



# Stream and well water samples from two rural communities in Ekiti State, Nigeria: assessment of physicochemical parameters, bacteriological quality and public health significance

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**ABSTRACT.** Infectious diseases caused by microorganisms are widespread health risks associated with drinking water. This study evaluated the physicochemical parameters and bacteriological quality of the stream and well water using standard protocols. The bacteria were identified by conventional and molecular methods. Antibiotic susceptibility and location of antibiotic resistance markers (ARMs) were determined using disc diffusion and acridine orange, respectively. The highest mean Total Heterotrophic Bacterial Counts (THBC), Total Coliform Counts (TCC) and Faecal Coliform Counts (FCC) from the stream water was  $4.3 \pm 0.3 \times 10^6$ ,  $8.9 \pm 0.0 \times 10^5$ , and  $3.5 \pm 0.1 \times 10^4$  (CFU mL<sup>-1</sup>), respectively. The well water had mean TCC ranging between  $2.8 \pm 0.0 \times 10^5$  and  $2.1 \pm 0.1 \times 10^4$  (CFU mL<sup>-1</sup>). Six bacterial genera: *Staphylococcus*, *Pseudomonas*, *Escherichia*, *Enterobacter*, *Klebsiella*, and *Shigella* were isolated. The mean temperature of the water ranged from  $26.0 \pm 0.3^\circ\text{C}$  to  $27.0 \pm 0.1^\circ\text{C}$ . The highest mean dissolved oxygen, total hardness, sulphate and magnesium was  $24.0 \pm 1.0$ ,  $40.1 \pm 0.8$ ,  $11.0 \pm 1.0$ , and  $67.0 \pm 1.5$  (mg L<sup>-1</sup>), respectively. The results showed that  $\geq 66.7$  % *S. aureus* were Levofloxacin and Streptomycin sensitive; between 45.5 and 68.1% of the isolates were Gentamycin and Chloramphenicol resistant, while 81.8% exhibited multidrug resistance. *Escherichia coli* EcSW3, *E. aerogenes* EeWW2, *K. pneumoniae* KpSW3, and *S. aureus* SaSW had their entire ARMs located on the plasmids with the molecular sizes  $< 2.027$  Kbp. This study showed that the stream and well water harboured bacteria with some ARMs on plasmids, indicating the possibility of horizontal transfer of antibiotic-resistant genes among the bacteria. In addition, it showed the necessity to enlighten the rural populace on the importance of cleaning the surroundings near water sources so as to prevent water-borne diseases.

**Keywords:** water; plasmid; antibiotic; resistance; physicochemical.

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## Introduction

Most rural communities in developing countries, especially in Nigeria, lack access to potable water supply and rely predominantly on river and stream sources for their household, domestic, and agricultural purposes (Ishaku, Rafee Majid, Ajayi, & Haruna, 2011; Okeola, Kolawole, & Ameen, 2010). The degradation in water quality is primarily attributable to anthropogenic sources (Scott, Milton, Erickson, Klopfenstein, & Stock, 2003; Hasan, Khalid-UR-Rashid, & Akter, 2019). In some rural areas in Nigeria, domestic wastes, sewage, faeces, effluents and other pollutants are being constantly discharged into streams and drinking water that serve as water sources for daily needs. These multiple sources of contamination are compounded by the limited environmental awareness in rural areas. The deterioration of the biological quality of water bodies that are subjected to pollution from runoff of agricultural and domestic waste, windblown debris, mine tailings, and industrial production sites has been reported (WHO, 2003; Uddin & Jeong, 2021). When the load of organic matter or waste discharged into the stream is excessively heavy, the stream's self-purification capacity becomes extremely challenging and difficult. Thus, there will be pollution of these water sources, and this can be detrimental to human health when consumed.

Water supplies in developing countries, especially in Africa, are devoid of treatment; thus, the communities have to make use of the most convenient supply (Moyo, Wright, Ndamba, & Gundry, 2004;

Odeyemi, Akinjogunla, & Ojo, 2011; Odeyemi, Dada, Akinjogunla, & Agunbiade, 2017). Many people in rural communities collect stream water using diverse, clean or unclean containers and transport the water to their homes (Sobsey, 2002). Contamination of the water by pathogenic micro-organisms frequently occurs between the point of collection and the point-of-use in the home owing to unhygienic practices and makes the water become a health risk (Gundry, Wright, & Conroy, 2004; Moyo et al., 2004). Monitoring of the physicochemical water quality parameters such as sulphate, nitrate, phosphates, and conductivity plays a fundamental role in evaluating the water environment, ecosystem, and hydrochemistry (Whitehead et al., 2019; Islam, Idris, Islam, Ali, & Rakib, 2021). Microbiological assessment of some well water samples has revealed *Escherichia coli* as the predominant organism (Odeyemi, Ayantola, & Peter, 2018), and other bacterial genera isolated were *Klebsiella*, *Enterobacter*, *Staphylococcus*, *Micrococcus*, and *Streptococcus* (Odeyemi et al., 2018).

