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Determination of Antibiotic-Resistant Genes in Bacteria from Borehole Water Samples in Kaduna Metropolis

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Abstract

The persistence of drug-resistant microorganisms in environmental water sources, particularly boreholes, presents growing challenges for public health, especially within densely populated urban locales. In Kaduna Metropolis, borehole water serves as a crucial means of potable water supply; however, the infiltration of bacteria with antibiotic resistance into these sources represents a concerning vector for advancing antimicrobial resistance (AMR). This investigation explored the genetic mechanisms of bacterial strains retrieved from borehole water across four administrative districts Chikun, Igabi, Kaduna North, and Kaduna South spanning dry and wet climatic periods. A cumulative total of 200 samples were acquired and subjected to bacteriological assessment using established culture-based methodologies. Polymerase Chain Reaction (PCR) techniques were utilized to screen for specific resistance-related genes, including *blaTEM*, *gyrA*, *sul1*, and *PBP2a*. Molecular screening identified multiple resistance determinants, with *sul1* being the most frequently encountered. These findings point to the role of borehole water systems in harboring resistant microbial populations and underline environmental contributions to AMR transmission. The identified geographical and seasonal disparities in bacterial occurrence further emphasize the impact of regional infrastructure and environmental dynamics, reinforcing the call for sustained monitoring and proactive public health strategies, even in the absence of seasonal or locational trends in resistance behavior.

Keywords: Antimicrobial resistance, polymerase chain reaction, multiple resistant determinants

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Introduction

Water is one of the most valuable resources not only for humans but all forms of life, even non-living things. The importance of water cannot be overemphasized. Good quality water is more important because it is one of the most needed resources and living organisms cannot do without it. The quality of water has a great effect on its end users. Water is vital to life, however, the cases associated with waterborne diseases and deaths recorded have been on the rise exponentially. This is due to poor access to quality potable water [1]. It was reported by The United Nations International Children's Emergency Fund (UNICEF) and World Health Organization (WHO) that about 2.1 billion individuals in the world at large cannot get potable drinking water in their homes [2]. Even the United Nations in its sixth of the proposed seventeen sustainable development goals (SDGs) clearly stated the advantage of water of good quality to better the future of humanity by the year 2030 [3].

The problem and challenge of water-borne illnesses continuously limit the hard work put into improving public health in the emerging world. Ground water makes potable water available to about 1.5 billion people globally daily and has shown the most consistent means of meeting rural water needs in sub-Saharan Africa [4].

Groundwater is usually considered a good source of drinkable water due to the decontamination quality of soil. However, underground water may be prone to contamination and may not be as safe as is generally assumed. Groundwater begins with rainfall that is absorbed gradually into the ground. The amount of water that goes in into the ground will differ from place to place, depending on the terrestrial gradient, intensity of rainfall as well as the kind of land surface. Permeable, or porous, land containing plenty of sand or gravel will permit as much as 50% of rain to enter the ground and become groundwater [5]. Potable water is supposed to be hygienic and free from all forms of pathogenic organisms. However, some studies have shown that there is the likelihood of contamination of even underground water as well as resistance genes in the contaminating organisms which can make such water unfit for drinking because of the danger it poses to public health [6].

Abundant incidences of waterborne diseases have been reported in Nigeria and there are growing indications of new occurrences of cholera, diarrhoea, and other waterborne diseases which are resistant to antibiotics in recent times [7].

Water is consumed by virtually every individual in the world, and is the second most valuable and necessary resource, after oxygen. Borehole water is currently safest for domestic and industrial uses in Nigeria, especially Kaduna. Research in many parts of Africa and Nigeria has shown that water, including borehole water, can be contaminated with antibiotic-resistant bacteria because of gene transfer and the kind of bacteria present in the environment. Addressing these problems related to potable water from boreholes is crucial for promoting public health and well-being, reducing diseases and the rate of mortality especially in children below the age of 5.

This research aims to identify their resistant genes of all the bacterial isolates in borehole water samples from Kaduna metropolis.

