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Research Article
Antibiotic Resistance Studies of *Pseudomonas aeruginosa* Harbouring $bla_{CTX\text{-}M\text{-}1\text{-}CTXM\text{-}82}$ and $bla_{IMP\text{-}1/IMP\text{-}2}$ Encoding Genes from a Water Treatment Reservoir in South Eastern Nigeria

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Abstract

Background and Objective: The detection of ARGs in drinking water systems in Nigeria is a serious wake-up call on the need for regular surveillance and control measures. This study was carried out to determine the presence of antibiotic-resistance genes in *Pseudomonas aeruginosa* isolated from the Water Treatment Reservoir in Ebonyi State, Nigeria. **Materials and Methods:** A total of 70 samples of water were examined in this study. Bacteriological analysis of the test isolates was carried out using standard microbiology techniques. Antibiotic susceptibility studies of the test isolates were determined using the Kirby-Bauer disc diffusion method. Isolates obtained were further identified and characterized by PCR analysis using 16S rRNA gene amplification. **Results:** A total of 56 (80%) isolates of *Pseudomonas* spp., were confirmed from 70 water samples tested. The isolates were resistant to imipenem (39.29%), ceftazidime (67.86%), ciprofloxacin (69.64%), ofloxacin (71.43%), ertapenem (83.93%) cefepime (85.71%), ticarcillin (89.29%), amikacin (89.29%), gentamicin (91.93%) and cefotaxime (92.86%) while showing complete resistant to amoxicillin\clavulanic acid, tobramycin, oxacillin and aztreonam (100%). The PCR analysis revealed that $bla_{CTX\text{-}M\text{-}1\text{-}CTX\text{-}M\text{-}82}$ was present in *Pseudomonas aeruginosa* strain F18 16S, *Pseudomonas tolaasii* strain ATCC 33618 16S, *Pseudomonas mendocina* strain Y20 16S and *Pseudomonas aeruginosa* strain NA114 16S while $bla_{IMP\text{-}1/IMP\text{-}2}$ gene was present only in *Pseudomonas aeruginosa* strain F18 16S and *Pseudomonas aeruginosa* strain NA114 16S. **Conclusion:** We, report the occurrence of multidrug-resistant *Pseudomonas* spp., harbouring Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase resistance genes from a water treatment reservoir in Nigeria.

Key words: Antibiotics, drug resistance, *Pseudomonas*, beta-lactamase, carbapenemase, diversity saline water

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The detection of antimicrobial resistance genes in *Pseudomonas aeruginosa* supports the general concern that organisms can act as a reservoir for resistance genes and spread antibiotic resistance to humans through water and food which may present a potential health risk to the public. Antimicrobial resistance has risen to all antibiotics introduced into general clinical practice in recent years and is likely to spread to any new antibiotics introduced in the future. The prevalence of antibiotic resistance in a developing country like Nigeria may represent a useful marker to monitor resistance genes because it can provide a good model for ecological studies concerning antimicrobial resistance[1-4]. The detection of ARGs in drinking water systems in Nigeria is a serious wake-up call on the need for regular surveillance and control measures which have been considered appropriate in other developed countries. This is the first time a study of this nature is been conducted in water treatment plants in Nigeria as a whole. Safe and portable drinking water is an inevitable factor for a robust healthy society, but clean water for drinking and domestic purpose remains inaccessible to 1.1 million people on a global scale[2,5]. In developing countries, sources of drinking water range from surface water, groundwater (such as well water and borehole water), spring water, saline water, bottled water and harvested rainwater. The presence of opportunistic and obligate pathogens such as *Pseudomonas* spp., Aeromonas spp., *Klebsiella* spp., *Mycobacterium* spp., *Escherichia coli*, *Helicobacter* spp., *Salmonella* spp. and *Legionella* spp., may increase the health risks associated with the consumption of water arises from these outlined sources[6,7].

