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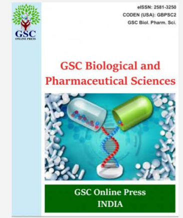

(Research Article)

Occurrence of antibiotic resistant bacteria and antibiotic resistant genes in rivers found in Anambra state, Nigeria

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Abstract

The study involves the analysis of water samples obtained through simple random sampling (SRS) technique from selected rivers in Anambra State, Nigeria for presence of antibiotic resistant bacteria and antibiotic resisistant genes. The high morbidity and mortality rate among the populace calls for this research. The most probable number (MPN) technique was utilized. Antibiotic susceptibility test, molecular characterization and plasmid profiling tests were carried out. The result of the MPN test indicated that coliform bacteria mostly Escherichia coli occurred predominantly in the water samples connoting effective confirmation of faecal contamination of the rivers. E. coli strains were distinguished by their ability to give colour changes on some lactose broth tubes impregnated with the water samples, surviving high temperatures of 45°C. Antibiotic susceptibility test results revealed that the E. coli isolates were resistant to all the seven antibiotics used namely, clindamycin, gentamycin, ciprofloxacin, erythromycin, sulfonamides, ampicillin and tetracycline. The results of molecular characterization indicated that the most prevalent resistant genes detected in the water samples were the tet A, tet B, sul1 and sul2 of the E. coli strains. The results of the plasmid profiling of the isolates revealed the presence of plasmids on the identified genes. The inferential statistics was employed during data analysis. It was therefore evident that antibiotic resistant bacteria and antibiotic resistant genes occurred in the rivers studied. This then calls for concerted efforts to checkmate this occurrence with a view to promoting the good health of all and sundry.

Keywords: Antibiotics; Bacteria; Genes; Resistance; Water analysis; River and Anambra

1. Introduction

Many sites in waterways all throughout the planet are loaded up with hazardously undeniable degrees of antimicrobial agents. This is a worldwide well-being crisis that could kill 10 million individuals by 2050 [1] as a portion of the world's most normal infections are becoming untreatable.

The World Health Organization has classified antimicrobial resistance as a widespread serious threat that is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country [2]. Antibiotic resistant bacteria and antibiotic resistant genes were recognized in a wastewater stream in the Netherlands in the year 2017 [3] and also in China [4]. Antibiotic resistant bacteria and antibiotic resistant genes were recognized in an unearthed lake in Port Harcourt, Rivers State [5], and in Mubi, Adamawa State [6]. The potential antibiotic resistant bacteria that were distinguished include; Escherichia coli, Helicobacter pylori and Staphylococcus aureus [7] while the resistant genes include mec A Methicillin-opposition quality and amp C Beta-Lactam obstruction quality [8].

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Escherichia coli and intestinal *Enterococci species* were isolated in Mubi [6]. In another study, *Staphylococcus aureus*, *Campylobacter* and *Salmonella* were distinguished [9]. The greater part of these microscopic organisms are gut bacteria, *Staphylococcus aureus* is present on individuals' skin, in their noses and throats.

Antibiotics like Tetracyclines, Ciprofloxacins, Sulphonamides, Sulfamethoxazoles, Trimethoprims and Metronidazoles which were intended to treat contaminations brought about by microorganisms have discovered their ways into the streams through sources like human/animal excreta, latrine outlets, compost/manure run-offs, mechanical side-effects, to give some examples, in this way presenting unexpected issues like medication obstruction, decrease of ordinary verdure, teeth/bone staining, harm of organs/tissues, stomach related issues, photosensitivity, drug communications, contagious diseases, accompanied with numerous economic losses.

The presence of the antibiotics in the streams has made certain water living beings change (transform) their DNA (deoxyribonucleic acid) formation. In some cases they secure anti-infection opposition qualities found on plasmids and transposons from different life forms through quality exchange or through mating interaction. The selections of antibiotics for the treatment of normal irresistible infections in people are turning out to be progressively costly and insufficient because of the rise of antibiotic resistant bacteria and antibiotic resistant genes on the conditions which find their ways into the surface waters. Waterways provide water utilized in farming, amusement parks, industry and home life (for instance: drinking, washing, cooking, latrine flushing and cleaning) yet these streams have been affirmed to be repositories for the spread and circulation of human related antibiotic resistant bacteria and antibiotic resistant genes [10].