Materials and Method

Study area

This study was conducted in Kaduna metropolis, Kaduna state, Nigeria. Kaduna state is located in the Northwest geopolitical zone of Nigeria and lies between longitude 6° and 9°E and latitude 9° and 30°N [8] as shown in Fig. 1. Kaduna metropolis has distinct wet season from April to October and dry season from November to March and is located within the Guinea savannah zone of Nigeria [8]. The state shares boundary with Katsina and Zamfara states in the North, Bauchi and Plateau states on the East, Nasarawa and Federal Capital Territory on the South, Niger state to the West and Kano state to the Northwest. As of 2020, Kaduna State, Nigeria, comprises 23 local government areas, spans an area of 46,053 km^2, and has an estimated population of approximately 8,324,285 people [9]. This study was carried out in Kaduna metropolis which covers 268.358 km^2 with built up areas covering 2,000 km^2. Water samples were collected from four different local government areas, namely; Chikun, Kaduna North, Igabi and Kaduna South local government areas.

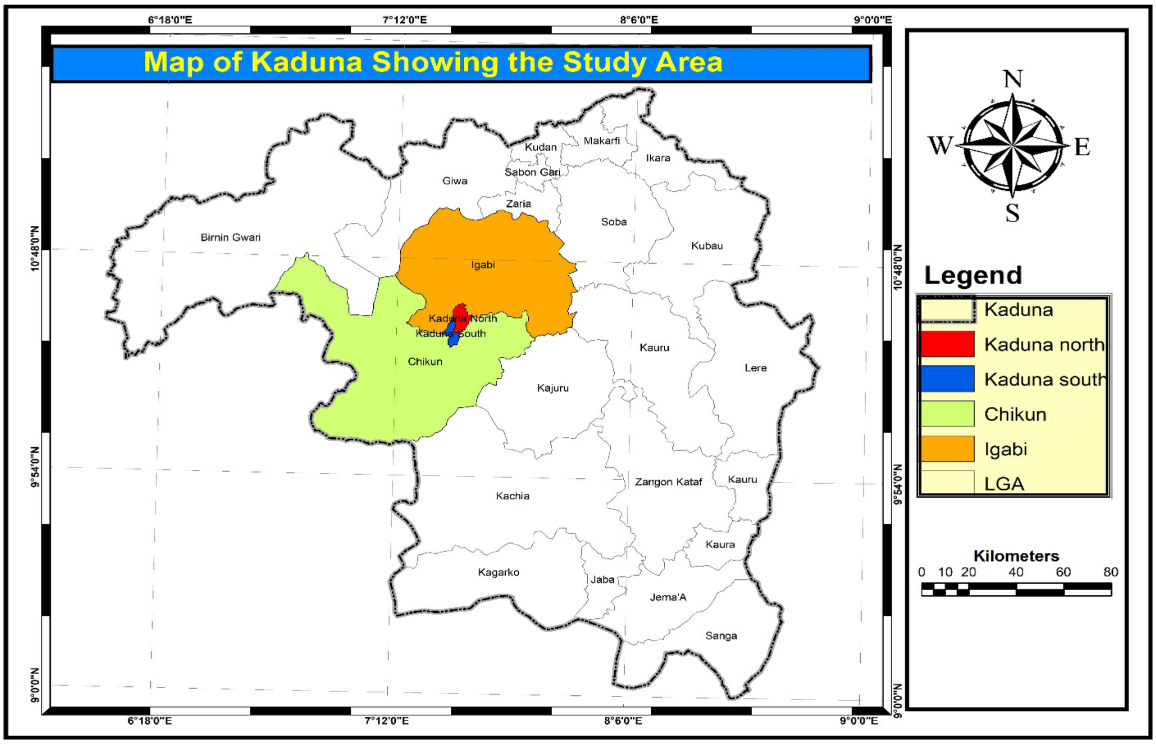


Figure 1: Map of Kaduna (the coloured portions indicate the metropolis where water samples were collected in this study) [10]

Study design

This is a cross-sectional study which was carried out within a duration of six months to identify the bacteria from borehole water samples in the study site and study their antibiotic resistance profiles.

Sample collection

Water samples were obtained from twenty (20) different boreholes located in four (4) respective LGAs namely, Chikun, Igabi, Kaduna North and Kaduna South local government areas within Kaduna metropolis at different intervals and taken to the Department of Biotechnology, Nigerian Defence Academy (NDA), Kaduna, Nigeria for bacterial isolation, identification and molecular analysis. Samples were collected over a period of six months, from December 2023 to May 2024 to capture both rainy and dry seasons. A total of one hundred (100) water samples were collected from different public boreholes during the research work in sterile well-labelled wide-mouthed bottles between the hours of 9:00 a.m. and 2:00 p.m. The samples were collected directly from the boreholes into sterile bottles, tightly corked and transported to the Laboratory of the Department of Biotechnology, Nigerian Defence Academy, Kaduna within 2 h of collection and processed shortly after their arrival at the laboratory.