The dissemination of antibiotic-resistant bacteria and the spread of antibiotic resistance genes have become a serious global public health concern (Akinjogunla, Eghafona, & Enabulele, 2011). Water environments such as streams, wells, rivers, and lakes are recognized reservoirs of antibiotic-resistant bacteria, and antibiotic resistance genes have been detected in much drinking water. Furthermore, an increasing number of people are becoming susceptible to water-borne infections and diseases caused by pathogenic bacteria, and the indiscriminate use of antimicrobial drugs has triggered the proliferation of antibiotic resistance by microorganisms (WHO, 2003). Over time, a bacterium can build up a whole range of antibiotic resistant genes which may be transmitted within a genus, species, and/or to other species (Akinjogunla, Adenugba, & Inyang, 2017). This study assessed the physico-chemical parameters and bacteriological quality of the stream and hand-dug well water samples from a rural settlement in Ekiti State, Nigeria.

## Material and methods

### Study area

The study area was Ayedun Ekiti (Odo Ayedun and Oke Ayedun), located within the Ikole Local Government Area, Ekiti State, Nigeria, with an estimated population of 170,414 (NPC, 2006). Geographically, both Odo Ayedun and Oke Ayedun are entirely within the tropics with a well-defined rainy season, which occurs from May to October, and a dry season from November to April. The two communities are located on the Equator between latitudes and longitudes of  $7.8163^{\circ}$  N,  $5.5854^{\circ}$  E and  $8.057^{\circ}$  N,  $5.5644^{\circ}$  E, respectively. The inhabitants of Odo Ayedun and Oke Ayedun are predominantly Yoruba, and they are mainly farmers, students, civil servants, and traders who depend on both stream and well water for domestic and agricultural purposes due to the lack of potable water in the area. The map showing the different sampling locations is presented in Figure 1.

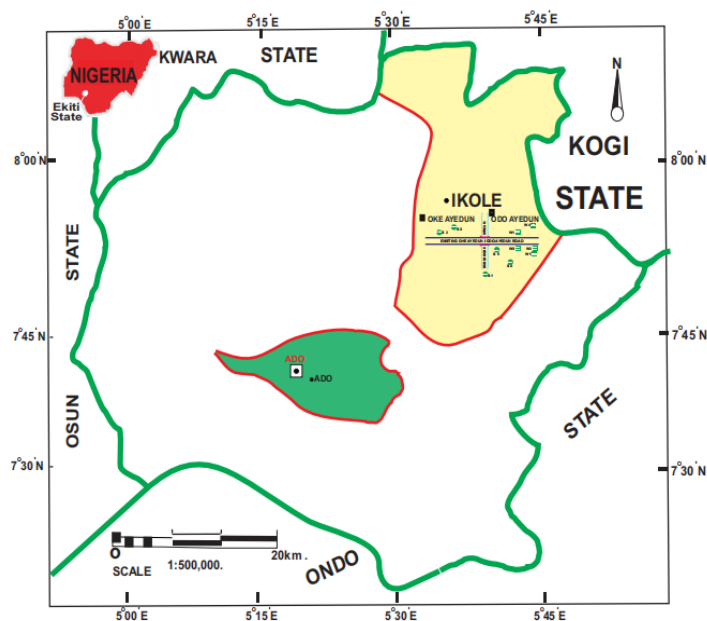


Figure 1. Map of Ekiti State, showing the study area.

## Sample collection

### Stream and well water samples

A total of fifteen water samples from Omoologbe (n = 3), Irutu (n = 3), Agbede (n = 3), Ilagbo (n = 3) and Imila (n = 3) streams were aseptically collected in sterilized wide mouth plastic bottles of 1000 mL holding capacity using the grab method. Water samples from the hand dug wells in Okeowa, Iloro, Upebi, Ere, and Ilumope quarters were aseptically collected in sterilized wide mouth plastic bottles of 1000 mL holding capacity. A total of five water samples were collected for this study in triplicate, totaling 15 water samples. All the water samples collected were appropriately labelled and stored in a cool box for transport to the laboratory at Ekiti State University for bacteriological and physicochemical analyses.

### Physicochemical analyses of stream and well water samples

The eight parameters selected for physico-chemical stream and well quality analysis were: temperature, pH, turbidity, conductivity, dissolved oxygen (DO), total hardness, sulphate ( $\text{SO}_4^{2-}$ ) and magnesium (Mg). The pH, temperature, and conductivity of the stream and well water samples were measured in situ using a pH-meter (KENT EIL 7020, Surrey, England); a simple mercury thermometer and conductivity meter (CDM83, Radiometer A/S Copenhagen, Denmark), respectively (APHA, 2005). Total hardness was determined by EDTA titration using an Erichrome Black-T-indicator (APHA, 2005). Turbidity and DO concentrations were determined with a turbidity meter and a DO meter with a luminescent probe, respectively. The Sulphate ( $\text{SO}_4^{2-}$ ) and magnesium (Mg) was carried out using ASTM standards, as approved by APHA (2005). Each analysis was carried out in triplicate, and the mean value was calculated.