Studies have shown that waterways were brimming with antibiotic resistant bacteria and antibiotic resistant genes which prompted the pervasiveness of medication obstruction among the general population leading to monetary misfortune, unexpected problems and even passing from utilization of the antibiotics stacked water from the waterways [7].

The contribution of ecological contaminations in oceanic framework is a typical marvel [5]. Hydroponics addresses one area that supplement wild fish because of the developing interest of fish protein in the populace. This has additionally contributed monstrously to the infectivity of the general population during helpless taking care of and eating of fishes from defiled waterways.

Thus, the broad utilization of antibiotics in medication and horticulture has brought about their perpetual discovery in the climate which straightforwardly or in a roundabout way impacts their quality in the rivers. Increment of fecal contamination in sources of water is an issue [9].

Free meandering creatures particularly canines and birds add to fecal contamination of surface water and lakes. Also, anti-toxins safe microorganisms and anti-toxins safe qualities can wind up in waterways by various courses, for instance through run-off of fertilizer from creatures treated with antibiotics to waterways.

Besides, drugs enter streams principally through human and animal excreta that contain the antibiotic resistant bacteria and antibiotic resistant genes and cause water contamination. Notwithstanding medical services, anti-infection agents use is alarmingly high in the farming business. Waste can enter straightforwardly into streams in low-pay nations, or through spills in wastewater offices. Sometimes, drug producing locales may likewise spill or unlawfully dump materials into watersheds. Another source might be the release of halfway treated or untreated waste water, for example, from clinics where individuals are treated with antibiotics. Likewise, surface water can be defiled with microorganisms through precipitation, and overflow blending in with sewage gushing and excrement from natural life, which render it unsatisfactory for human utilization [11, 12].

Indeed, horticulture which includes crop reproducing and animals cultivating, these increase the contamination hazards [13, 14].

In this manner, when individuals come into contact with the defiled waterways, they are faced with the hazard of openness to microscopic organisms that are impervious to at least one antibiotic treatment. This may happen for example during entertainment, drinking, cooking or through water utilized for water systems. In this way, various dangers can show themselves in unexpected ways. Initially, after openness to antibiotic resistant bacteria and additionally antibiotic resistant genes, individuals may foster illnesses which may be difficult to treat.

Also, individuals may not turn out to be sick themselves, but may move the antibiotic resistant bacteria and genes to individuals who are more defenseless/immune-compromised, like clinic patients, babies and the old. Consequently, this

classification of individuals can foster sickness or their infections may turn out to be difficult to treat. The event of antibiotic resistant bacteria and antibiotic resistant genes in streams is a significant worry for the spread of these agents, as organic entities blend.

As indicated by world wellbeing association [15], observing and reconnaissance of antibiotic resistant bacteria and antibiotic resistant genes in sources implied for human utilization is significant. The world wellbeing association on creature suggested the persistent observing and reconnaissance of obstruction microorganisms in oceanic creatures. The drawn out impact of antibiotic resistant bacteria and antibiotic resistant genes on waterway creatures and the regular biological system involved the obliteration of numerous natural bonds, as it influences the microclimate of this climate in the midst of different difficulties.

Restorative disappointment prompts monetary harm as the patient not just waste more than is required to treat a specific contamination, yet additionally prompts more unexpected problems and even demise.

Presently, scientists are calling for more worldwide researches and improvement methodologies to foster the advancement of broad-spectrum antibiotics alongside effective treatments to antibiotics.

The decision of Anambra State for this research was a direct result of reasons that incorporated the proximity of the vicinity to the researcher and the broad investigation of the rivers nearby to link the city to the world map where studies has so far been done.

At last, this study is aimed to give general information on the occurrence of antibiotic resistant bacteria and antibiotic resistant genes in rivers of Anambra State which were important to gauge and control this health hazard in the rivers [16, 17, 6]. It is required that all hands should be on deck so as to checkmate the devastating impact of this menace on the health of the populace.