Serial dilution and isolation of bacteria

The samples were each diluted in test tubes at serial dilutions of 0-5 folds (10^{-1} to 10^{-5}) respectively. Each sample was analyzed in duplicate in order to isolate heterotrophic bacteria. After the water samples had been serially diluted, 0.1 ml of the 2-fold (10^{-2}) and 4-fold (10^{-4}) was inoculated onto nutrient agar plates respectively and 4-fold (10^{-4}) was inoculated on to the MacConkey agar plates, using spread plate method. The plates were all incubated at 37^0C for 24 h. The colonies were thereafter enumerated and recorded.

Distinct colonies were sub-cultured to obtain pure colonies. The pure colonies were grown on agar slants and stored at 4^0C in the refrigerator.

Identification of bacterial isolates

Bacterial isolates were identified by the following methods:

Gram staining: Gram staining of bacterial isolates from each positive plate was carried out according to Tripathi and Sapra [11]. A smear of distinct bacterial colony was prepared on a clean, grease free glass slide by suspending a loopful of the bacterial isolate on it, air dried and heat fixed for 60 seconds. This smear was then allowed to cool before flooding with crystal violet (primary stain) for about 60 seconds before washing with distilled water. The slide was then be flooded with iodine solution and left to stand for about 60 seconds after which it was washed with distilled water and then decolorized with 75% ethanol for 30 seconds. Safranin was then added for 2 min and then washed with distilled water and then blot dried. This was then examined under the microscope using the oil immersion objective lens (100x magnification). Bacterial cells that appeared blue/purple were recorded as Gram positive, while those that appeared reddish were recorded as Gram negative [12]. Their morphology (spiral, rod-shaped or circular) was also observed and recorded.

Biochemical characterization of bacterial isolates: The bacteria in the samples were characterized using various biochemical tests which include: Triple sugar ion (TSI), Indole, Methyl red, Citrate, Oxidase, Catalase, Coagulase and Hydrogen Sulphide [13].

Catalase test: A drop of 3% Hydrogen Peroxide (H_2O_2) was placed on a clean slide and a sterile inoculating loop was used to pick an inoculum and then mixed with the 3% (v/v) H_2O_2 rapidly, the slide was observed for bubbling. A bubbling reaction indicates that the isolate is catalase positive (e.g *Staphylococcus, Micrococcus*) while a non-bubbling reaction means that the isolate is catalase negative (e.g. *Streptococcus*).

Coagulase test: Two drops of blood plasma were placed on an uncontaminated slide and the isolate of interest was inoculated into it and observed for visible agglutination. A positive result showed visible clumping indicating *Staphylococcus aureus* and a negative result without visible clumping indicates the presence of other Staphylococci.

Methyl-red test: Peptone broth was prepared and distributed into several test tubes, the test tubes were sterilized in an autoclave at 121°C, the test tubes were allowed to cool and the isolates of interest were inoculated into the test tubes and incubated at 37°C for 48 hours, five drops of methyl red was then added to the tubes after incubation, shaken and examined after 5 minutes, a colour change was observed. A positive reaction was indicated by the appearance of a red ring on the broth, while a yellow ring signified a negative result [14].

Citrate test: Some bacteria are capable of utilizing citrate as their major and sole source of carbon. Here, Simmon's citrate agar plates were inoculated with the bacterial isolates and incubated at 37°C for 24 h. Colour change from green to blue on the plates indicated citrate utilization by the test organisms [14].

Oxidase test: Two (2) drops of oxidase reagent agent was placed on a filter paper, a colony of the isolate from the water samples were smeared across the same area. A positive oxidase test was indicated by a dark purple coloration of the oxidase reagent, which became visible on the filter paper [14].

Triple sugar iron (TSI) test: Different bacteria have the ability to utilize certain sugars and ferment them. This characteristic varies and is a great way of identifying them. This test is used to distinguish bacteria as a result of their capability to ferment sugars and produce gas and hydrogen sulfide. Isolates of interest were stabbed and streaked on the surface of TSI respectively and incubated at 37°C for 18-24 hours. After 24 h, the media were examined for color changes in the slant, butt, for gas production and H_2S production [15].

