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# Investigation And Characterization Of Pathogens Isolated From Water Sources In Umudi, Nkwerre Local Government Area

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**Abstract** **Original Research Article**

Access to safe drinking water remains a major challenge in rural Nigerian communities where residents rely heavily on streams, hand-dug wells, boreholes and rainwater. Consumption of microbiologically contaminated water contributes significantly to waterborne morbidity and mortality, a risk exacerbated by rising bacterial resistance to standard antibiotics. To evaluate the physical, chemical, and microbiological quality of water sources in Umudi, bacterial pathogens were characterized using biochemical tests and 16S rDNA molecular sequencing after physicochemical analysis. A cross-sectional descriptive design was employed. Water samples were collected from stored rainwater, boreholes, hand-dug wells, and rivers (Aju-miri and Ofeiyi). Samples underwent physicochemical analysis using a multiparameter meter, cultural enumeration on nutrient agar, biochemical characterization (Gram staining, Catalase, Oxidase, Coagulase, Indole, MR, motility, TSI, and H2S tests) and antibiotic resistance test using Kirby-Bauer disc diffusion method. Other tests employed include, Genomic DNA extraction, 16S rRNA amplification using 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers and sequencing on an ABI 3510 sequencer. Physicochemical parameters varied across sources (e.g., pH ranged from 5.82 in rivers to 7.96 in boreholes). Cultural and biochemical characterization revealed the presence of multiple pathogenic bacteria, Staphylococcus aureus (isolated from 7 samples), *Salmonella* spp. (8 samples), *Shigella* spp. (3 samples), Proteus spp. (2 samples), *Klebsiella* spp. (1 sample), and Escherichia coli (1 sample). Antibiotic susceptibility profiling demonstrated resistance to commonly used antibiotics and sensitivity to good number of antibiotics. Molecular analysis via 16S rRNA gene sequencing confirmed the genetic identity of *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., Proteus spp., *Klebsiella* spp. and *Escherichia coli*. The water sources in Umudi are heavily contaminated with multidrug-resistant enteric and pathogenic bacteria, posing severe health risks to the local population. Government of the state should support urgent water treatment, encourage improvement of sanitation and strict antibiotic stewardship in Umudi.

**Keywords:** Drinking water quality, bacterial contamination, antibiotic resistance, pathogenic bacteria, 16S rRNA sequencing.

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## INTRODUCTION

Water is a clear, odorless liquid made of hydrogen and oxygen. It is critical for all living things. It covers most of the Earth. It can be a liquid, ice, or gas. It acts as a great solvent and helps control temperatures because of its strong molecular bonds. It is an essential resource for human survival, domestic activities, agriculture, and industrial processes. Safe drinking water is still hard to find in many developing areas, especially in villages without treated tap water. People in these places use streams, rivers, wells, boreholes, and rain. These water sources often have germs and cause sickness (Babra and Adrian, 2025). According to the World Health Organization (WHO), potable water should be free from pathogenic microorganisms and harmful chemical substances that may pose health risks to consumers.

Water contamination occurs when harmful substances or microorganisms enter water bodies, making them unsafe for use. Major sources of contamination include domestic sewage, agricultural activities, industrial effluents, and surface runoff. Contaminated drinking water is a primary way microbial germs spread in developing countries, though inadequate sanitation and contaminated food are also major contributors to intestinal infections (Nicholas, 2004).Surface water is easily polluted because it stays open to the air and land. Groundwater in wells can also get dirty when waste from toilets or trash pits leaks into the water, mostly if the wells are shallow or built the wrong way (Prashant *et al*., 2025).