1.1 Statement of Problem

Over the years, some factors like human, animal and industrial processes have contributed to the surge of antibiotic resistant bacteria and genes in rivers. Many researchers have found out that there are antibiotic resistant bacteria and antibiotic resistant genes in rivers [6, 5].

This study is meant to either support or dispute the fact that there are antibiotic resistant bacteria and antibiotic resistant genes in waterways around the globe and at the end join the studies of other researchers to proffer lasting solutions to the problems of drug resistance (treatment failures), teeth and bone stains, damage of organs and tissue, reduction of normal flora, to name but a few thereby salvaging the lives of the populace.

Aim of the Study

The aim of this study is to isolate antibiotic resistant bacteria and antibiotic resistant genes with a view to saving lives that are lost through treatment failures caused by drug resistance.

Objectives of the Study

The following objective was delineated for this study:

To analyze water samples collected from different rivers in Anambra State in order to investigate the presence of antibiotic resistant bacteria and antibiotic resistant genes in them.

1.2 Research Question

The following research question guided the study:

Are there antibiotic resistant bacteria and antibiotic resistant genes in the rivers found in Anambra State?

1.3 Hypothesis

The research hypothesis stated that the presence of antibiotic resistant bacteria and antibiotic resistant genes in rivers cause drug resistance and other health problems

Scope of Study

In respect to the significant health implications and problems occasioned by the occurrence of antibiotic resistant bacteria and antibiotic resistant genes in rivers, [13]; it now demands further probe and research which will then proffer ways to curb the menace and in turn help to salvage the health of the populace.

The scope of this study was limited to the presence of antibiotic resistant bacteria and antibiotic resistant genes in rivers found in Anambra State in conjunction with other researches made by fellow scientists to buttress or dispute the fact that they occur in rivers around the globe.

This research also covered the problems associated with the occurrence of antibiotic resistant bacteria and antibiotic resistant genes in rivers like drug resistance as well as proffer solutions.

2. Material and methods/ Sample and Sampling Techniques

A total of six (6) rivers were randomly sampled. Simple Random Sampling (SRS) technique was employed to select the six (6) rivers based on several factors that included the size of the rivers, community rivers' authority and proximity to the researchers.

Table 1 Names of the Rivers and their Samples' Identifications

S/N	Names of Rivers	Identification
1	Eze	A
2	Omambala	B
3	Ele	C
4	Idemili	D
5	Ubu	E
6	Agulu	F

2.1. Sample Collection

Water samples were aseptically taken from six (6) different rivers using sterile screw-capped cans which were purified using boiled water-containing detergents and finally rinsed with the particular water sample. The water cans (1 litre for each river) were opened at about 15cm depth, allowed to full, closed under water, quickly cork-sealed with aluminum foil and then wrapped in black nylon bags.

The water samples were labelled properly (A to F) and then transported to the Laboratory of Chukwuemeka Odumegwu Ojukwu University (COOU), Uli for analysis within 48h. Aliquot of the samples will be analyzed using standard microbiological procedures.

2.2. Physical Parameters

2.2.1. Procedure

The following analyses were done on three aliquots of each sample and the average results noted.The pH, temperature and turbidity were measured using standard procedures [18].

2.3. Identification and Confirmation of Antibiotic Resistant Bacteria

The identity of bacterial isolates present in the water samples were obtained using serial dilution method [19] and the Most Probable Number (MPN) method while the confirmation of the sensitivity of the bacterial isolates were gotten by Kirby Bauer's disc diffusion method [20].

2.3.1. Procedure

This research followed the procedure as described by Cullen et al., 2016 [19] as follows: Dilution was made in the 1st tube by taking 2ml normal saline in a tube and inoculating the desired culture in it; 10 tubes and plates were labelled 1, 2, 3……..,, 10; 9 ml of normal saline was added in each test tube; 1 ml (known volume) of the culture was transferred from the previously made dilution into the 1st tube having 9ml normal saline; 1ml was transferred from 1st tube into 2nd test tube and repeated steps till 10th test tube; 1ml was discarded from the 10th test tube and lastly, the remaining diluted 10th test tube was covered for further analysis.

2.3.2. The Most Probable Number (MPN) method

2.3.3. Procedure of MPN

This study followed the procedure as described by UNEP/WHO, 1996 [21].