Antibiotic susceptibility testing: To ensure standardized bacterial concentrations, a 0.5 McFarland turbidity benchmark was prepared using a mixture of 0.05 ml of 1.175% barium chloride and 9.95 ml of 1% sulfuric acid, stirred consistently to yield a bacterial cell density approximating 1.5×10^8 CFU/ml and an optical density of 0.132 at 600 nm. This suspension was portioned into sterile screw-cap tubes, sealed, and kept at ambient temperature in darkness to preserve stability. Prior to use, each tube was agitated thoroughly. A sterile loop was employed to transfer a small inoculum from a 24-hour-old bacterial culture into sterile 0.89% normal saline. The turbidity of the resulting suspension was visually compared against the McFarland standard to confirm uniform density.

Subsequent antimicrobial sensitivity profiling of the isolates was executed via the Kirby-Bauer disc diffusion approach, utilizing Mueller-Hinton agar, aligned with the Clinical and Laboratory Standards Institute [16] protocols. The isolates were assessed for susceptibility against multiple antibiotics including: Septrin (SXT), Chloramphenicol (CH), Ciprofloxacin (CPX), Amoxicillin (AM), Augmentin (AU), Gentamicin (GM), Pefloxacin (PEF), Ofloxacin (OFX), Sparfloxacin (SP), Streptomycin (S), Ampiclox (APX), Zinnacef, {Cefuroxime} (Z), Rocephin, {Ceftriaxone} (R), Erythromycin (E), and Imipenem (IM).

Sterile swabs were used to inoculate Mueller-Hinton plates with the adjusted bacterial suspension, and antibiotic discs were carefully placed on the agar surface based on whether the organism was Gram-positive or Gram-negative. The plates were inverted and incubated at 37°C for 24 h. Post incubation, the diameters of inhibition zones were measured in millimeters, and results were interpreted in line with CLSI guidelines, including assessments of sensitivity, resistance, and critical zone thresholds [16].

Molecular detection of antibiotic-resistant genes

DNA extraction

The DNA Extraction of the isolates was performed using Accuprep^{TM}Genomic DNA Extraction Kit protocol (K-3032), following the manufacturer's instruction.

The enzyme, Proteinase K (20 µl) was introduced into a sterile 1.5 ml tube and 200 µl of the cultured cell of about 10^4 CFU was introduced into the tube containing the enzyme. Binding buffer solution (200 µl) was added into the tube containing the sample and instantly mixed by vortexing. The whole mix was incubated at 60°C for 10 min. Then 100 µl of isopropanol was added to the tube containing the sample and properly mixed. The lysate was cautiously removed into the upper reservoir of the binding column tube. The tube was closed and then centrifuged for 1 minute at 8,000 rpm. The tube was opened and the binding column was removed to a new 2 ml tube for filtration. The first washing procedure was carried out by adding 500 µl of washing buffer (W1) and then centrifuged for 1 min at 8,000 rpm. The solution was decanted into a disposal bottle; the second washing was done by adding another 500 µl of washing buffer (W2) and then centrifuged for 1 min at 8,000 rpm. The solution was decanted and centrifuged again at 12,000 rpm for 1 min to carefully get rid of ethanol. The binding column was then transferred to a new 1.5 ml tube for the elution process, which was done by adding 200 µl of the elution buffer into the binding column and allowed for 1 min at room temperature (15-25°C). This was to allow the elution buffer to be fully absorbed into the glass fibre of the binding column. Finally, the tube was centrifuged at 8,000 rpm for 1 min to elute the DNA. The above procedure was carried out for all the four different bacterial isolates selected for further molecular analysis. All the four eluted genomic DNA was then refrigerated at 4^oC for storage prior to analysis.

Polymerase chain reaction for the detection of antibiotic-resistant genes

This study used multiplex PCR to simultaneously detect antibiotic resistance genes, β-lactamase TEM gene (blaTEM), DNA gyrase subunit A gene (gyrA), Sulfonamide resistance gene 1 (sul1) and Penicillin-Binding Protein 2a (PBP2a) in four different bacterial isolates (*E. coli, S. aureus, E. aerogenes* and *S. pyogenes*). For *E. coli* and *E. aerogenes*, resistant genes blaTEM, gyrA and sul1 were targeted, while resistant genes blaTEM, gyrA and PBP2a were targeted for *S. aureus* and *S. pyogenes.*

All gene primers were selected on the basis of high multi-drug resistance of the organisms isolated and the class of antibiotics they exhibited multiple resistance to. The procedure was optimized to ensure specificity and reproducibility across the different targets [13].

