20.667±0.577^{d}	25.667±0.577^{f}	25.667±0.577^{f}	23.667±0.577^{e}	19.667±0.577^{d}

Keys: R-Resistance, S-Susceptible, I-Intermediate, SXT-Septrin, CH-Chloramphenicol, SP-Sparfloxacin, CPX-Ciprofloxacin, AM-Amoxacillin, AU-Augmentin, CN-Gentamycin, PEF-Pefloxacin, OFX-Ofloxacin, S-Streptomycin, E.C- *Escherichia coli*, K.P- *Klebsiella pneumoniae*, P.A- *Pseudomonas aeruginosa*, P.V- *Proteus vulgaris,* S.T-*Salmonella typhi*, 1- Isolates.

Table 6: Deduced Antibiotics Sensitivity Patterns of Multiple Antibiotics Bacteria after Plasmid curing

1	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
E.C	S	S	S	S	S	S	S	S	S	S
K.P	I	I	S	S	I	S	S	S	I	I
P.A	I	I	I	I	I	I	S	I	S	I
P.V	I	R	S	S	I	I	S	S	I	R
S.T	S	S	S	S	S	S	S	S	S	S

Keys: R-Resistance, S-Susceptible, I-Intermediate, SXT-Septrin, CH-Chloramphenicol, SP-Sparfloxacin, CPX-Ciprofloxacin, AM-Amoxacillin, AU-Augmentin, CN-Gentamycin, PEF-Pefloxacin, OFX-Ofloxacin, S-Streptomycin, E.C- *Escherichia coli*, K.P- *Klebsiella pneumoniae*, P.A- *Pseudomonas aeruginosa*, P.V- *Proteus vulgaris,* S.T-*Salmonella typhi*, 1- Isolates.

The water sources with the exception were positive for presumptive and confirmatory tests, which indicates that these water sources contain coliforms especially *Escherichia coli*. This is in agreement with the research of Odeyemi [14] which presented *E. coli* as a common encounter in different water sources; be it rivers, streams, rain water, well water, underground water and even pipe borne water. The stream water also ranked higher in microbial contamination compared to other sources of water. This could be based on the fact that it is categorized as a surface water hence subject to influx of bacteria isolates. This study has been able to establish the correlation between ESBL production and the MDRIs screened out by the antibiotic sensitivity test. All the MDRIs were implicated for ESBL production as it was observed in *A. baumanni, E. aerogenes, E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. typhi, S. typhimurum* and *S. dysenteri.* According to Souha and Zeina [15], the production of Beta-lactamase enzymes by bacteria is one of the most common causes of bacterial resistance to the Beta-lactam antibiotics and Gram negative bacteria producing these enzymes are formidable adversaries to microbiologists and researchers [15]. The antibiotic resistance patterns in this present study is in agreement with the findings of Odeyemi [16] which reported most of the tested isolates to be least resistance to Ofloxacin.

The isolates were resistant to Amoxacillin, which corroborated the findings of Rahal [17] confirming that most strains of *Pseudomnas, Klebsiella, Enterobacter, Citrobacter, Serratia, Salmonella, E. coli* and indole positive *Proteus* species are resistant to Ampicillin. Incidence of multiple antibiotic resistant bacteria (MDRIs), and especially that they possess plasmids in this study is in agreement with the study of Akinyemi [18]. Five (5) out of twelve (12) isolates on which plasmid analysis were carried out contained plasmid band whose molecular weight ranged from 1564bp to 2027bp. This might be responsible for the initial antibiotic resistance exhibited by the isolates before plasmid analysis in this study while the resistance observed in other isolates might have been chromosomal mediated and this is in agreement with the findings of Kroll [19] who submitted that plasmid have encoded genes that provide resistance to occurring antibiotic in competitive environmental niche.

The post-curing antibiotics sensitivity analysis carried out on the MDRIs bacterial isolates revealed that the test isolates were susceptible to those antibiotics that they were previously resistant to. This implies that the presence of the plasmids in the five isolates were responsible for the multiple antibiotic resistance pattern exhibited by the isolates initially. This finding agrees with the work done by Afolami [20] who reported that plasmid-mediated mechanisms might increase like-hood of horizontal spread of antibiotic resistances in bacteria. More so, efflux pump mechanisms or other factors like mutation of gene encoding ribosomal protein, which decreases permeability of the cell envelope in enteric bacteria might also be responsible for antibiotic resistances [21].

4. CONCLUSION

Shortage of water for drinking and household use has been a major concern to public health in developing countries; this work has been able to characterize Gram negative bacteria associated with different water sources in Akure south local government area of Ondo State, Nigeria. Worrisome is the fact that majority of these water samples contain Gram negative bacteria that are resistant to antibiotics. This work further confirms the emergence of resistance of microorganism to current antibacterial. More worrisome is the fact that some of these Gram negative bacteria contain plasmid(s) which ease the transfer of resistant genes to other members of the population. Thus, there is a need for an inexpensive medium of purification of water prior to human intake to avoid deleterious effect of these pathogens in the area of study.

Comment [F2]: Restrict to six lines.

COMPETING INTERESTS

No competing interest exist

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