Preparation of the medium and dispensation into test tubes

Media (lactose broth) was prepared according to prescriptions on product's manual; sterilization was done by autoclaving at 15 lbs pressure, 121°C for 15 minutes and then the sterilized media was dispensed into test tubes (10 mL and 5 mL) in numbers required.

Presumptive test

A series of lactose broth test tubes were inoculated with measured amounts of water samples to be tested following the steps below: 5 tubes of lactose broth were taken and inoculated respectively with 10mL of water sample to be tested; another set of 5 tubes of lactose broth were inoculated each of them with 1mL of water sample and lastly, 5 tubes of lactose broth were inoculated each with 0.1mL of water sample.

All the tubes were incubated at 37°C for 24 h; when no tube appeared positive, they were re-incubated up to 48 hr. The numbers of tubes that gave positive reaction were compared to a standard chart and recorded as the number of bacteria present in them.

For example, a water sample tested showed a result of 3–2–1 (3 × 10 mL positive, 2 × 1 mL positive, 1 × 0.1 mL positive) so an MPN value of 17 was delineated in the standard chart indicating that the water sample contained an estimated 17 viable coliforms per 100 ml.

Confirmed test

A loopful of growth from the above presumptive tubes were transferred into another tube of lactose broth and incubated at 35°C for 48 hours; plates of EMB agar were streaked with a loopful of growth from the positive tubes and incubated at 35°C for 18-24 hours.

Completed test

For this, inoculum from each of the positive tubes from the confirmed test was streaked on two plates of EMB (Eosin Methylene Blue) agar; one plate was incubated at 37°C and another at 44.5 ± 0.2 °C for 24 hours. After incubation, all plates were examined for the presence of typical colonies.

2.3.4. Purification of the bacterial isolates

The pure forms of the isolates were gotten by twice sub-culturing on Nutrient agar plates using the streak plate method and then into Nutrient agar slants in Bijou bottles by stabbing. They were incubated for growths and then stored in the refrigerator at 4°C until used for further analysis like Gram stain and molecular characterization.

2.3.5. Antibiotic susceptibility profile of the bacteria

Procedure of Kirby-Bauer disc diffusion

All procedures and results were as described in the Medical Laboratory Manual for tropical countries [22] and with reference to the Bergey's Manual of Systematic Bacteriology [23]; the results were analyzed in accordance with the British Society for Antimicrobial Chemotherapy- BSAC [24] and Clinical Laboratory Standard Institute.

A total of 10 antibiotics that are mostly used in the treatment of human and animal diseases were employed in this study. The antibiotics and their needed concentrations were as follows: CLIN: Clindamycin (2µg), GENT: Gentamycin (10µg), CIP: Ciprofloxacin (5µg), ERY: Erythromycin (15µg), SULF: Sulfonamides (250µg), AMP: Ampicillin (10µg), TET: Tetracycline (30µg).

The antibiotics were placed on the discs. Using sterile forceps, the antibiotic impregnated paper discs were then aseptically placed on the surface of the Mueller-Hinton Agar (MHA) plates at equal distance to each other; 0.1ml of the broth culture of each isolate was uniformly inoculated on the MHA (or nutrient agar) plates through a streak-plate method over the entire plates, in at least three planes, using a swab stick. The plates were then allowed to dry for 10mins and were incubated at 37°C for 24h; after incubation, diameters of clear zone of inhibitions were observed and measured in millimeter.

2.3.6. Identification and Confirmation of Antibiotic Resistant Genes

These were achieved through molecular characterization and plasmid profiling. Molecular characterization identified the genes while plasmid profiling confirmed sensitivity of the genes.

Molecular characterization

Polymerase chain reaction (PCR)

This research followed the procedure as described by Mullis, 1993 [25]. 5µl of the supernatant (extracted genomic DNA) was used as template for the PCR; the PCR ingredients (reaction mixtures) were mixed together in about 8 PCR tubes with each containing about 100µl of the reaction mixtures The reaction mixtures were as follows: 5µl extracted DNA, 2 mm MgCl2 (Magnesium Chloride), 0.8 mm dNTPs, 0.2µl Primer 1, Primer 2 and 1x PCR buffer

The PCR tubes were subjected to the following temperature variations: Denaturation- Temperature was raised to 95°C for 1m followed by 30 cycles of 96 °C for 30s; annealing- Temperature was cooled down to 55 °C for 30s, followed by 60°C for 30s; elongation-Temperature was then raised to 72 °C for 10m at the end of which the final product was collected and kept for further studies. The three (3) steps above were done for all the six (6) water samples respectively.