The earlier extracted bacterial DNA from the four selected isolates were subjected to a PCR setup. For each DNA, a 25 µl PCR reaction was prepared containing 12.5 µl of 2×PCR master mix, 0.5 µl each of forward and reverse primers for each target (final concentration 0.2 µM per primer), 2 µl template DNA and nuclease-free water to volume. All primer sets were validated to have similar annealing temperatures to facilitate multiplexing. PCR was carried out with an initial denaturation at 95°C for 3 min, then denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds and extension for 1 min at 72°C; for 35 cycles.

Table 1 shows the resistant genes targeted in the selected isolates.

Agarose gel electrophoresis

Agarose gel electrophoresis is a standard molecular biology method used to separate DNA fragments according to their length. To begin, a 1.5% agarose solution was prepared by dissolving 1.5 grams of agarose powder in 100 milliliters of 1X Tris-Acetate-EDTA (TAE) buffer. The mixture was heated using a microwave until the agarose completely melted, then allowed to cool to around 50°C. At this point, a DNA-intercalating dye, either ethidium bromide was added to facilitate later visualization of DNA bands under ultraviolet illumination.

Table 1: Antibiotic resistant genes targeted in selected isolates

Target gene	Primer name	Primer sequence ($5^1 – 3^1$)	Amplicon size (bp)	References
blaTEM	TEM-F	F: TCAGCGAAAAACACCTTG	861	[17]
	TEM-R	R: CCCGCAGATAAATCACCA		
sul1	Sul1-F	F: CGGCGTGGGCTACCTGAACG	433	[18]
	Sul1-R	R: GCCGATCGCGTGAAGTTCCG		
gyrA (QRDR)	gyrA-F	F: CAGTCGAGAGGTCGTTGTCC	441	[19]
	gyrA-R	R: TTGTCGCCGTCGTAAGTAGC		
PBP2a (mecA)	PBP2a-F	F: GTGAAGCAACCATCGTTAC	500	[20]
	PBP2a-R	R: CCTTCTACACCTCCATATCAC		

bp – Base pair, **F** – Forward, **R** – Reverse

The cooled agarose solution was poured into a gel casting tray fitted with a comb to create wells and left undisturbed at room temperature until the gel solidified. Once set, the gel was placed inside an electrophoresis tank containing 1X TAE buffer, ensuring the gel was entirely immersed. The comb was carefully removed to expose the sample wells. DNA samples were mixed with loading dye, which helps track their migration and ensures proper sinking into the wells. A DNA ladder (molecular size marker) was also loaded into one well to allow for comparison of fragment sizes.

Electrophoresis was performed using the Bio-Rad PAC 300 power unit (serial H5 00183116). A voltage of 100 volts was applied, and the gel was run for t 45 min, until the tracking dye moved far enough across the gel. Once the run was complete, the gel was transferred onto a UV transilluminator (DOC 200, Bio-Rad, USA), where DNA bands were made visible. These bands, which correspond to DNA fragments, were captured using a gel documentation system. Shorter DNA pieces migrated further through the gel than longer ones, due to the sieving properties of the agarose matrix [21].

This procedure is commonly used to check for the presence, size, and quality of DNA fragments, especially PCR products, in molecular diagnostics and research.

Molecular identification of bacterial isolates

PCR amplification of the 16S rRNA gene

Extracted DNA of the four selected bacterial isolates were respectively amplified by Polymerase Chain Reaction. A 25 µL PCR reaction containing 12.5 µL of 2× PCR master mix, 1 µL of forward primer (27F: 5’- AGAGTTTGATCCTGGCTCAG- 3’), 1 µL of reverse primer (1492R: 5’- GGTTACCTTGTTACGACTT- 3’), 2 µL of template DNA, 8.5 µL of nuclease-free water was prepared and placed in a thermal cycler with initial denaturation at 95°C for 3 min and 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 90 seconds and final extension at 72°C for 5 min.

Afterwards, 5 µL of each of the four PCR products were run on a 1% agarose gel containing ethidium bromide to verify the presence of a ~1500-bp band [22].