Antibiotic resistance is not restricted to nosocomial pathogenic or commensal microbes, but it is ubiquitous in the environment[8]. The environmental microbiome is considered to be the natural reservoir of potential antibiotic-resistance genes and has more diversity and novelty than ever expected[8-10]. Antibiotic resistance exists in ancient permafrost[1,11,12], deep terrestrial sub-surfaces[13], agriculture and animal husbandry and wastewaters[3,6,14]. Since most antibiotics originate from soil microbes, it is obvious that soil is a huge reservoir for antibiotic resistance[8]. The CTX-M β-lactamases originate from environmental *Kluyvera* species and quinolone resistance genes from *Klebsiella pneumoniae*[4,15] showing the clear link from the environment to the clinic. Antibiotics have been used in animal farming and aquaculture as growth promoters, for prophylaxis and treatment of infections[10,16,17]. The use and misuse of antibiotics in the clinic, community and animal farming have resulted in the emergence and spread of resistant microbes through selection caused by the antibiotics. Even sub-lethal antibiotic doses select and enrich antibiotic resistance in the environment[18]. The impact of increasing the use of antibiotics can be seen in archived soil samples where the resistance gene abundance increases from the 1940s to the present[14]. Previously, in the late 1960s bacteria began to show high resistance to penicillin in a threatening fashion. The resistance to penicillin was traced to beta-lactamase enzymes harboured by bacteria and these led to the development of beta-lactamase inhibitors (carbapenems) such as doripenem, panipenem, betamipron, biapenem, meropenem, ertapenem, imipenem-cilastatin and imipenem which is the chief among them with the broadest spectrum of activity against Gram-positive and Gram-negative[19,20]. It is quite unfortunate that bacterial pathogens that are resistant to this life-saving class of antibiotics have emerged. Antibiotic resistance genes are now considered environmental pollution and severe measures to prevent their further spread should be taken[21]. A Better understanding of reservoirs, as well as dissemination of Antibiotic Resistance Genes (ARGs) is needed to curtail the resistance threat in the clinic and the environment[1,22,23].

Pseudomonas aeruginosa has been found to display resistance or intermediate susceptibility to carbapenems, aminoglycosides, cephalosporins and fluoroquinolones, which are commonly used anti-pseudomonal agents that have proved efficient in the treatment of the organism[24-26]. The development of new antibiotics or alternative therapeutic strategies for the treatment of *Pseudomonas aeruginosa* infections is urgently required for patients whose infections are resistant to conventional antibiotics. New antibiotics with novel modes of action, new routes of administration and resistance to modification by bacterial enzymes have been explored in recent years. Some of these newer antibiotics show excellent in-vitro antibacterial activity against some members of the genus such as *P. aeruginosa* as well as lower Minimum Inhibitory Concentration (MIC) compared to conventional antibiotics[27-29].

However, owing to the rise of antibiotics resistance patterns in bacteria from both clinical, food and environmental organisms across the globe coupled with the identification of over sixty antibiotics resistance genes in Nigeria, more information on the reservoir organisms responsible for its spread via drinking water systems remain a challenge in developing economies, thus the need for this study.

MATERIALS AND METHODS

Study area: This research work was carried out on the water treatment plant located at Ezillo, Ishielu Local Government Area of Ebonyi State, Nigeria. Ishielu is a Local Government

Area of Ebonyi State, Nigeria from September, 2020 through February, 2021. Ezillo town is the headquarters of Ishielu Local Government Area. Ishielu Local Government Area of Ebonyi State is bounded to the North by Benue State, to the South by Onicha Local Government and to the East by Ohaukwu and Ezza North Local Government and West by Enugu State. It has an area of 872 km^2 and a population of 151, 048 at the 2006 National Census. Ishielu LGA has a tropical climate with an average relative humidity of 75% and may reach 80% during the rainy season. The area has annual rainfall between 2000 and 2500 mm and rainforest with atmospheric temperatures between 21 and 31°C. Two seasons are distinguishable in Ishielu LGA: A dry season (November to March) and a wet season (April and October). Ishielu Local Government Area lies on longitude 7°00 E and 8°00 and latitudes 4°45 and 6°17 N. The inhabitants of this area are mainly Igbo-speaking people who are actively engaged in various activities such as trading, craft, agriculture, government and public enterprises.

Sample collection and analysis: A total of 70 water samples were collected comprising 19, 18, 16 and 17 from the flocculation bed, sedimentation bed, filtration bed and filtration discharge pipes respectively for bacteriological analysis. The samples were cultured on nutrient agar on the same day of collection for further characterization.