Waterborne diseases are diseases spread by drinking or touching bad water. Pathogens like bacteria, viruses and parasites can cause them. Frequent bacterial sicknesses from water are cholera, typhoid fever, dysentery, and stomach flu (Ashbolt, 2015). Waterborne diseases caused by pathogenic microorganisms remain a significant cause of morbidity and mortality worldwide. Pathogen like *E. coli*, *Salmonella*, *Shigella*, *Vibrio*, and *Staphylococcus aureus* live in dirty water. They cause severe diarrhea, typhoid, cholera, and stomach sickness. Bad sanitation, open toilets, farm runoff, and poor trash disposal cause this dirty water. Bacterial pathogens are the most commonly studied due to their frequent association with waterborne diseases. The presence of indicator organisms such as coliform bacteria, especially *Escherichia coli*, is often used to assess the microbiological quality of water and the likelihood of fecal contamination (Amir and Zhengjian, 2019).

Consumption of microbiologically unsafe water is a leading cause of morbidity and mortality worldwide, especially in developing countries. Waterborne diseases disproportionately affect children, pregnant women, the elderly and immunocompromised individuals. In rural Nigerian communities with poor safe water access, studies show high levels of these germs in raw water sources. Drinking this dirty water causes repeated outbreaks of water-related illnesses (Anna *et al*., 2025). How clean water is kept, proper toilet use, and good personal habits directly control how often waterborne sickness spreads. Young kids, older adults, and people with weak immune systems face the highest risk of severe illness or danger from these diseases (Fewtrell *et al*., 2005).

Bacterial infections usually need antibiotics, extra care, and rehydration if diarrhea occurs. Doctors pick the specific antibiotic based on the exact pathogen and what drugs it fights off best. (Christopher, 2010). Rising rates of drug-resistant bacteria make waterborne illnesses harder to treat. This growth shows a clear need for careful antibiotic use, better clean water management and prevention steps like vaccines and water purification (WHO, 2020). Water bodies contaminated with resistant bacteria can act as reservoirs and dissemination routes for antibiotic resistance genes. Pathogens such as E. *coli, Salmonella, and Pseudomonas aeruginosa* isolated from water sources have been reported to exhibit multidrug resistance, making infections difficult to treat (Rizzo *et al*., 2013).

## AIM

To investigate and characterize pathogenic bacteria isolated from selected water sources in

Umudi, Nkwerre Local Government Area in Imo State.

## OBJECTIVE OF THE STUDY

The objectives of this research are to:

- Evaluate Umudi water quality and safety by measuring their physical properties and chemical composition.
- Isolate bacteria pathogens from water sources in Umudi.
- Identify and characterize bacterial pathogens using standard biochemical test.
- Identify the bacteria pathogens at molecular level using 16S rDNA sequencing.
- Evaluate the resistance patterns of the isolated bacteria pathogens.

## METODOLOGY

### STUDY AREA

The study area is Umudi, Nkwerre Local Government Area, Imo State, Nigeria. Umudi is a rural community where residents largely depend on streams, hand-dug wells, boreholes, and rainwater for domestic activities such as drinking, cooking, and washing. . It is primarily known for housing Hezekiah University (UNIHEZ), the first private university in the eastern heartland. Local development is accelerating as government initiatives drive vital infrastructure improvements.

### STUDY DESIGN

This study adopted a cross-sectional descriptive design aimed at investigating and characterizing bacteria pathogens present in selected water sources in Umudi, Nkwerre LGA.

### MEDIA PREPARATION

The media (Nutrient agar) used for the research was sterilized in an autoclave at 121$^{o}$C for 15 mins at Psi. All glassware was also sterilized in hot air oven at 160$^{o}$C for 1 hour. All sterilization was done based on producer's specifications.

### SAMPLE COLLECTION

Borehole water samples were collected very early in the morning using sterile bottle before they are being used by people. On collection, it was allowed to flow for few minutes before collection. While river water samples were collected using same sterile bottle and were collected away from offshore. Well water were collected using a different container for each well and were used to fill up sterile bottles use for the study. The samples were randomly collected from different collection points located in different villages in Umudi Community, Nkwere.

### SAMPLE ANALYSIS

#### Culture and Preliminary Identification

The samples collected were cultured on nutrient agar after 10 folds serial dilution and incubated at 37$^{0}$C for 24hours. The colony forming units were counted in each of the incubated culture.

