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Antimicrobial Susceptibility Testing and Molecular Identification of Bacteria Isolated from Treated Water Sources for Municipal Consumption in Sokoto, Sokoto State, Nigeria

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ABSTRACT

This study evaluated the microbiological quality of treated municipal water sources in Sokoto, Nigeria, to assess their suitability for consumption and potential public health risks. A total of 15 water samples were collected from five diverse locations these includes; Water board post treatment, Minannata, Runjin Sambo, Mabera and Tsohuwar Kasuwa area and analysed for microbiological quality assessment which includes; bacterial isolation and identification, antimicrobial susceptibility testing, biochemical test and antibiotic sensitivity test by using Kirby-Bauer method as well as molecular identification of antibiotics resistance bacterial pathogens. Seven bacterial species were isolated and identified, with the highest frequencies attributed to Escherichia coli (25%), Staphylococcus aureus (18.75%), S. typhi (18.75), Bacillus spp (12.5), Pseudomonas aeruginosa (12.5%), Proteus spp and Shigella flexineri (6.25) respectively. Antimicrobial susceptibility testing showed alarming resistance, particularly in E. coli and Proteus spp which was highly resistant to Septrin (100%), ciprofloxacin (80%), levofloxacin (80%), and amoxicillin/augmentin (60-75%) . Conversely, S. aureus and S. typhi were generally more sensitive to tetracycline, ampicillin, and gentamycin. Further molecular analysis of multi-drug-resistant strains identified emergent pathogens such as Proteus terrae, and Providencia stuartii. This study underscores the significant public health risk posed by contaminated water after treatment, driven by the widespread presence of multi-drug resistant (MDR) bacteria. Continuous monitoring, urgent infrastructure upgrades, and enhanced water treatment strategies are imperative to mitigate the rising threat of water-related illnesses in the region.

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INTRODUCTION

Almost every living organism need water either directly or indirectly for their survival. Over 70% of the earth`s surface is covered with water. This is the reason why water is the most abundant naturally occurring chemical substance found on the earth crust (Chakraborty, 2021). It is estimated that Hundred percent (100%) of the earth population depend on water for survival while food production heavily depends on water; as such the minimum standard of quality treatment is required (Mathew & Krishnamurthy, 2017). The quality of water is determined by its physical, chemical and biological properties. As such, water quality should be evaluated before its usage. Therefore, quality parameters most likely to affect

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the quality of water are to be assessed (Shah, 2017). Despite advancement in water treatment technologies, many communities (such as the study area) struggle with accessing enough, clean and safe potable water, leading to a range of health problems and economic burden (Tella *et al.*, 2025).

Heavy metals contamination in earth dam water bodies also poses significant environmental and public health challenges (Mahas, 2025, Tazri *et al.*, 2025). Heavy metals, such as lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), and nickel (Ni), can enter water bodies through natural geological processes or human activities such as industrial discharge, mining, agricultural runoff, and improper waste disposal (Chen *et al*., 2025; Sultana *et al.*, 2025). Once these metals are introduced into an earth dam, they often persist in the ecosystem due to their non-biodegradable nature (Chen *et al*., 2025). This persistence leads to the accumulation of heavy metals in sediments, water, and aquatic organisms, causing long-term environmental degradation and posing risks to human and ecological health (Datta, *et al.,* 2025).

Only recently has the environment been recognized for its role in the global spread of clinically related antibiotic resistance. Aquatic environments specifically, may receive water for urban sewage and runoff from agricultural facilities. The occurrence of antibiotic resistance in aquatic environments has been reported since the 1970s, when *Enterobacteriaceae* was observed in rivers in the USA (Feary *et al*., 2021). Furthermore, it has been demonstrated that rivers and lakes that are contaminated with wastewater effluent also contain ARBs (Iwane *et al*., 2021; Czekalski *et al.*, 2019). Antimicrobial-resistant microorganisms in water may originate from waste, including human and animal faeces (Burgmann *et al.,* 2018). This is generally due to the fact that the microbes from these organisms had, at some stage, been exposed to antibiotics, either for therapeutic purposes (infection control or as a prophylactic) or as growth promoters in the case of animal-rearing practices (Arsene *et al.,* 2021).