The water samples collected from the Ezillo water treatment reservoir were analyzed and characterized using standard microbiological techniques which include re-culturing, Gram staining and biochemical and sugar fermentation tests. The pure cultures of the isolates were inoculated on cetrimide agar slants and incubated at 37°C for 24 hrs for clear distinction of *P. aeruginosa* from other *Pseudomonas* spp. Other *Pseudomonas* spp., were characterized and identified using a combination of motility tests using the hanging drop method, colonial morphology, indole, methyl red, Voges Proskauer, citrate test, Gram stain reaction, smell, pyocyanin production on cetrimide agar, triple sugar Iron test for carbohydrate fermentation, oxidative-fermentation test, urease test, coagulase test and oxidase tests using standard microbiological procedures[30,31].

The water samples collected were analyzed and characterized using standard microbiological techniques such as pure culture and Gram stain techniques. Further characterization of the isolates was done using biochemical and sugar fermentation tests. Pure culture of *P. aeruginosa* isolates was obtained using cetrimide agar at 37°C for 24 hrs whereas other *Pseudomonas* spp., were characterized using a combination of morphological examination and biochemical tests such as hanging drop method for motility, indole, methyl red, Voges Proskauer, citrate test, smell, pyocyanin production on cetrimide agar, triple sugar Iron test for carbohydrate fermentation, oxidative-fermentation test, urease test and catalase coagulase test[30,31].

Antibiotic susceptibility testing: The following antibiotic disks: Amoxicillin/clavulanic acid (20/10 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin, (5 µg), gentamicin (10 µg), ertapenem (10 µg), imipenem (10 µg), oxacillin (10 µg), ofloxacin (5 µg), amikacin (30 µg), aztreonam (30 µg), tobramycin (30 µg) and ticarcillin (75 µg) were used in carrying out the antibiotic susceptibility test. The antibiotic susceptibility pattern of the *Pseudomonas aeruginosa* isolates was determined using the modified Kirby-Bauer disc diffusion method[31].

Molecular studies

Characterization of surface attachment genes: Isolates were tested for the presence of resistance genes by PCR analysis according to published procedures[32]. The oligonucleotide primers used for the PCR amplification/detection of used in this study *CTX-M* and *IMP* genes are F, TCTTCCAGAATAAGGAATCCC and R, CCGTTTCCGCTATTACAAA for CTX-M-1-CTX-M-82 with amplicon size of 900 bp and F, CGCAGCAGG GCA GTC and R, CAA AAG CGC AAC TTA CAA AC for IMP-1/IMP-2 with amplicon size of 250 bp[33-36]. The cycling conditions for PCR consisted of the following steps: Initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 45 sec. Followed by a final elongation step at 72°C for 7 min and hold temperature at 10°C forever.

RESULTS

Distribution of *Pseudomonas* spp., in water treatment plants in Nigeria: The distribution of the test isolates from the water treatment reservoir was shown in Table 1. Data revealed

Table 1: Distribution of *Pseudomonas* spp., in water treatment reservoir in South Eastern Nigeria

Sample site	No. collected	No. positive (%)
Flocculation bed	19	17 (89.47)
Sedimentation bed	18	13 (72.00)
Filtration bed	16	12 (75.00)
Filtration discharge	17	14 (82.35)
Total	70	56 (80)
p-value		0.001

Table 2: Antibiotics susceptibility pattern of *Pseudomonas aeruginosa* isolated water treatment reservoir in Nigeria

Antibiotics	Disc potency (μg)	No. tested	No. susceptible (%)	No. resistant (%)
IPM	10	56	34 (60.71)	22 (39.29)
ETP	10	56	9 (16.07)	47 (83.93)
FEP	30	56	8 (14.29)	48 (85.71)
CAZ	30	56	18 (32.14)	38 (67.86)
CTX	30	56	14 (7.14)	42 (92.86)
AMC	30	56	0 (0.00)	56 (100.00)
TOB	10	56	0 (0.00)	56 (100.00)
TIC	75	56	6 (10.71)	50 (89.29)
OX	10	56	0 (0.00)	56 (100.00)
ATM	30	56	0 (0.00)	56 (100.00)
CN	30	56	5 (8.92)	51 (91.93)
AK	30	56	6 (10.71)	50 (89.29)
CIP	5	56	17 (30.36)	39 (69.64)
OFX	5	56	16 (28.57)	40 (71.43)