#### Physiochemical Test

Multiparameter meter were used to test the physiochemical parameters of the water samples.

#### Biochemical Characterization

The isolates were subjected to biochemical tests. The tests include the following: Gram staining, Coaugulase test, Citrate test, Catalase test, Indole test, Hydrogen sulphide test, Oxidase test, Methyl red test and TSI test.

### MOLECULAR IDENTIFICATION OF THE ISOLATES

#### Bacterial Genomic DNA Extraction of the Isolates

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the

pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer into a ZR Bashing Bead Lysis tubes, 750 microliter of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tubes were centrifuged at 10,000x*g* for 1 minute (Zymo Research, 2023).

Four hundred (400) microliters of supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 x*g* for 1 minute. One thousand two hundred (1200) microliters of fungal/bacterial DNA binding buffer were added to the filtrate in the collection tubes bringing the final volume to 1600 microliter, 800 microliter was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000x*g* for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microliter of the DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000x*g* for 1 minute followed by the addition of 500 microliter of fungal/bacterial DNA Wash Buffer and centrifuged at 10,000x*g* for 1 minute (Singh *et al*., 2022).

The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, 100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000x*g* microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction.

### DNA Quantification of the Isolates

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer at 260nm wavelength according to manufacturer's instructions. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

### 16S rRNA Amplification of the Isolates

The 16s RRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator.

### DNA Sequencing of the Isolates

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ulBigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min. all these were carried out base on manufacturer's guidelines.

## RESULTS

The investigation started with physiochemical parameters analysis of the water samples to evaluate water quality and safety by measuring its physical properties and chemical compositions. The samples used for the analysis include, borehole samples (Bw), stored water in

a rubber tank (GP), underground/well water (Well) and river samples. The result of the physiochemical parameters analysis is shown below:

**TABLE ONE: Physiochemical parameters result of the samples**

| Samples | pH | EC (µS/c) | TDS | SG | ORP |
|---|---|---|---|---|---|
| **GP1** | 6.73 | 44 | 73 | 1.002 | +112 |
| **GP2** | 6.75 | 36 | 72 | 1.002 | +110 |
| **GP3** | 7.46 | 39 | 72 | 1.002 | +112 |
| **GP4** | 7.40 | 45 | 73 | 1.001 | +116 |
| **Bw1** | 7.80 | 10 | 6 | 1.001 | +88 |
| **Bw2** | 7.74 | 10 | 6 | 1.001 | +94 |
| **Bw3** | 7.77 | 9 | 6 | 1.001 | +82 |
| **Bw4** | 7.96 | 12 | 6 | 1.002 | +84 |
| **Well1** | 7.40 | 46 | 52 | 1.002 | +108 |
| **Well2** | 7.34 | 46 | 35 | 1.002 | +104 |
| **Well3** | 7.66 | 40 | 18 | 1.002 | +101 |
| **Well4** | 7.50 | 43 | 25 | 1.002 | +106 |
| **Oj** | 5.82 | 68 | 98 | 1.002 | +82 |
| **Of** | 5.81 | 66 | 96 | 1.002 | +83 |
| **Hez1** | 7.31 | 54 | 28 | 1.002 | +94 |
| **Hez2** | 7.28 | 44 | 23 | 1.002 | +93 |
| **Hez3** | 7.15 | 48 | 26 | 1.002 | +96 |
| **Hez4** | 7.34 | 45 | 24 | 1.002 | +94 |
| **Hez5** | 7.42 | 46 | 23 | 1.002 | +95 |
| **Hez6** | 7.35 | 44 | 23 | 1.002 | +96 |
| **Hez7** | 7.65 | 33 | 16 | 1.002 | +88 |
| **Hez8** | 7.61 | 33 | 16 | 1.002 | +92 |