Results and Discussion

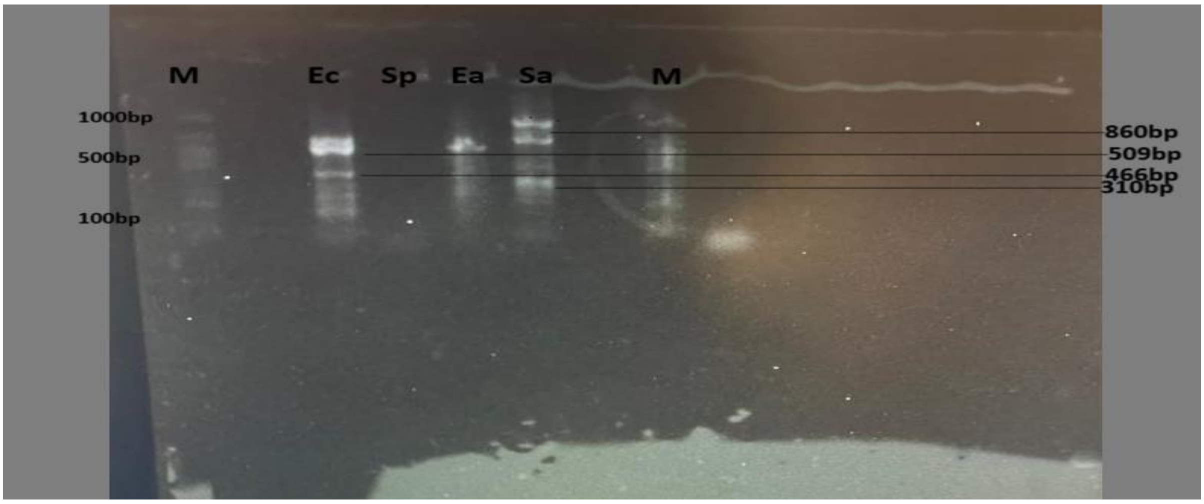
Molecular detection of resistance genes (blaTEM, gyrA, sul1 and PbP2a)

The molecular detection of the antibiotic-resistant genes in isolates by PCR as shown in Fig. 2, revealed the presence of different resistant genes with varying bands for the four different isolates. Isolate *S. pyogenes* showed no band at all for all the primers used for its amplification. Isolates *E. coli*, *K. aerogenes* and *S. aureus* all had a band around 509 bp in common which corresponds to the band size of sul1 gene. The sul1 gene encodes a sulfonamide-resistant dihydropteroate synthase, conferring resistance to sulfonamide antibiotics. Its presence in environmental bacteria, such as *E. coli, S. pyogenes,* and *K. aerogenes*, isolated from borehole water is a critical population level-risk signaling the presence of undesirable impurities and the spread of antibiotic resistance as seen in this study and similar to the finding conducted in Ghana by Agyekum [23] which revealed that sul1 gene was identified in Gram-negative bacteria from drinking water sources through PCR analysis.

Isolates *S. aureus* showed a band around 860 bp and 310 bp as well, similar to blaTEM and PBP2a genes respectively. The resistant gene blaTEM encodes TEM-type β-lactamases, enzymes that make organisms insensitive to β-lactam antibiotics such as penicillins and cephalosporins in. The dissemination of this gene among bacterial pathogens is a significant public health concern, particularly when these organisms are present in water sources like boreholes. This study detected the blaTEM gene in *Staphylococcus aureus* similar to the work of Adekanmbi and Falodun [24].