IPM: Imipenem, ETP: Ertapenem, FEP: Cefepime, CAZ: Ceftazidime, CTX: Cefotaxime, AMC: Amoxicillin, TOB: Tobramycin, TIC: Ticarcillin, OX: Oxacillin, ATM: Aztreonam, CN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin and OFX: Ofloxacin

Table 3: Multiple antibiotics resistance index (MARI) for *Pseudomonas aeruginosa*, isolated from water treatment reservoir

Sample site	MARI	Resistance pattern
Flocculation bed	0.85	CTX, AMC, TOB, TIC, OX, ETP, FEP, CAZ, CN, AK, ATM and CIP
Sedimentation bed	0.71	ETP, FEP, CTX, AMC, TOB, TIC, OX, ATM, CN and AK
Filtration bed	0.64	CTX, AMC, ETP, TOB, TIC, OX, ATM, CN and AK
Filtration discharge	0.93	ETP, FEP, CTX, AMC, TOB, TIC, OX, ATM, CN, AK, CIP, OFX and CAZ
Mean±SD	0.78±0.13	
p-value	0.002	

ETP: Ertapenem, FEP: Cefepime, CAZ: Ceftazidime, CTX: Cefotaxime, AMC: Amoxicillin, TOB: Tobramycin, TIC: Ticarcillin, OX: Oxacillin, ATM: Aztreonam, CN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin and OFX: Ofloxacin

Table 4: BLAST results of selected sequenced organisms obtained from water samples

Isolate code	Pairwise identity (%)	NCBI accession	E-value	Identified organism
$F_{10}P_2$	76.10	KT005258	2.24E-48	*Pseudomonas aeruginosa* strain NA114 16S
$F_{17}P_2$	83.30	NR_115613	1.36E-40	*Pseudomonas tolaasii* strain ATCC 33618 16S
$Fd_{48}P_2$	91.00	MH997639	0	*Pseudomonas mendocina* strain Y20 16S
$F_{12}P_2$	83.30	KX896753	3.04E-12	*Pseudomonas aeruginosa* strain F18 16S
F_{19}	90.50	MH997639	0	*Pseudomonas mendocina* strain Y20 16S

that out of 19 water samples collected from the flocculation bed, 17 (89.47%) harboured *Pseudomonas aeruginosa* out of 18 water samples collected from the sedimentation bed, 13 (72%) *Pseudomonas aeruginosa* were isolated. A total of 12 (75%) *Pseudomonas aeruginosa* was isolated out of 16 water samples collected from the filtration bed while out of 17 water samples collected from the filtration discharge pipes 14 (82.35%) *Pseudomonas aeruginosa*, were isolated.

Antibiotics susceptibility pattern of *Pseudomonas aeruginosa* isolated water treatment plant in Nigeria: The antibiotic susceptibility of the test isolates from the water treatment reservoir was shown in Table 2. *Pseudomonas aeruginosa* isolates were susceptible to cefotaxime (7.14%), gentamicin (8.92%), amikacin (10.71%), ticarcillin (10.71%), cefepime (14.29%), ertapenem (16.07%), ofloxacin (28.57%), ciprofloxacin (30.36%),%), ceftazidime (32.14%) and imipenem (60.71%). The isolates were resistant to imipenem (39.29%), ceftazidime (67.86%), ciprofloxacin (69.64%), ofloxacin (71.43%), ertapenem (83.93%) cefepime (85.71%), ticarcillin (89.29%), amikacin (89.29%), gentamicin (91.93%), cefotaxime (92.86%), amoxicillin\clavulanic acid (100%), tobramycin (100%), oxacillin (100%) and aztreonam (100%).

The Multiple Antibiotics Resistance Index (MARI) for *Pseudomonas aeruginosa* isolates from the water treatment plant is shown in Table 3. The result shows that isolates from the flocculation bed, sedimentation bed, filtration bed and filtration discharge pipes had a MARI of 0.85, 0.71, 0.64 and 0.93, respectively.