MATERIALS AND METHODS

Collection of Samples

A total volume of 7500 ml of water samples was collected, comprising 15 samples in total. Fifteen (15) samples were collected from treated water sources. All samples were collected in sterile 500 ml bottles across five sampling sites. (a) Water Board (post-treatment), (b) Mabera, (c) Minannata, (d) Tsohuwar Kasuwa, and (e) Runjin Sambo. Each water sample was labeled appropriately with the area code number and transported in ice pack cooler within 2-3 hours to the microbiology research laboratory of Usmanu Danfodiyo University, Sokoto for analyses (Lazarus, 2018). Water Samples were cultured on Nutrient agar and MacConkey agar and incubated at 37°C for 24 hours. Significant bacterial isolates were defined as $\geq 10^5$ CFU/mL. Isolates were identified using standard biochemical tests and confirmed by Gram staining.

Antibiotic susceptibility testing

The antibiotic susceptibility profiles of the bacterial isolates were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK), following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2022).

Disc Diffusion Test Procedure

This was carried out after inoculum preparation using McFarland turbidity standard, test plate inoculation and application of the discs.

McFarland Turbidity Standard Preparation

In order to standardize the inoculums for susceptibility test, $BaSO_4$ turbidity standards, equivalent to 0.5 McFarland standards was used. A $BaSO_4$ 0.5 McFarland standards were prepared as follows: A 0.5-ml aliquot of 0.05ml $BaCl_2$ (1.175%w/v $BaCl_2.2H_20$) was added to 99.5ml of 0.18 mol/L H_2S0_4 (1% v/v) with constant stirring to maintain a suspension. The correct density of the turbidity standard was verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.08 to 0.10

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for the 0.5 McFarland standards. The tubes were tightly sealed and stored in the dark at room temperature. The barium sulfate turbidity standard was vigorously agitated on a mechanical vortex mixer before each use and inspect for a uniformly turbid appearance. The barium sulfate densities standards were verified monthly in the course of the research (CLSI, 2022).

Inoculum Standardization

Direct Colony Suspension Method preparation was performed according to CLSI, (2022). Inoculations were prepared by making a direct saline suspension of isolated colonies selected from 18-24hrs agar plate. The suspension was adjusted to match the 0.5 McFarland turbidity standards (equivalent to 150×10^6 cfu/ml) by a vortex mixer. This was visually confirmed by comparing the inoculum against a McFarland turbidity standard under adequate lighting conditions.

Antibiotic susceptibility test

Within the period of fifteen (15) minutes of suspension, a sterile swap stick was dipped into the adjusted inoculum suspension. The swap was streaked on Mueller Hinton agar plates using streaking for confluent growth method. The same procedure was repeated by streaking two more times, rotating the plate approximately 60^0 each time to ensure an even distribution of inoculums. The lid was left partially open for 5 minutes, to allow for any excess surface moisture to be absorbed before impregnating the antibiotic discs (CLSI, 2022).

The antimicrobial discs containing various proportions of Ciprofloxacin (25µg), Septrin (25µg), Gentamicin (10µg), Tetracycline (30µg), Ampicillin (10µg), Augmentin (20µg), Amoxicillin (20µg), Oxaciline (5µg) and Levofloxacin (5µg) were used for bacterial isolates. The disc in each case was picked and placed onto the inoculated agar plate surface. Inoculated discs were gently pressed down to ensure even radial diffusion of antibiotic contents. The plates were incubated at 35^0C for 24hrs. The diameters of zone of inhibition were measured by a transparent calibrated ruler to the nearest millimeter (mm) (CLSI, 2022). Results were interpreted as sensitive, intermediate and resistant according to the guidelines of Clinical Laboratory Standard (CLSI, 2022). Multiple Antibiotic Resistance were defined as resistance to more than two class of antibiotics.

Identification of Antibiotic Resistance Bacteria

Bacterial isolates that grew around the antibiotic in reference to their standard measurement were considered as resistant and were subculture into new plate. Using a sterile wire loop, resistant isolates were subculture on Nutrient agar slant for stocking and subsequent molecular analysis.

Molecular Detection and Characterization of Multi-Drug-Resistant Bacterial Isolates

Molecular detection and identification of multi-drug-resistant bacteria involve several techniques to identify and understand the mechanisms of resistance, which includes whole genome sequencing that provide comprehensive data on resistant genes, 16s rRNA which is used to identify and classify bacteria and PCR used to detect specific DNA sequence ((Ramasamy *et al*., 2018)).