KEY: *pH= Potential of Hydrogen, EC= Electrical conductivity, TDS= Total Dissolved Salts, SG= Specific gravity, ORP= Oxidation Reduction Potential, GP= Stored rain water, Bw= Borehole water, Well= Well water, Aj= Aju-miri river, Of= Ofeiyi river, Hez= Hezekiah university borehole.*

The water samples were cultured from the 5$^{th}$ fold diluents in Nutrient agar and the samples exhibited different morphology characteristics as shown below:

**TABLE TWO: CULTURAL CHARACTERISTICS OF THE ISOLATES**

| Samples | Colony morphology | | | | Number of colony (*cfu/ml*) $10^{-3}$ |
|---|---|---|---|---|---|
| | **Size** | **Type** | **Shape** | **Color** | |
| **GP1** | Small | Smooth | Circular and convex | Golden yellow | 39x $10^{-5}$ |
| **GP2** | Large | Rough | Circular and Concave | Milky | 30 x $10^{-5}$ |
| **GP3** | Large | Smooth | Circular and convex | Milky | 46 x $10^{-5}$ |
| **GP4** | Very small | Smooth | Rhizoid | Whitish | 36 x $10^{-5}$ |
| **Bw1** | Small | Smooth | Circular and Concave | Milky | 40x $10^{-5}$ |
| **Bw2** | Large | Rough | Circular and Raised | Milky | 44 x $10^{-5}$ |
| **Bw3** | Large | Rough | Circular Raised | Milky | 35 x $10^{-5}$ |
| **Bw4** | Small | Smooth | Circular and Concave | White | 5 x $10^{-5}$ |
| **Well1** | Small | Smooth | Irregular and flat | White | 2 x $10^{-5}$ |
| **Well2** | Very small | Smooth | Circular and Raised | Whitish | 2x $10^{-5}$ |
| **Well3** | Small | Smooth | Circular and Flat | Whitish | 3x $10^{-5}$ |
| **Well4** | Small | Smooth | Circular and Flat | Whitish | 5x $10^{-5}$ |
| **Oj*A*** | Large | Smooth | Irregular and raised | Whitish | 88x $10^{-5}$ |
| **Oj*B*** | Large | Smooth | Circular and raised | Milky | ,, |
| **Of** | Large | Smooth | Circular and Raised | Milky | 60x $10^{-5}$ |
| **Hez1** | Very small | Smooth | Not cleared | Whitish | 5x $10^{-5}$ |
| **Hez2** | Small | Smooth | Irregular and raised | Milky | 10x $10^{-5}$ |
| **Hez3** | Small | Smooth | Circular and flat | Whitish | 4x $10^{-5}$ |
| **Hez4** | Large | Rough | Raised | Milky | 27x $10^{-5}$ |
| **Hez5** | Large | Smooth | Raised | Milky | 15x $10^{-5}$ |
| **Hez6** | Large | Rough | Raised | Grayish white | 26x $10^{-5}$ |
| **Hez7** | Large | Smooth | Raised | Milky | 24x $10^{-5}$ |
| **Hez8** | Very small | Smooth | Flat | Whitish | 3x $10^{-5}$ |

KEY: *cfu/ml= colony forming unit per mil, GP= Stored rain water, Bw= Borehole water, Well= Well water, Oj= Aju-miri river, Of= Ofeiyi river, Hez= Hezekiah university borehole.*

The biochemical characteristics of the isolates was done with the WHO endorse standardized. Isolates were identified by comparing biochemical results with Bergey's Manual of Determinative Bacteriology. Most similar and dissimilar colonies showed different biochemical characteristics. The result of

biochemical tests is shown in the table below:

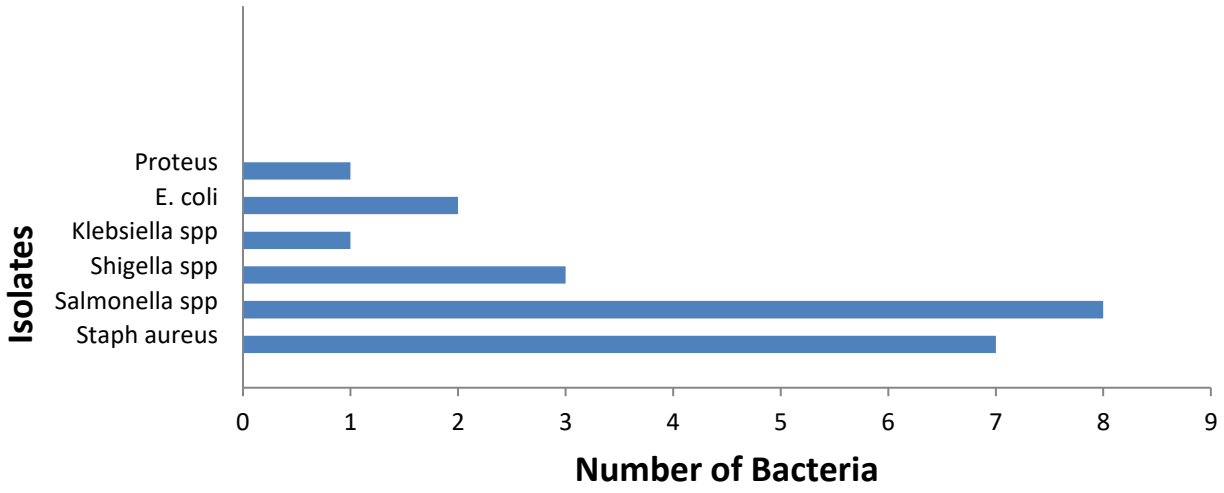
**Table Three: Biochemical Characterizations of the isolates**

| Samples | Gram Stain | Catalase | Oxidese | Coagulase | Indole | MR | Motility | TSI | $H_2S$ | Probable organism |
|---|---|---|---|---|---|---|---|---|---|---|
| GP1 | +cocci | - | - | + | - | - | - | + | - | *Staphylococcus aureus* |
| GP2 | +cocci | - | - | + | - | - | - | + | - | *Salmonella spp* |
| GP3 | -rod | + | - | - | - | + | + | - | - | *Shigella* |
| GP4 | -rod | + | - | - | - | + | - | - | + | *Klebsiella spp* |
| Bw1 | -rod | + | - | - | - | + | + | - | - | *Staphylococcus aureus* |
| Bw2 | -rod | + | - | - | - | + | + | - | - | *Salmonella spp* |
| Bw3 | -rod | + | - | - | - | + | + | - | - | *Salmonella spp* |
| Bw4 | -rod | + | - | - | - | + | + | - | - | *Staphylococcus aureus* |
| Well1 | -rod | + | - | - | - | + | + | - | - | *Salmonella spp* |
| Well2 | -rod | + | - | - | - | + | + | - | - | *Salmonella spp* |
| Well3 | -rod | + | - | - | - | + | + | - | - | *Salmonella spp* |
| Well4 | -rod | + | - | - | - | + | + | - | - | *Salmonella spp* |
| Aju-miri river | -rod | + | - | - | - | + | + | - | - | *Staphylococcus aureus* |
| Ofeiyi river | -rod | + | - | - | - | + | + | - | - | *Shigella* |
| Hez 1 | -rod | + | - | - | - | + | + | - | - | *Staphylococcus aureus* |
| Hez 2 | -rod | + | - | - | - | + | + | - | - | *Staphylococcus aureus* |
| Hez3 | -rod | + | - | - | - | + | + | - | - | *Escherichia coli* |
| Hez 4 | -rod | + | - | - | - | + | + | - | - | *Proteus* |
| Hez 5 | -rod | + | - | - | - | + | + | - | - | *Salmonella spp* |
| Hez6 | -rod | + | - | - | - | + | + | - | - | *Escherichia coli* |
| Hez7 | -rod | + | - | - | - | + | + | - | - | *Staphylococcus aureus* |
| Hez 8 | -rod | + | - | - | - | + | + | - | - | *Shigella* |