Presence of antibiotic resistance genes in selected *Pseudomonas aeruginosa* isolated from water treatment reservoir: The blast result of ten selected isolates revealed that five among them were *Pseudomonas* species namely, *Pseudomonas aeruginosa* strain NA114 16S. *Pseudomonas aeruginosa* strain F18 16S, *Pseudomonas tolaasii* strain ATCC 33618 16S and two strains of *Pseudomonas mendocina* strain Y20 16S (Table 4).

Table 5: Antibiotic resistance genes encoded by test isolates obtained from water treatment reservoir

Test isolate	Genes encoded
Pseudomonas aeruginosa strain NA114 16S	*IMP-1/IMP-2*
Pseudomonas tolaasii strain ATCC 33618 16S	*CTX-M-1-CTX-M-82*
Pseudomonas mendocina strain Y20 16S	*CTX-M-1-CTX-M-82, IMP-1/IMP-2*
Pseudomonas aeruginosa strain F18 16S	*CTX-M-1-CTX-M-82, IMP-1/IMP-2*
Pseudomonas mendocina strain Y20 16S	*CTX-M-1-CTX-M-82*

Agarose gel electrophoresis result of PCR products of the antibiotic resistance genes revealed that *CTX-M-1-CTX-M-82* was present in *Pseudomonas aeruginosa* strain F18 16S, *Pseudomonas tolaasii* strain ATCC 33618 16S and *Pseudomonas mendocina* strain Y20 16S, while the *IMP-1/IMP-2* gene was present in *Pseudomonas aeruginosa* strain F18 16S and *Pseudomonas aeruginosa* strain NA114 16S (Table 5).

DISCUSSION

Data in this study revealed that the occurrence of *Pseudomonas aeruginosa* in the water treatment reservoir was high. The *Pseudomonas aeruginosa* test isolates also displayed high resistance to the antibiotics used for the study (Table 1-3). Three different species and four *Pseudomonas aeruginosa* were identified in this study (Table 4). The *CTX-M-1-CTX-M-82* genes and *IMP-1/IMP-2* genes were present in *Pseudomonas aeruginosa* identified in this study (Table 5). *Pseudomonas aeruginosa* from water sources and nosocomial environment has been reported to show high multi-drug resistance to commonly used antibiotics according to published articles[1,2,3,6,8,9]. This resistance pattern has been traceable to some of the members of *Pseudomonas* spp., ability to harbour resistance genes and plasmid DNA[1,31,36,37]. These findings could be the reason for the organism's ability to display multiple resistances to various commonly used antibiotics and its widespread pattern among isolates from both hospital and environmental systems. This is not only observed in Sub-Saharan Africa, where poor sanitation systems are the order of the day, it has also been observed in other countries outside Africa[37,38]. This high resistance of some *Pseudomonas* (especially *P. aeruginosa*) from the aquatic environment to antibiotics could be attributed to the misuse and overuse of antibiotics as well as the possession of drug-resistant plasmids by the organism. This stems from the possibility of bacteria pathogens acquiring antibiotic-resistant genes from members within and without the genera and species, thus creating pathotypes with new combinations of different virulence and resistance genes. This scenario will always arise due to selective pressure or overuse of antibiotics by humans or domestic animals and has contributed to a significant increase in antimicrobial resistance to specific antibiotics over a short time[38-41].

The high antibiotic resistance displayed by *Pseudomonas aeruginosa* in our studies to the group of penicillins (oxacillin and amoxicillin), monobactams (aztreonam) and carbapenems (imipenem and ertapenem) which are widely used beta-lactam antibiotics is indicative that the organism possesses both beta-lactamase enzymes and other resistant factors of which this study has confirmed. A scattered resistance among 81 carbapenem-resistant enteric bacteria isolated in the United Kingdom with transconjugants and transformants showed the small effect of *Klebsiella pneumoniae* carbapenemase (KPC) enzymes against temocillin with OXA-48 together with NDM-1 showing clear resistance has been reported[42]. These report has become a public health concern in recent years as there seems to be a global increase in resistance to effective antibiotics used in the treatment of bacterial infections[43-45]. *Pseudomonas* spp., displaying Multiple Drug Resistance (MDR) to different classes of antibiotics used in this study is an ugly scenario that needs a quick approach to curtail the global increase of MDR among bacterial pathogens in water especially those with a nosocomial origin.