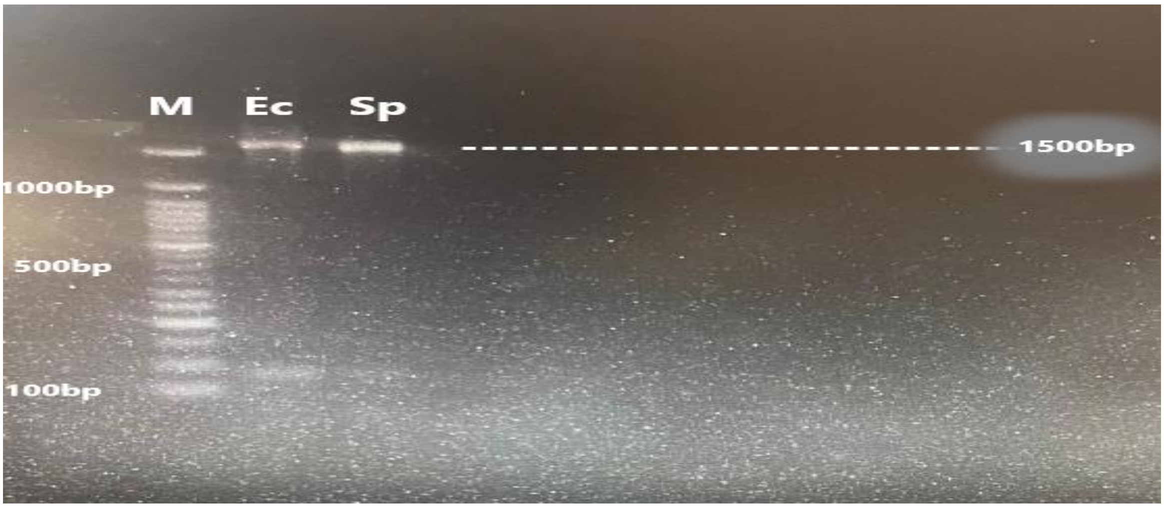
The **PBP2a** gene was detected in *Staphylococcus aureus* in this study. This gene facilitates the production of penicillin-binding protein 2a (PBP2a), a transpeptidase that imparts resistance to β-lactam antibiotics, including methicillin. This mechanism remains a hallmark of methicillin-resistant *Staphylococcus aureus* (MRSA). The identification of MRSA in groundwater drawn from boreholes raises considerable health concerns, as it suggests the presence of drug-resistant bacteria within drinking water supplies. Supporting these findings, prior research undertaken in Awka, located in Anambra State, Nigeria, explored the prevalence of community-associated MRSA among personnel, students, and environmental surfaces within the Faculty of Pharmaceutical Sciences. Among 261 collected samples, 142 isolates of *S. aureus* were recovered, 8.4% of MRSA isolates carried the PBP2a gene, all of which were obtained from nasal specimens. While this research did not focus on water sources, it highlights the prevalence of MRSA in community settings [25]. Another study in Sokoto Town, Nigeria, examined *S. aureus* isolates from in-patients and their caregivers and the researchers found a 21.9% prevalence of *S. aureus* among the samples, with 70.5% of these being MRSA. PBP2a was detected in 27.3% of the *S. aureus* isolates. Although the study focused on clinical samples, it underscores the presence of MRSA in the setting [26].

However, Isolate *E. coli* showed another band at 466 bp for gyrA gene. The resistant gene, gyrA on the other hand was detected in *E. coli* through PCR analysis in this study. In *E. coli*, the **gyrA** gene is responsible for producing the A subunit of DNA gyrase, a critical enzyme that catalyzes the introduction of negative supercoils into DNA. This activity is essential for DNA replication and transcription. Alterations in the quinolone resistance-determining region (QRDR) of the **gyrA** gene have been associated with reduced sensitivity or outright resistance to fluoroquinolone-class antibiotics. These mutations often involve amino acid substitutions at positions 83 and 87, which alter the enzyme's structure and reduce drug binding affinity. This agrees with the work of Patoli *et al.* [27] who detected gyrA in *E. coli* in drinking water samples from Hyderabad, India.