DNA Extraction

The extraction was carried out using DNA extraction kit (Qiagen, USA), following manufacturer instructions. Before the procedure, all samples and reagents were equilibrated to room temperature. To the 200µl bacteria suspension in a 1.5 ml nuclease free micro tube, 400µl of Buffer AVL was added , mixed by vortex for 15 Seconds, Incubated at room temperature for 10 min. Thereafter, 300uL of absolute ethanol was added, incubated at -20^0C for 1hrs. Carefully, the solution was added to the QIAamp Mini column in a 2ml collection tube.

The cap was closed and centrifuge at 8000 rpm for 1 min. The QIAamp Mini column was transferred to a clean 2ml collection tube, and the tube containing the flow-through was discarded. Subsequently, 500µl Buffer AW1 was added to the spin column and centrifuge at 8000rpm for 1min, and discarded the flow-through. Furthermore,

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500µl Buffer AW2 was added, centrifuged at full speed (13,000 rpm) for 3 min and discarded the flow-through. The tubes were spun dried by centrifuging again for 3 minutes without adding any reagent. The QIA-amp Mini column was placed in a clean nuclease free 1.5 ml micro-centrifuge tube and the old collection tube containing the filtrate was discarded. Carefully, 60 µl Buffer AE equilibrated to room temperature was added directly to the silica membrane of the spin column and incubated at room temperature for 5 min and centrifuged at 13,000 rpm for 1 minute. The spin column was discarded and the micro tube containing the DNA was caped and stored at -20 ^{o}C until required (Ye & Lei, 2023).

Amplification of 16SrRNA gene

PCR targeting 16SrRNA gene was performed using primers previously designed. The 25 µL PCR mix was prepared containing the following: Master mix 12.5µL (Qiagen, USA), 2.5µL of coral load, 1 µL of 10mM each of 27-F and 1492-R (Table 1), 6 µL of nuclease free water and 2 µL of DNA template. The tubes were carefully mixed and transferred to Bio-Rad MyCycler programmed with the following cycling condition: Initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing specific to 16sRDNA gene at 60°C for 1 min and the extension at 72°C for 1 min 30 seconds, followed by final extension at 72°C for 10 min. The PCR products thus obtained were kept at 4°C until required.

Table 1: Primer Sequence for Amplification of Bacterial Specific Gene

Primer Name	Sequences	Tm	Expected Size	Amplicon	Reference
27-Forward	AGAGTTTGATCMTGGCTCAG	55.2	1465-bp		Sabat *et al.*, 2000
1492-Reverse	TACGGYTACCTTGTTACGACTT	57.4			

Detection of PCR Products Using Gel-electrophoresis

Agarose gel (1%) was prepared by weighing 0.5 gm Agarose in 50 ml 1XTris- Acetate EDTA (TAE) buffer and subjected to heat until the Agarose was completely dissolved and appeared as a clear transparent solution. The Agarose solution was allowed to cool to 50°C and then 2µL of ethidium bromide (0.5µg/ml) dye was added to it. Thereafter, the gel was poured into the gel casting tray with sealed edges and a gel comb was placed into the slots on the tray. The gel was allowed to solidify 30 min and then the comb was gently removed, and the gel slab along with the running tray was submerged carefully into the electrophoresis tank containing 1X TAE buffer.

A total volume of 8 µl amplicon was transferred on a clean Para film and mixed with 2 µl of 6X gel loading dye (Biolabs, UK) and loaded carefully into the wells of Agarose gel. To determine the size of the amplified PCR product, 100-bp DNA ladder (Biolabs, UK) were loaded in the first well. Electrophoresis was performed at 70 V for 1 hour and the mobility were monitored by the migration of the dye in the gel. After appropriate migration, the Agarose gel was visualized under UV trans-illuminator in a Bio-Rad gel documentation device and the results documented (Ramasamy *et. al.,* 2018).

Sequences Analysis

The nucleotide sequence primarily was determined by NCBI Blast and CLUSTALW. BioEdit Sequence Alignment Editor and MEGA 5.2 software that was used to confirmed and rearrange the sequences. MEGA 5.2 software was utilized for the construction of the selected bacterial phylogenetic analysis (Combet *et. al.,* 2000).

Phylogenetic Analysis

The DNA result from the sequence company was prepared as text documents an open NCBI site and click BLAST and copy the text document result and pasted on enter accession

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number and blast result was seen after running 10-30 seconds (Horiike, 2016).