Gel electrophoresis

This study followed the procedure as described by Kryndushkin et al., 2003 [26]. Here, water samples were stacked into the wells with a pipette; the force supply of the chamber was turned on, which applied an electric field to the cushion. The electric field made contrarily charged atoms move through the gel toward the anode. (DNA and RNA are adversely charged; proteins should be treated with a cleanser to give them a negative charge).

The particles' development was affected by the permeable gel network to such an extent that bigger, heavier atoms moved somewhat leisurely, while more modest, lighter particles moved all the more rapidly. The thickness of pores and the sort of substance used to make the gel further impact the pace of atom relocation.

A colored "stepping stool," or marker with various atoms of known and shifting sub-atomic loads, was run close by exploratory examples to fill in as a kind of perspective for size. The color empowered the representation of the marker as it travelled through the gel; tests ordinarily were additionally colored for perception. Ethidium bromide, which fluoresces under bright light, was utilized for fresh representation of DNA tests.

2.3.7. Gene sequencing

Procedure of sequencing

The Sanger sequencing method [27] was completed following the six (6) steps below:

The double-stranded DNA (dsDNA) was denatured into two single-stranded DNA (ssDNA); a primer that corresponds to one end of the sequence was attached; four polymerase solutions with four types of dNTPs but only one type of dd NTP was added; the DNA synthesis reaction was initiated and the chain extends until a termination nucleotide was randomly incorporated. The resulting DNA fragments were denatured into ssDNA; the denatured fragments were separated by gel electrophoresis and the sequence was determined.

Procedure of Plasmid profiling

Harvest of bacterial and suspended cells

This research followed the procedure as described by Neupane *et al.,* 2016 [8]. A single colony from a freshly streaked selective plate was chosen and inoculated into a starter culture of 2-5 ml LB medium containing the appropriate selective antibiotic. It was then incubated for approximately 8h at 37°C with vigorous shaking (approx. 300 rpm); a diluted starter culture 1/500 to 1/1000 was put into 3 ml selective LB medium. Grown at 37°C for 12-16h with vigorous shaking (approx. 300 rpm).

The bacterial cells were harvested by centrifugation at 6000 x g for 15 min and removed as much of the supernatant as possible. The bacterial pellet was resuspended in 0.1-0.5 ml of resuspension buffer (50mM Tris-Cl, 10 mM EDTA, 100 μg/ ml RNase A, pH 8.0). The bacteria were resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

Cell lysis

0.25 ml of lysis buffer was added, mixed thoroughly by vigorously inverting the sealed tube 4–6 times, and incubated at room temperature (15-25°C) for 5 min. it was not vortexed, as this will result in shearing of genomic DNA. The lysate appeared viscous. The lysis reaction was not allowed to proceed for more than 5 min.

Neutralization

0.3 ml of neutralization buffer was added, mixed immediately and thoroughly by vigorously inverting 4–6 times, and incubated on ice for 5 min. Precipitation was enhanced by using chilled neutralization buffer and incubating on ice. After addition of neutralization buffer, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS (potassium dodecyl sulphate). The lysate was mixed thoroughly to ensure even potassium dodecyl sulphate precipitation. When the mixture still appeared viscous, more mixing was done to completely neutralize the solution. A homogeneous colourless suspension indicated that the SDS (Sodium dodecyl sulphate) was effectively precipitated.

Load lysate on column

Before loading the column, the supernatant was carefully removed and then transferred to a collection tube containing the column and centrifuged at 13,000 rpm for 1 minute; the flow-through liquid was discarded and supernatant containing plasmid DNA promptly removed. After centrifugation, the supernatants were clear. When the supernatant was not clear, a second, shorter centrifugation was carried out to avoid applying any suspended or particulate material to the column.