KEY: +rod= positive rod (Gram staining), -rod= negative rod (Gram staining), +rod= positive cocci (Gram staining), += positive, - =negative, A= Acid, A/G= Acid and gas, TSI= Triple sugar indole *GP= Stored rain water, Bw= Borehole water, Well= Well water, Aj= Aju-miri river, Of= Ofeiyi river, Hez= Hezekiah university borehole*

*Staphylococcus aureus* were isolated from 7 different samples, *Salmonella spp* were isolated from 8 different samples and *Shigella* spp were isolated from 3 different samples. While, *Klebsiella* spp, *Proteus and E. coli;* 1, 2 and 1 respectively, where isolated from the water samples from different water sources in Umudi. The percentage occurrence of the isolates are shown in the figure 1

**Figure 1: Percentage occurrence of the isolates from different water samples**




The resistant pattern prior to the resistance gene identification of the pathogens were carried out according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI, 2018) and Kirby-Bauer disc diffusion technique as recommended by the World Health Organization(WHO). Result of the test with the diffusion disc containing popularly use antibiotics in Imo State is shown in the table four below:

**Table Four: Sensitivity pattern of the isolates**

| Isolates | OFX | CN | PEF | CPX | AU | AZ | LEV | CF | AM | SP |
|---|---|---|---|---|---|---|---|---|---|---|
| *Staph aureus* | 5(18.5%) | 0 0(0%) | 5(18.5%) | 4(14.8%) | 0(0%) | 1(3.7%) | 5(18.5%) | 2(7.4%) | 4(14.8%) | 2(7.4%) |
| *Salmonella* spp | 5(15.2%) | 1(3.0%) | 5(15.2%) | 5(15.2%) | 0(0%) | 2(6.1%) | 5(15.2%) | 2(6.1%) | 3(9.1%) | 5(15.2%) |
| *Shigella* spp | 5(14.3%) | 4(11.4%) | 5(14.3%) | 5(14.3%) | 1(2.8%) | 2(5.7%) | 5(14.3%) | 5(14.3%) | 1(2.8%) | 5(14.3%) |
| *Klebsiella* spp | 4(13.3%) | 1(20%) | 2(6.7%) | 4(13.3%) | 1(3.3%) | 2(6.7%) | 3(10%) | 4(13.3%) | 5(16.7%) | 2(6.7%) |
| *Proteus* | 1(2.9%) | 4(11.7%) | 3(8.8%) | 4(11.7%) | 4(11.7%) | 2(5.9%) | 5(14.7%) | 5(14.7%) | 1(2.9%) | 5(14.7%) |
| *E. coli* | 4(13.8%) | 1(3.4%) | 2(6.9%) | 3(10.3%) | 1(3.4%) | 2(6.9%) | 5(17.2%) | 5(17.2%) | 1(3.4%) | 5(17.2%) |

**KEY**: CF = Cefuroxime, CEP= Ceporex, CTZ= Ceftazidime**,** S = Streptomycin, CN = Gentamycin, AU = Augmentin, OFX = Ofloxacin, CPX = Ciprofloxacin, PEF = Peflacinen, LEV= Levofloxacin, AZ= Azithromycin, Cefuroxime, AM= Amoxicillin

Molecular characterization of the isolates was done using 16S rRNA sequencing. The obtained 16s rRNA sequence from the isolates produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. Agarose electrophoresis showing 16S RNA fragment of Salmonella typhi is shown in figure 2.