RESULTS

Table 2 shows the frequency and percentage occurrence of bacteria isolated from treated water samples the table provides quantitative proof that the treated water is microbiologically unsafe. The combined frequency of fecal-borne pathogens and indicator organisms is high (25%: *E. coli*, *S. typhi* 18.75%, *S. aureus* 18.75%, *Shigella flexineri* 6.25%, *Bacillus spp* 12.5%, *P. aeruginosa* 12.5% and *Proteus* spp 6.25% respectively), necessitating immediate intervention to improve the treatment efficacy and the integrity of the distribution system to safeguard public health.

Table 2: Frequency and percentage of occurrence of bacteria isolated from treated water sources for municipal consumption.

Name of Isolates	Frequency	% Occurrence
E. coli	4	25
S. aureus	3	18.75
S. typhi	3	18.75
Shigella flexineri	1	6.25
Bacillus spp	2	12.5
Proteus spp	1	6.25
P. aeruginosa	2	12.5
Total	16	100

The antibiotic susceptibility patterns of bacteria isolated from treated water showed in Table 3. The presence of resistant bacteria in the treated, potable water poses a direct and immediate danger to human health. The finding that 25% of the *E .coli* isolates are resistant to Ciprofloxacin (CPX) means that one quarter of the fecal contamination is caused by a strain that would be difficult to treat with one of the most reliable oral antibiotics for diarrheal disease. Multidrug Resistance Potential: The 100% resistance of *Proteus sp* to Oxacillin and the 33.3% resistance of *S. aureus* to STX in the *treated water* indicate that any infection contracted from this source may require the use of more potent of antibiotics. This indicated that water treatment process failed on two levels: It failed to physically inactivate the bacteria and failed to prevent the distribution of antibiotic-resistant strains to the public.

Table 3: Antibiotic susceptibility response of bacteria isolated after water treated in (%)

Bacteria sp (n)	CPX	STX	GN	AMP	AUG	OXA	TET	AMX	LEV
E. coli 4	75	50	50	50	75	75	75	75	75
S. aureus 3	80	50	75	75	80	75	75	80	75
Bacillus spp 2	50	80	80	80	80	80	80	80	80
S. typhi 3	100	50	50	50	50	50	50	50	50
Shigella 1	100	100	100	100	100	100	100	100	100
Proteus spp 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P. aeruginosa 2	100	100	100	50	50	100	50	50	50

Key: Sensitive ≥ 20mm Intermediate 17-19 Resistant ≤ 12 mm

The DNA extraction, polymerase chain reaction and subsequent gel electrophoresis is given on plate 1. The Agarose gel image shows amplification of isolated bacteria with antibiotic resistant gene of 2 specimens, Negative control (NC), Positive control (PC) and Molecular Weight Marker (M). Samples 1, 2, 3and 4 were positive with PCR product size corresponding to the band with molecular weight 16S (1465bp) while the other samples were negative with no bands

Plate 1: Agarose gel electrophoresis image showing amplification of bacterial gene.

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Key: M Lanes: Molecular ladder
NC Lane: Negative control
PC Lane: Positive control
Lanes 1, *Proteus specie*, Lane 2, *E. coli*, Lane 3, and 4 which were happen to be positive sample with PCR product size corresponding to the band with molecular weight 16S (1464-bp).

Amplicons of the two positive samples from gel electrophoresis were successfully sequenced (PX418005 and PX418006) and presented on table 4. Four enteric bacterial i.e *Proteus terrae* and *Providencia stuartii* genotypes were identified after blasting the sequence on National Center for Biotechnology Information (NCBI) USA website. All the sequence obtained were officially registered and assigned a specific accession number by the Genbank of National Center for Biotechnology Information (NCBI USA).

Table 4: Identity of antibiotic-resistant bacteria circulating in treated water samples in Sokoto

Bacterial type	Accession number	Base match accession number	% Similarity	E-value	Query cover%
P. terrae	PX418005	NR-146019.1	99.12	0	100
P. stuartii	PX418006	NR-114964.1	96.38	0	100

Key: *Proteus terrae* and *Providencia stuartii*

Figure 4.1: The phylogenetic tree showing the antibiotic-resistant *Proteus terrae* of treated water in Sokoto and other from National Centre for Biotechnology Information (NCBI) USA data base as shown in Figure 4.1, phylogenetic tree that illustrates the evolutionary and genetic relationships among various strains in the bacterial genus *Proteus*, based on their 16S ribosomal RNA (rRNA) partial gene sequences. The sample PX418005 is shown to be the closest relative to the strain *Proteus myxofaciens* strain ATCC 19692.