Bind and wash

0.7 ml of wash buffer was added to the column placed in the collection tube and centrifuged for 10 minutes at 13000 rpm for 1 minute. 1ml equilibration buffer (750 mm NaCl, 50 mm MOPS, pH 7.0, 15% isopropanol) was applied and the column was allowed to empty by gravity flow. Flow of buffer was initiated automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. The supernatant from step 6 was applied to the column and allowed to enter the resin by gravity flow.

Plasmid elution

DNA was eluted with 0.8 ml elution buffer (1.23 mm NaCl, 50mm Tris-Cl, pH 8.5, 15% isopropanol). The product was collected in a 1.5 ml or 2 ml micro-centrifuge tube; DNA was precipitated by adding 0.7 volumes (0.56ml per 0.8ml of elution volume) of room-temperature isopropanol to the eluted DNA. Mixed and centrifuged immediately at ≥10,000 rpm for 30 min in a micro-centrifuge. The supernatant was carefully decanted. All solutions were at room temperature in order to minimize salt precipitation.

DNA pellet was washed with 1 ml of 70% ethanol and centrifuged at 10,000 rpm for 10 min; the supernatant was carefully decanted without disturbing the pellet; the 70% ethanol removed precipitated salt and replaced isopropanol with the more volatile ethanol, making the DNA easier to re-dissolve.

The pellet was air-dried for 5–10 min, and the DNA was re-dissolved in a suitable volume of buffer (for example, TE buffer, pH 8.0, or 10mm Tris-Cl, pH 8.5). The DNA pellet was re-dissolved by rinsing the walls to recover all the DNAs.

Determination of yield

DNA concentration was determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel.

To quantitate the nucleic acid concentration, the plasmid DNA was diluted as 1:100 or 1:50 (depending on the plasmid copy number) in TE buffer and the absorbance (optical density) was measured at 260 nm (A260) and 280 nm (A280).

TE buffer was used as the blank. This measurement permits the direct calculation of the nucleic acid concentration using the formula:

$$[DNA]\ (\mu g/mL) = A260 \times \text{Dilution factor} \times 50$$

Where, 50 is the extinction coefficient of DNA. The ratio A260/A280 provided a reasonable estimate of the purity of the preparation.

2.3.8. Method of Data Analysis

In this study, the inferential statistics was employed during the data analysis whose goal was to draw conclusions and make inferences from a sample in order to generalize them to a population of rivers in Anambra.

3. Results

3.1. Physical Parameters

The outcomes of the pH measurement, temperature and turbidity carried out on the water samples were enumerated on the table below.

Table 2 The Results of the Physical Parameters on the Water Samples

S/N	Isolates	pH	Temperature (^oC)	Turbidity
1	A	4.0	34.0	Clear
2	B	6.0	37.0	Unclear
3	C	6.5	35.0	Clear
4	D	5.5	38.0	Clear
5	E	7.0	37.0	Unclear
6	F	6.5	35.0	Clear

Table 3 The Results of the MPN Test

S/N	Samples	Patterns	MPN/CFU/Viable cells
1	A	5-3-1	110
2	B	5-1-2	63
3	C	4-3-0	27
4	D	5-4-1	170
5	E	5-0-2	43
6	F	4-1-0	17

Table 4 The Results of the Antibiotic Susceptibility Test (mm)

Antibiotics							
Isolate Identity	**CLIN**	**GENT**	**CIP**	**ERY**	**SULF**	**AMP**	**TET**
Isolate A	03	05	10	05	08	08	10
Isolate B	10	10	05	05	06	11	08
Isolate C	05	08	07	10	05	04	12
Isolate D	08	11	10	12	13	05	05
Isolate E	12	12	15	08	05	10	11
Isolate F	05	10	11	09	10	09	07

KEY: CLIN: Clindamycin (2µg), GENT: Gentamycin (10µg), CIP: Ciprofloxacin (5µg), ERY: Erythromycin (15µg), SULF: Sulfonamides (250µg), AMP: Ampicillin (10µg); TET:Tetracycline (30ug)

3.2. Molecular Test

Primer sequences used for identification of the genes were the Shiga toxins (stx1 & stx2), intimin (eaeA) and hemolysin (hlyA) using multiplex PCR technique.