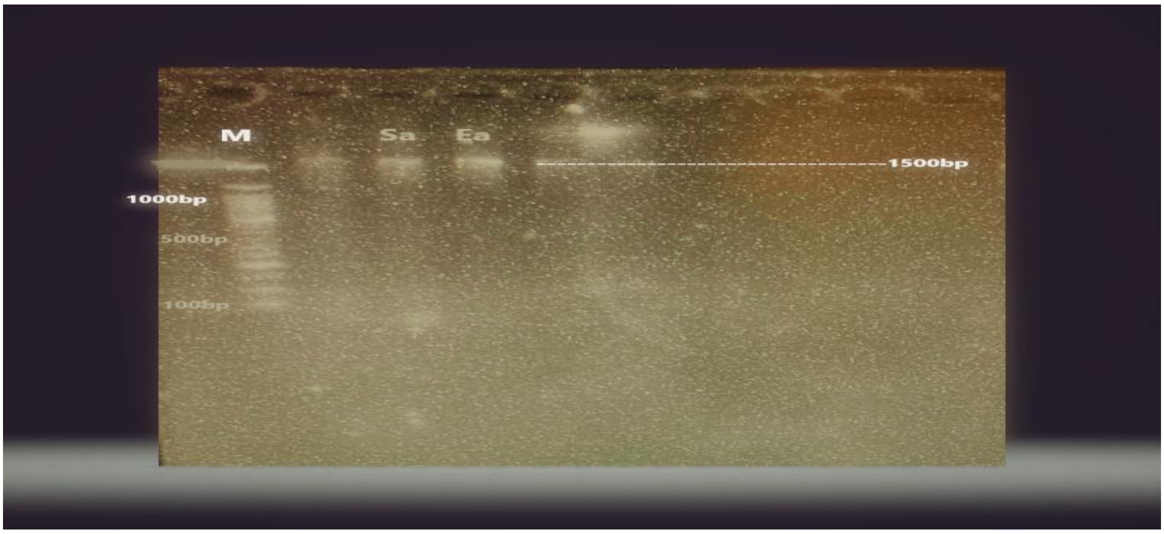
M – Molecular marker, Ec – *Escherichia coli*, Ea – *Klebsiella aerogenes*, Sa – *Staphylococcus aureus*, Sp – *Streptococcus pyogenes*, 860 bp – blaTEM, 509 bp – PBP2a, 466 bp – gyrA, 310 bp – sul1

Figure 2: Electrophoregram of antibiotic resistant genes (blaTEM, gyrA, sul1 and PBP2a)



M – Molecular marker, Ec – *Escherichia coli*, Sp – *Streptococcus pyogenes*

Figure 3a: Electrophoregram of the amplification of 16S rRNA gene in resistant isolates



M – Molecular marker, Ea – *Klebsiella aerogenes*, Sa – *Staphylococcus aureus*

Figure 3b: Electrophoregram of the amplification of 16S rRNA gene in resistant isolates

Amplification of 16S rRNA gene in resistant isolates

All the four isolates, *E. coli, S. aureus, K. aerogenes and S. pneumoniae* showed clear bands around the 1500 bp respectively, when amplified through PCR and viewed after gel electrophoresis, using the 16s rRNA gene primer as shown in Fig. 3a and b, which is the band size of the 16s rRNA gene.

The appearance of clear bands around the ~1500 bp size for all four isolates confirms successful molecular detection of the conserved ribosomal sequence in each isolate. The expected length of the full 16S rRNA gene (~1500 bp) has been documented in numerous studies as the canonical target size for universal bacterial primers [28, 29].

The successful amplification across both Gram-negative (*E. coli, K. aerogenes*) and Gram-positive (*S. aureus, S. pneumoniae*) isolates demonstrates the utility of the primer set in spanning phylogenetically diverse bacteria. This aligns with contemporary work showing that full-length 16S amplification enhances taxonomic resolution and ensures that both conserved and variable regions are included [29, 30].

Moreover, the clear and singular banding pattern indicates that the DNA templates were of sufficient purity and quantity, and that inhibitory contaminants or primer-dimer formation were minimal. In clinical diagnostics, the presence of a discrete ~1500 bp fragment is often considered evidence of robust template quality and appropriate PCR conditions [31].

Conclusion

This study provides compelling evidence that borehole water in Kaduna Metropolis serves as a reservoir for antibiotic-resistant bacteria, revealing the silent, yet significant role environmental sources play in propagating antimicrobial resistance. Through the application of molecular tools such as PCR and 16S rRNA sequencing, we detected key resistance genes, *blaTEM*, *gyrA*, *sul1*, and *PBP2a* across bacterial species including *Escherichia coli*, *Staphylococcus aureus*, and *Enterobacter aerogenes*. Notably, *sul1* emerged as the most frequently detected gene, underscoring widespread sulfonamide resistance even in presumed safe water sources. The absence of resistance markers in *Streptococcus pyogenes* further illustrates the diversity of bacterial responses to environmental pressure.

These findings expose a critical vulnerability in public health systems which the unchecked transmission of resistant pathogens through community water supplies. Rather than isolated laboratory curiosities, these genes represent a living threat circulating, evolving, and adapting in real time. As antibiotic resistance escalates into a global crisis, this study reinforces the urgency of integrating environmental monitoring into broader AMR strategies. Clean water is not only a human right but a frontline defense against microbial threats. We must begin to treat our boreholes not merely as water points, but as potential battlegrounds in the fight for future antibiotic efficacy.

Conflict of interest: All the authors declare that they have no conflicting interest.

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