Figure 4.2: The phylogenetic tree showing the diversity of antibiotic-resistant *Providencia stuartii* isolated from treated water sources in Sokoto and others from National Centre for Biotechnology Information (NCBI) USA data base is presented in Figure 4.4, revealed that phylogenetic tree constructed using the 16S ribosomal RNA (rRNA) gene sequence illustrate the evolutionary and genetic relationships among various bacterial strains from the genera *Pectobacterium* and *Providencia*. The sample PX418006 is shown to be most closely associated with two strains from the genus *Pectobacterium*: *Pectobacterium quasiaquaticum* strain A477-S1-J17 and *Pectobacterium aroidearum* strain SCRI 109.

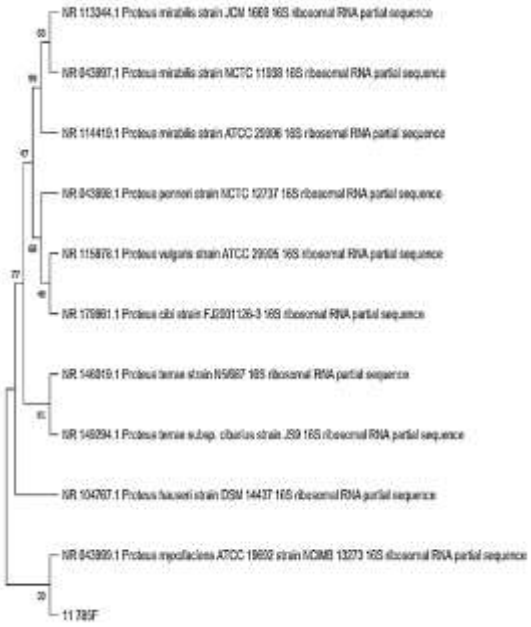

Figure 1: Shown a Phylogenetic tree of Proteus terrae constructed using maximum likelihood tree integrated into MEGA6 Program.

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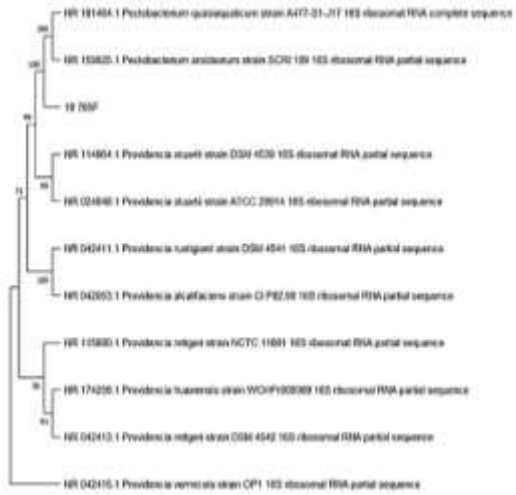

Figure 2: Present a Phylogenetic tree of Providencia stuartii constructed using maximum likelihood tree integrated into MEGA6 Program.

DISCUSION OF FINDINGS

The bacteria isolated from treated water sources in this study, are; *Escherichia Coli* 4(25%), *Staphylococcus aureus* 3(18.5%), *Salmonella typhi* 3(18.5%) *Bacillus Specie* 2(12.5%), *P. aeruginosa* 2(12.5%), *Shigella flexineri* 1(6.25%) and *Proteus Specie* 1(6.25%) are in agreement with similar work done by Akinluyi, (2019) conducted in Niger-Delta. He isolated similar pathogenic bacteria such as *klebsiella Pneumoniae* 4(25%), *Salmonella typhi* 4(25%) *Pseudomonas specie* 3(18%) and *Shigella specie* 2(12.5%), which are of public health significance. The difference with this work is that wells which are a source of drinking water sources and type of people living in a rural village like Gwallameji community is being analysed.

The identification of *E coli* using such properties as their cultural and macroscopic and biochemical properties like showing circular, distinct metallic green sheen colonies on culture with eosine methelene blue agar, gram-negative short rods and production of indole and being a lactose fermenter with the production of acid and gas is used. The incidence of this pathogen in the drinking water sources strongly suggest that the outbreaks of gastrointestinal infections in the areas are strongly related to poor human activity around the drinking water sources. *Staphylococcus aureus* is also due to human activities such as bathing, washing etc. Its clinical importance is in the outbreak of boil and dysentery to the public is well known. *Salmonella specie* also results as a result of human actives too and this introduces typical coliforms into the drinking water sources resulting in typhoid fever, malaria and cholera etc. This result closely agreed with Bouki *et al.,* (2023). When they analysed river water and discovered *staphylococcus aureus* 7(25.5%) and other microorganisms and also concluded that its occurrence could be due to human activities as can be seen from the project *Escherichia coli* is present in wells and tap water.