Table 5 The Primer Sequences used for Identification of the Genes

Primer	Oligonucleotide sequence (5'-3')	Product size (bp)	PCR cycling
stx1 (F)	ACACTGGATGATCTCAGTGG	614	35 X 94°C for 45s
stx1 (B)	CTGAATCCCCCTCCATTATG		57oC for 45s
			72°C for 1m
stx2 (F)	CCATGACAACGGACAGCAGTT	779	35 X 94°C for 45s
stx2 (B)	CCTGTCAACTGAGCAGCACTTTG		58°C for 45s
			72°C for 1m
eaeA (F)	GTGGCGAATACTGGCGAGACT	890	30 X 95°C for 2m
eaeA (F)	CCCCATTCTTTTTCACCGTCG		55°C for 1m
			72°C for 1m
hylA (F)	ACGATGTGGTTTATTCTGGA	165	30 X 95°C for 2m
hylA (B)	CTTCACGTGACCATACATAT		55°C for 1m
			72°C for 1m

Keys: stx= shiga toxins; intimin= eae; haemolysin= hly; F=front; B= back

The identities of the presumptive Escherichia coli were confirmed through the amplicons of stx1, stx2 and eaeA while the presumptive Aeromonas species were confirmed through the amplicons of hlyA.

Table 6 The Average Number of Presumptive Antibiotic Resistant Genes

S/N	Samples Identity	tetA	tetB	sul1	Sul2
1	A	55	75	65	25
2	B	45	85	70	40
3	C	85	50	45	35
4	D	60	45	80	30
5	E	50	65	55	20
6	F	75	55	75	15

3.3. Plasmid Profiling

To investigate the genomic relation behind the antibiotic resistance, plasmid profiling was done on the isolates from the water samples. All the isolates of *E. coli* harbored at least a single plasmid.

Of the 6 isolates, 5 isolates contained more than 1 plasmid. The plasmid sizes ranged from app 3.4 approximately 1.5 to 15 kb molecular weight, the most common plasmid of size approximately 11 to 12 kb being detected in all the isolates.

All the isolates showed similar patterns which then revealed that they contain plasmids with different molecular weights due to their migration patterns on agarose gel electrophoresis.

For example, plasmid of lane 1 to lane-8 had similar migration pattern on agarose gel electrophoresis whereas plasmid in lane-3 and lane-4 migrate more quickly than others. That means lane 3 has low molecular weight plasmid (1.5kb). Lane 1, lane 4, lane 5, lane 6 showed more than 1 band indicating that these isolates have more than 1 plasmid.

4. Discussion

The water analysis showed the antibiotic resistance of mostly the Escherichia coli and the antibiotic resistant genes- tet A, tet B, sul1 and sul2 which may have resulted from poor hygienic lifestyle and environments, this is in agreement with studies of Umeaku *et al.,* 2019; Stella *et al.,* 2018 and Fu *et al.,* 2017 [28,12,11].

The presence of multiple plasmids that were observed during the profile analysis may act as possible sources to transfer highly resistant genes to pathogenic organisms and humans that could be a threat for the treatment of diseases by commercially available antibiotics [10, 6].

The Escherichia coli were isolated, they are coliform bacteria that ferment lactose and are always present in the digestive tracts (guts) of animals leading to their presence in their faecal matters. As a result, faecal pollution of our rivers is not far-fetched owing to the presence of these indicator organisms- E coli. This conforms with studies of Umeaku *et al* [28].

When these bacteria are consumed by drinking the contaminated water, they cause problems like diarrhea, vomiting, to mention but a few. The best ways to eliminate these coliforms could be by chlorination, boiling/filtering, ultra filtration and ozonation.

The tet A and tet B were the resistant genes for tetracycline antibiotic drug while the sul1 and sul2 were the resistant genes for sulphonamides antibiotic drug. These antibiotic resistant genes could be carried by the coliform bacteria or transferred to more pathogenic bacteria which when consumed could bring about drug resistance, teeth stains, photosensitivity, to name but a few. People of developing countries often bear these antibiotic resistant bacteria in their faecal matters which when disposed indiscriminately in the environment, find their ways into our rivers. So, poor areas where people have no basic knowledge about antibiotic usage may have contributed to the surge of these E. coli isolates. This research proved that antibiotic pollution is now growing at an alarming increase in our environment which leads to the build-up of antibiotic resistant bacteria and their genes in waterways thereby posing great danger to human health which is in agreement with the findings by earlier scientists; Olukosi *et al.,* 2016; Zhang *et al.,* 2016 and Abu *et al.,* 2017 [7, 10, 5].