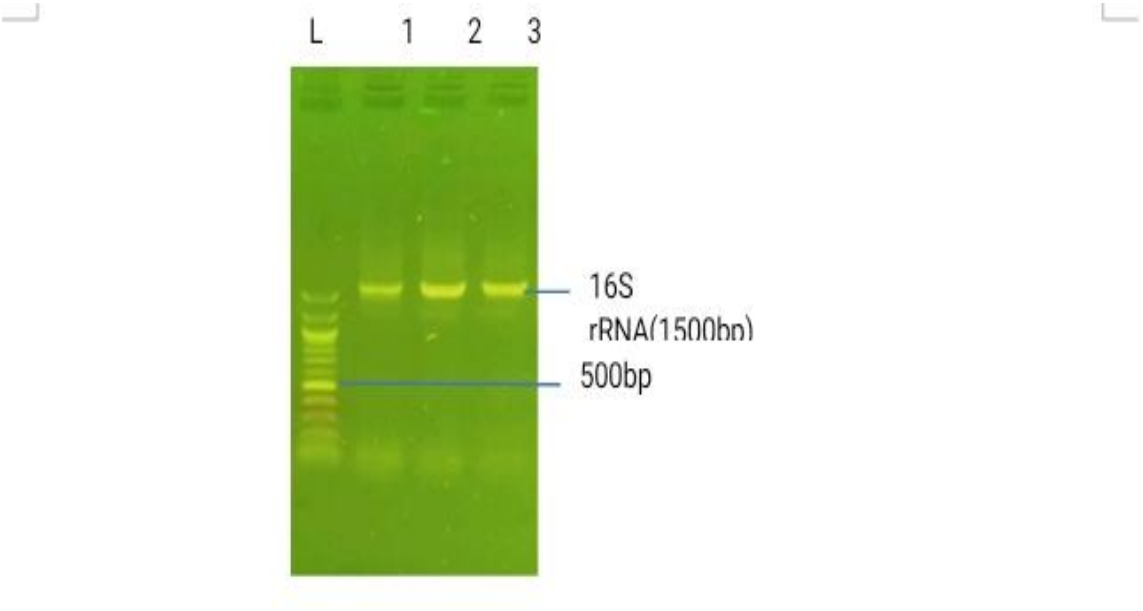



Figure 2: Agarose gel electrophoresis sowing the amplified 16S rRNA fragment. Lanes 1-3 represent the amplified 16SrRNAbands at 1500bp while L represents the 100bp molecular ladder.

## DISCUSSION

Table One displays the physicochemical properties of various water sources in Umudi Nkwere, primarily from stored rainwater (GP), borehole water (Bw and Hez), well water, and two rivers (Oj and Of). These parameters indicate how different environmental and geological factors impact the overall quality and microbial risk of the water. For pH (Potential of Hydrogen), most samples (GP, Bw, Well, and Hez) are slightly alkaline to neutral (pH 7.15 to 7.96), falling well within the standard drinking water range. However, the river samples (Oj and Of) are distinctly acidic (pH 5.81 and 5.82). Acidic water is typically softer and may leach heavy metals if passing through metal pipes. For the EC (Electrical Conductivity) and TDS (Total Dissolved Salts), EC and TDS correspond closely, measuring the concentration of dissolved ions. Borehole (Bw) and Hezekiah University (Hez) samples show very low Electrical Conductivity and Total Dissolved Salts, indicating low mineral content. In contrast, the rivers show the highest Electrical Conductivity and Total Dissolved Salts, likely due to suspended matter, runoff, or mineral dissolution. SG (Specific Gravity) values for all samples are nearly identical (1.001 to 1.002), indicating that density differences between the various water sources are statistically insignificant. ORP (Oxidation Reduction Potential) measured the ability of water to act as an oxidizing or reducing agent, typically used to evaluate microbial activity and disinfection. All samples display positive ORP values (+82 mV to +116 mV), pointing to oxidizing environments. However, these levels fall below the +200 mV to +600 mV threshold typical for effective microbial control in drinking water. Low positive or negative ORP can indicate an environment where certain contaminants or microorganisms may persist without oxidants.