Antimicrobial susceptibility testing was carried out on bacterial isolates obtained from treated water samples against selected antibiotics. The results showed varying levels of sensitivity among the isolates: *Escherichia coli* (E. coli), was highly sensitive to amoxicillin (75%), ciprofloxacin (75%), and ampicillin (50%). Staphylococcus aureus (S. aureus), was highly sensitive to ampicillin (75%), tetracycline (75%), augmentin (75%), and amoxicillin (60%). Bacillus species exhibited complete sensitivity (100%) to ciprofloxacin, tetracycline, augmentin, and amoxicillin. These findings are in agreement with the study of Nuru *et al*. (2019), which documented similar results. *Salmonella typhi* was found to be highly sensitive to subtrix (100%), metronidazole (80%), ampicillin (100%), and tetracycline (75%). *Enterobacter species* were highly sensitive to erythromycin (75%), ciproxin (100%), levofloxacin (80%), and amoxicillin (75%). *E. coli* was reported to be highly sensitive to tetracycline (100%), augmentin (75%), and ampiclox (75%).

All of the isolates in this study showed multi-drug resistance, with partial resistance to at least nine different antibiotics. This finding suggests that the isolates may have acquired resistance to the tested antibiotics, either through exposure to antibiotics in the environment or through the presence of intrinsic resistance genes. Several studies have highlighted that trace amounts of antibiotics are commonly present in the environment, particularly in places such as domestic wastewater, sewage systems, and

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septic tanks. These environments act as potential reservoirs where bacteria are frequently exposed to antibiotics, creating opportunities for them to develop or acquire resistance genes (Carlson & Yang, 2015; Sui *et al*., 2015). This environmental exposure plays an important role in the spread of antimicrobial resistance, as it provides bacteria with constant selective pressure to survive, adapt, and eventually resist multiple classes of antibiotics.

Antimicrobial resistance among members of the *Enterobacteriaceae* family, to which the isolates from this study belong, is well documented (Arthur *et al*., 2019; Losch *et al.*, 2018; Lynch *et al*., 2023). *Enterobacter* species (especially *E. cloacae* and *E. aerogenes*) are well-known opportunistic pathogens responsible for nosocomial infections. These bacteria exhibit intrinsic resistance to amoxicillin and third-generation cephalosporins such as rocephin (Bosi *et al*., 2019; Mezzatesta *et al.*, 2022; Pontron *et al.*, 2023). This resistance is primarily due to the overexpression of AmpC β-lactamase**s**, enzymes encoded on their chromosomes (Pontron *et al*., 2023). AmpC β-lactamases are not limited to *Enterobacter* species. They can also be transferred to other organisms that either weakly express chromosomal AmpC genes, such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis*, through transmissible plasmids (Jacoby, 2019).Additionally, resistance in other clinically important pathogens such as *Escherichia coli*, *Vibrio cholerae*, and *Salmonella typhi* has also been documented (Arthur *et al*., 2019).

Similarly, Ateba and Maribeng (2021) reported that 75–100% of *Enterococcus* species, including *Enterococcus faecalis* (a fecal streptococcus), isolated from groundwater sources were resistant to erythromycin and several other antibiotics. In line with the trend observed in this study, Mulamattathil *et al.* (2024) also reported that all environmental isolates including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus vulgaris* were completely sensitive to neomycin, streptomycin, and ciprofloxacin. However, they also recorded resistance to erythromycin, trimethoprim, and amoxicillin. Likewise, Onuoha (2015) found that *Escherichia coli* isolated from well water sources showed sensitivity to ciprofloxacin and pefloxacin.

The antibiotic-resistant species identified at molecular level in this study are *Proteus terrae* and *Providencia stuartii*. These species are recognized as a significant global public health concern in water bodies and are considered high-risk bacterial pathogens. This study agrees with the findings of Popoola *et al*. (2025), who also reported similar isolates, specifically *Aeromonas spp.* and *Proteus spp* isolated from tropical fresh water eco system. However, these findings disagree with the results of Ghadigaonkar & Rath (2023), who reported different predominant isolates (*E. coli, Klebsiella pneumoniae*, and *Comamonas spp.*). This highlights a notable variation in the predominant species isolated in this study compared to most other literature.