5. Conclusion

Finally, persistent exposure of various antibiotics to environmental settings expedites the development of superbugs (multi-drug resistant bacteria) which could be the greatest threat of public health in times to come. Our study strongly supports the fact that antibiotic resistant bacteria and antibiotic resistant genes are present in our rivers.

Recommendations

The following recommendations are given to the various strata of the society as they directly and indirectly support the occurrence of antibiotic resistant bacteria and antibiotic resistant genes in our environments cum rivers.

- Individuals should consume pipe-borne water and not from the surface water.
- Individuals should boil, filter and chlorinate water before consumption.

- Individuals should avoid indiscriminate defecation/urination and improper sewage/waste disposal but construct/make use of toilet facilities and dump refuse at right sites.
- Individuals should only use antibiotics that are prescribed by a certified health professional.
- Individuals should never demand for antibiotics if the health worker there is no need for them.
- Individuals should always follow the health worker's advice when using antibiotics.
- Individuals should never share or use leftover antibiotics.
- Individuals should prevent infections by regularly washing hands, preparing food hygienically, avoiding close contact with sick people, practicing safer sex, and keeping vaccinations up to date.
- Individuals should prepare food hygienically, following the following the keys to safer food, namely keep clean; separate raw/cooked; cook thoroughly; keep food at safe temperatures; use safe water/raw materials and choose foods that have been produced without the use of antibiotics for growth promotion or disease prevention in healthy animals.
- Policy makers should enact laws against the construction of houses without toilet facilities.
- Policy makers should also enact laws against indiscriminate defecation/urination cum sewage disposal and punish offenders.
- Policy makers should consider dredging of the rivers in the budget to remove debris.
- Policy makers should also include the construction of public toilets in their budget.
- Policy makers should ensure that a robust national action plan to tackle antibiotic resistance is in place.
- Policy makers should improve surveillance of antibiotic resistant infections.
- Policy makers should strengthen policies, programs, and implementation of infection prevention and control measures.
- Policy makers should regulate and promote the appropriate use and disposal of quality medicines.
- Policy makers should make information available on the impact of antibiotic resistance.
- Health professionals should prevent infections by ensuring their hands, instruments, and environment are clean and by so doing, avoid transferring them to their patients.
- Health professionals should only prescribe and dispense antibiotics when they are needed, according to current guidelines.
- Health professionals should report antibiotic resistant infections to surveillance teams.
- Health professionals should advise their patients about water safety techniques, on good hygiene and on how to take antibiotics correctly, the dangers of misuse of antibiotics and about the surge of antibiotic resistance.
- Health professionals should also talk to their patients about preventing infections (for example, vaccination, hand washing, safer sex, and covering nose/mouth when sneezing).
- The healthcare industry should invest in research and development of new antibiotics, vaccines, diagnostics and tools used in health management.
- The healthcare industry should engage in the mass production of broad-spectrum antibiotics.
- The healthcare industry should ensure that proper information about a drug is well stipulated on the drug's leaflet/manual.
- The agricultural sector should: construct and use ranches for animal rearing to avoid indiscriminate defecation by the farm animals.
- The agricultural sector should utilize pipe-borne water in animal feeding but not surface water.
- The agricultural sector should only give antibiotics to animals under veterinary supervision.
- The agricultural sector should not use or rather reduce the use of antibiotics for growth promotion or to prevent diseases in healthy animals.
- The agricultural sector should vaccinate animals to reduce the need for antibiotics as alternatives to antibiotics.
- The agricultural sector should promote and apply good practices at all steps of production and processing of foods from animal and plant sources.
- The agricultural sector should improve biosecurity on farms and prevent infections through improved hygiene and animal welfare.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors hereby declare that there was no conflict of interest.

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