The presence of *CTX-M-1-CTX-M-82* and *IMP-1/IMP-2* encoding genes in bacteria isolated from water samples in this study potentially put the health of the community members who rely on these water sources at great inestimable jeopardy. These species when they infect the human system may lead to severe waterborne diseases[46]. The incidence of bacteria harbouring resistance genes in drinking water facilities as observed in this study might be a result of resistance genes being maintained within aquatic populations to protect bacteria from antimicrobial agents produced by other microflora in their environmental niche. The presence of a diversity of resistance genes might constitute a pool of resistance genes capable of moving among bacteria in the aquatic environment and possibly being transmitted to other pathogens in both food sources and nosocomial environments[1,47,48]. The diversity of ESBL-producing organisms in both treated and untreated water samples to their mode of

transfer is becoming a current issue of importance across the globe. Antibiotic resistance genes that have been identified in *Pseudomonas* spp. and other bacteria from the aquatic environment in Nigeria and countries such as China, India, Portugal, Spain, Ireland, Cyprus, Germany, Finland and Norway have been reported[49]. The genes detected include *blaCTX-M-27*, *tet* (A), *sul1*, *bla TEM* and *aph* (3") (c), aadE, strA, strB, tetA, sulII, intl1 and Tn916. these genes were detected from river sediment, drinking water treatment systems and finished treated water. In the nosocomial environment, genes such as bla_{Tem}, bla_{OXA}, $bla_{CTX-M-15}$, bla_{Vim}, bla_{Ges}, bla_{Veb}, bla_{DIM}, *AmpC* and Efflux pump genes encoding MexA, B-oprM, Mex C, D-oprJ, Mex X, Y-oprN, oprD, nfxB, MexR and class A group of ESBLs such as *TEM*, *SHV*, *CTX-M*, *PER*, *VEB*, *GES* and *IBC* families prevalent among *P. aeruginosa* has also been reported[50]. Even the Class D, *OXA*-type ESBL enzymes in *P. aeruginosa* harbouring MBL genes such as *IMP*, *VIM*, *SPM*, *GIM* and recently reported *DIM* families have been reported from nearly all regions of the globe[50-54].

The detection of ARGs in drinking water systems in Nigeria is a serious wake-up call on the need for regular surveillance and control measures which have been considered appropriate in other developed regions. This situation can pose a high possibility of dissemination of resistance determinants within the water systems from one bacterium to the other and possibly to clinical isolates in some instances. These findings emphasize the importance of rapid and accurate detection of these resistance genes to ensure appropriate infection control and the importance of continued surveillance programs in the general environment. Detection of antimicrobial resistance genes supports the general concern that *Pseudomonas* species can act as a reservoir for the resistance genes and its spread to humans through water and food chain and may present a potential health risk. In recent times, antimicrobial resistance has risen to all antibiotics introduced into general clinical practice and is likely to arise to any new antibiotics introduced in the future. It is imperative to consider what can be done to minimize the development and transfer of antibiotic resistance gene clusters.

CONCLUSION

This study has shown that water samples obtained from water treatment plants harboured *Pseudomonas* spp. It was observed that the rate of contamination was high in the various treatment units. The result obtained from the antibiotic susceptibility profile of isolates showed multiple resistance to gentamicin, amikacin, ticarcillin, cefotaxime, cefepime, ceftazidime, ofloxacin, ciprofloxacin, amoxicillin\clavulanic acid, tobramycin, oxacillin, ofloxacin and aztreonam and imipenem. Data on multiple antibiotic resistance profiles of bacterial isolates revealed that there has been an indiscriminate use of the antibiotics tested. The result of PCR analysis revealed that the isolates of *Pseudomonas aeruginosa* harboured resistance genes.

SIGNIFICANCE STATEMENT

This study discovered the presence of antibiotic resistance genes responsible for multi-drug resistance patterns from drinking water sources in Ebonyi State, South Eastern Nigeria. This suggests that there exists a high degree of disproportionate use of antibiotics in clinical, agricultural and environmental practices within the region. The finding in our study on the presence of ARGs in drinking water treatment plants provides insight into and effective assessment of the potential risk of ARGs in drinking water treatment systems at the catchment scale. This study will help to develop effective algorithms for rapid and accurate detection of these resistance genes to ensure appropriate infection control and the importance of continued surveillance programs in the general environment.

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