CONCLUSION

Antimicrobial resistance profile, the bacterial isolates exhibited a concerning level of antimicrobial resistance (AMR), suggesting significant challenges for empirical treatment of waterborne infections. High resistance: 100% resistance was observed against Septrin and Tetracycline, rendering these agents ineffective for treating infections caused by these isolates. Moderate resistance: 50% resistance was detected for Ampicillin, Ciprofloxacin, and Gentamicin, indicating a need for susceptibility testing before using these antibiotics, as half of the infections may fail treatment. Highly resistant organisms: *Pseudomonas aeruginosa, proteus species,* and *Escherichia coli* were the primary drivers of the observed high resistance levels. High susceptibility: *Serratia marcescens* and *Klebsiella pneumoniae* were the most sensitive organisms tested.

Molecular confirmation, molecular analysis of four prevalent isolates identified species with clinical relevance: *Proteus terrae*, and *Providencia stuartii,* confirming the presence of complex, potentially drug-resistant flora in Sokoto water system. The phylogenetic analysis of revealed that PX418005 and PX418006 as Germany cluster.

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REFERENCES

Adebawore, A. A., Awokunmi, E. E., Akinyeye, R. O. & Olanipekun, E. O. (2016). Physicochemical and bacteriological assessment of hand-dug well water from Ile-Oluji, Nigeria. *Journal of Innovative Research and Applied Sciences*. **3**(1):433-440.

Adesakin, T. A., Oyewale, A. T., Bayero, U., Mohammed, A. N., Aduwo, I. A. & Ahmed, P. Z. (2020). *Assessment of bacteriological quality and physicochemical parameters of domestic water sources* in Samaru community, Zaria, Northwest Nigeria. Heliyon. **6**:1-13.

Adetunde, L.A. & Glover, R. L. K. (2019). Bacteriological Quality of Borehole Water Used by Students' of University for Development Studies, Navrongo Campus in Upper-East Region of Ghana. Current Research *Journal of Biological Sciences*, **2**(6):361-364.

Adetunde, L.A. & Glover, R. L. K. (2019). Bacteriological Quality of Borehole Water Used by Students' of University for Development Studies, Navrongo Campus in Upper-East Region of Ghana. Current Research *Journal of Biological Sciences*, **2**(6):361-364.

Arsène, M. M., Davares, A. K., Andreevna, S. L., Vladimirovich, E. A., Carime, B. Z., Marouf, R., & Khelifi, I. (2021). The use of probiotics in animal feeding for safe production and as potential alternatives to antibiotics. *Veterinary world*, *14*(2), 319.

Arthur, M., Andremont, A. & Courvalin, P. C. (2019). Distribution of Eryhtromycin Esterase and rRNAMethylase Genes in Members of the Family Enterobacteriacea Highly Resistant to Erythromycin.Antimicrob. *AgentsChemother.* **31**: 404-409

Ateba, N. & Maribeng, D. (2021). Detection of Enterococcus Species in Groundwater from some rural communities in the Mmabatho area, South Africa: A Risk Analysis. *African Journal of Microbiology. Res.* **5**: 3930-3935.

Chakraborty, S. K. (2021). Water: Its properties, distribution, and significance. In *Riverine Ecology Volume 1: Eco-functionality of the Physical Environment of Rivers* (pp. 23-55). Cham: Springer International Publishing.

Chen, X., Ren, Y., Li, C., Shang, Y., Ji, R., Yao, D., & He, Y. (2025). Pollution Characteristics and Ecological Risk Assessment of Typical Heavy Metals in the Soil of the Heavy Industrial City Baotou. *Processes*, **13**(1), 170.

Clinical & Laboratory Standards Institute (CLSI). (2022). Performance Standards for Antimicrobial Susceptibility Testing (32nd ed., CLSI supplement M100). *Clinical and Laboratory Standards Institute* **31**(1).

Combet, C., Blanchet, C., Geourjon, C., & Deleage, G. (2000). NPS@: network protein sequence analysis. *Trends in biochemical sciences*, *25*(3), 147-150.

Czekalski, N. Berthold, T. Caucci, S. Egli, A., & Buergmann, H. (2019). Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into Lake Geneva, Switzerland. *Journal of Front Microbiology*. **34** (3):106

Datta, K., Swarnavo, C. & Aryadeep, R. (2025). "Management of soil, waste and water in the context of global climate change." *Journal of Environmental Nexus for Resource Management*. **5** 1-26.