The viable colony count in this study was done and interpreted according to Fibre Test Biology

Dilution work sheet. Based on serial dilution data and the result of number of colony forming units, Aju-miri (88x $10^5$) and Ofeiyi (60x $10^5$) rivers water samples have the highest microbial load, followed by the borehole water $Bw_2$(44x $10^5$), borehole water $Bw_2$ (40x $10^5$) and stored tank water GP4 (36x $10^{-5}$). This makes perfect sense, given that surface streams are constantly vulnerable to environmental runoff, animal waste, human interference, and the breaking down of organic matter according to (Anna *et al*., 2013). The bacterial count rises slightly from (35x $10^5$) in raw borehole water to (36x $10^5$) in the stored plastic tank water. This increase highlights a common issue, secondary contamination. The result supports Onuorah (2022**)** 'bacteria can easily multiply within the plastic tanks during storage'**.** According to World Health Organization (WHO) and local regulatory standards for drinking water, potable water must contain 0 CFU per 100 mL of total coliforms or fecal indicator bacteria(WHO, 2023). Therefore, none of the water sources in Umudi Nkwere is safe for direct consumption without heavy treatment.

The detection of *Salmonella typhi*, *E. coli*, *Shigella*, *Klebsiella*, *Proteus*, and *Staphylococcus aureus* across multiple water sources in Umudi Nkwere indicates severe, widespread fecal contamination. This renders the borehole, stored rainwater, and river water unsafe for drinking or domestic use without immediate, rigorous purification. The presence of these pathogens points to a mix of human waste, animal waste, and poor environmental hygiene. *E. coli* is a primary indicator of fecal contamination and exposure to it can lead to severe gastrointestinal distress, stomach cramps, and diarrhea. *Salmonella typhi* is the bacteria responsible for typhoid fever, causing high fever, weakness and severe abdominal pain. While *Shigella* is highly contagious pathogen that causes shigellosis, an intestinal infection resulting in severe diarrhea, fever, and stomach cramps. *Klebsiella* and *Proteus* are opportunistic bacteria often found in both environmental runoff and clinical environments; they frequently cause urinary tract infections (UTIs) and wound infections. However, according to the Nigeria Centre for Disease Control (NCDC), relying on these untreated sources presents a severe health crisis.

Studies across Nigeria consistently reveal widespread fecal contamination in rural and semi-urban water sources, with *E. coli* acting as the dominant microbial contaminant in 70% of reviewed literature. Similar research mirrors findings of co-contamination with *Salmonella typhi*, *Shigella*, *Klebsiella*, *Proteus*, and *Staphylococcus aureus* across surface and groundwater systems by Stephen and Kennedy, 2018). The findings of this research in Umudi Nkwere are heavily supported by Overview of Major Bacterial Contaminants of Drinking Water in Nigeria, which highlights *Klebsiella* appearing in roughly 60% of analyzed studies. This study is in correlation with Oche (2019), bacteria typically thrive in shallow boreholes and unprotected wells due to poor proximity to pit latrines.

According to WHO (2024), antibiotic resistance in *Salmonella typhi*, *E. coli*, *Shigella*, *Klebsiella*, *Proteus*, and *Staphylococcus aureus* is a critical global health concern. Therefore, the result of susceptibility pattern of the pathogens in this study confirmed that *Salmonella typhi*, *E. coli*, *Shigella*, *Klebsiella*, *Proteus*, and *Staphylococcus aureus* exhibit multidrug resistance (MDR), hence, rendering standard, first-line treatments like fluoroquinolones, and early-generation cephalosporins ineffective as established by Mayrin *et al*., (2020).

## CONCLUSION

All the water sources in Umudi Nkwere contain pathogens that are responsible for severe illnesses like cholera, typhoid and dysentery. The 16SrRNA sequencing confirmed the highest identified bacteria in this study to be *Salmonella typhi*, therefore typhoid infection is rampant in Umudi Nkwere. The confirmations do not contradict oral information about typhoid fever occurrence among the students of Hezekiah University located in Umudi as well as Umudi indigenes. None of these water sources are safe for direct consumption without heavy treatment; therefore, consuming water

with these microbial concentrations risks waterborne disease outbreaks.

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