Feary, T. W., A. B. Sturtevant, Jr., & Lankford, J. (2021). Antibiotic resistant coliforms in fresh and salt water. *Journal of Applied Environment and Health*. **34** (25):215-220.

Ghadigaonkar, D. & Rath, A. (2023) Isolation and Identification of Waterborne Antibiotic-Resistant Bacteria and Molecular Characterization of their Antibiotic Resistance Genes. *Journal of Visualized Experiments* **3,** 2-28

Horiike, T. (2016). An introduction to molecular phylogenetic analysis. *Reviews in Agricultural Science*, *4*, 36-45.

Iwane, T., Urase, T., & Yamamoto, K. (2021). Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. *Journal of Water Science and Technology*. **45** (3): 91– 99.

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Kutlu, B., Sesli, A., Tepe, R. & Mutlu, E. (2015). Assessment of physicochemical water quality of Birecik Dam, Şanlıurfa, South-Eastern Anatolia Region, Turkey. *Turkey Journal of Agriculture, Food Science and Technology*. **3**(7):623-628.

Lazarus, A. M., (2018) Analysis of water consumption pattern among residential areas in Gombe Metropolis. *Continental Journal of Applied Science*, (**3**) 77-84

Mahas, N. S. (2025). India and China's Approach to Transboundary River Pollutants and Their Impact on Human Health. In *Intersecting Realities of Health Resilience and Governance in India: Emerging Domestic and Global Perspectives* (pp. 177-197).

Mathew, B. B., & Krishnamurthy, N. B. (2017). *Water constituents and treatment methods Water:* June. 53-60.

Obi, C. N. & Okocha, C. O. (2017). Microbiological and Physcio-Chemical Analysis of Selected Bore-hole Waters in World Bank Housing Estate, Umuahia, Abia State, *Nigeria Journal of Engineering and Applied Sciences*, **2**(5):920-929.

Orji, M.U., Ezenwaje, E. E. & Anyaegbunam, B. C. (2016). Spatial appraisal of shallow well water pollution in Awka, Nigeria. *Journal of Environmental Biology* 16 101-106

Ozabor, P. T., Oluwajide, O. O., Akeju, A. O., Onifade, S. J., Olaniyan, S. O. & Olaitan, J. O. (2022). Isolation and Characterization of Resistant Bacterial Species Isolated from Shallow Well Water Situated Close to Graves as a Public Health Menace in Osogbo, Osun State. *Stanford Journal of Microbiology* 12, 47-53

Patricia U. O., Rebecca O. S. & Bolajoko A. A. (2021) Home Management of Acute Diarrhoea by Under-five Caregivers in Ikorodu LGA, Lagos State. *West African Journal of Pharmacy* 33 (**1**) 151-164

Popoola, B. M., Oluwasanmi, A. A., & Oyindamola, J. S. (2025) Antibiotic-resistant bacteria in tropical freshwater ecosystems: A review of occurrence, distribution and environmental implications. *International Journal of Microbiology* **4,** 1946-2950

Ramasamy, R., Subha, A., Thanga, S. R., Abinaya, R., Karthikadevi, M., Jesteena, J. & Ragunathan, R. (2018). *International Journal of Applied Science and Biotechnology*. **6**(*3*): 279-284

Rodrigues, L. and Pacheco, A. (2010). Groundwater contamination from cemeteries—Cases of study: Environmental 2010. Situation and perspective for the European Union, May 6–10, 2003, Porto, Portugal, Paper C **03.** p.,6-9

Sabat, G., Rose, P., Hickey, W. J., & Harkin, J. M. (2000). Selective and sensitive method for PCR amplification of *Escherichia coli* 16S rRNA genes in soil. *Applied and environmental microbiology*, *66*(2), 844-849.

Shah, C. R. (2017). Physical, Chemical and Biological Parameters of water determine its quality *Journal of Environmental Science* 69. 35-40.

Tazri, Z., Sakib, S., Das, R., Faruque, M. H., Hossain, A., Hosen, M. M. & Habibullah-Al-Mamun, M. (2025). Heavy metal contamination in a transboundary river: ecological and human health risk assessment. *International Journal of Environmental Analytical Chemistry*, 1-25.

Tella, T. A., Festus, B., Olaoluwa, T. D., & Oladapo, A. S. (2025). Water and wastewater treatment in developed and developing countries: Present experience and future plans. In *Smart Nanomaterials for Environmental Applications* (pp. 351-385). Elsevier.

Ye, X., & Lei, B. (2023). The current status and trends of DNA extraction. *BioEssays*, *45*(8).

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