### Bacteriological analyses of stream and well water samples

The methods described by Odeyemi, Oluyeye, Fagbohun, and Adebayo (2015) were used for the isolation of bacteria from stream and well water samples. To dislodge adhered bacteria, 1 mL of each well water sample was added to 9 mL of peptone water and vigorously shaken. Serial dilutions were made to obtain  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  dilutions. Then, 1 mL of the serially diluted sample was transferred onto plates of Nutrient Agar (NA), MacConkey Agar (MCA), and Eosine Methylene Blue (EMB) agar (Merck, Germany) and incubated aerobically at 37°C for 24 hours. After incubation, the colonies on the positive plates were counted to obtain the Total Heterotrophic Counts (THC), Total Coliform Counts (TCC), and Faecal Coliform Counts (FCC), respectively. Then, the colonies were subcultured onto freshly prepared plates of nutrient agar and incubated at 37°C for 24 hours. Pure cultures of isolates were streaked onto nutrient agar slants, incubated at 37°C for 24 hours, and stored in the refrigerator at 4°C for characterization and identification.

### Identification of bacteria by conventional biochemical tests and microbact™ kits

Bacteria were identified using Gram staining and conventional biochemical tests such as catalase, coagulase, Vogues Proskauer, methyl red, indole, urease, motility, citrate, hydrogen sulphide, oxidase, opsochin, spore, fructose, mannitol, maltose, galactose, lactose, glucose and sucrose. Microbial identification kits (Microbact™ GNB 12A and 12B) (Oxoid, UK) were also used for identification. The characteristics of the bacteria were evaluated using Bergey's Manual of Determinative Bacteriology (Holt, Krieg, Sneath, Stanley, & William, 1994).

### Molecular identification of bacterial isolates

The genomic DNA (gDNA) of bacteria was extracted using a Column-Pure Bacterial Genomic DNA Isolation Kit. The isolated bacterial DNA (1 µL) was amplified using universal 16S rRNA primers 27 F forward primer with primer sequence (5'-AGAGTTTGTATCMTGG CTCAG-3') and 1492 R reverse primer with primer sequence (5'-GGTTACCTTGTTACG ACTT-3'). A total of 25 µL PCR reaction mixture was prepared with 2 µL of template DNA, 1 µL of 10 pmol µL<sup>-1</sup> each of universal forward and reverse primers, 12.5 µL of Go Taq Green Master Mix and 0.5 µL of 2X Taq DNA polymerase (Promega, USA). The volume make-up was done with nuclease free water. The automated thermal cycler (BioRad PTC-200, Hercules, California, USA) was used for PCR amplification. The PCR reaction conditions were as follows: initial denaturation at 94°C for 5 min. followed by 30 cycles of amplification/denaturation at 94°C for 30 seconds; annealing at 52°C for 60 seconds; extension at 72°C for 2 min. and a final elongation/extension at 72°C for 10 min. The amplified PCR products of 16S ribosomal gene were electrophoresed on a 1.5 % agarose gel, stained with gel red, in 1 x TAE buffer (40 mM Tris-HCl, 2 mM acetate and 1 mM EDTA) at 100 V for 50 min. Then, the amplified PCR products were

visualized under UV transilluminator. The PCR amplicons obtained were sequenced and matched using the BLAST algorithm to confirm the identity of the isolates.

### Antimicrobial susceptibility testing

*In vitro* antibiotic susceptibilities of bacterial isolates from stream and well water samples were performed using the Kirby-Bauer disk diffusion method (CLSI, 2020). All the bacterial inocula used were standardized using the McFarland standard. Briefly, 10  $\mu$ L of each isolate was streaked homogenously onto each plate containing Mueller-Hinton Agar (Oxoid, UK) using a sterile cotton swab. Streptomycin (S), Cotrimoxazole (SXT), Chloramphenicol (CH), Sparfloxacin (SP), Ciprofloxacin (CPX), Amoxicillin (AM), Augmentin (AU), Pefloxacin (PEX), Ofloxacin (OFX), Norfloxacin (NB), Erythromycin (E), Levofloxacin (LEV), Gentamycin (CN), Ampicloxacin (APX), Ampicillin (AMX) and Rimfapicin (RD) (Oxoid, UK) were aseptically placed on the surfaces of the plates and the plates were incubated at 37°C for 18h. After incubation, the diameters of inhibition zones were measured in millimetres and the results were interpreted using standard values according to the Clinical and Laboratory Standards Institute (CLSI, 2020). Bacterial isolates that were resistant to at least one agent in  $\geq 3$  antibiotic classes were regarded as multidrug resistant isolates (Akinjogunla, Umo, Alozie, Oshosanya, & Saturday, 2021).

### Plasmid curing (elimination)

The plasmid curing method was used to determine the location of the antibiotic resistance markers (ARM). The curing of the resistant plasmids of the resistant bacterial isolates was done using Acridine Orange (Rasool, Ahmad, Khan, & Wahab, 2003; Akinjogunla et al., 2017). Each resistant bacterial isolate was incubated at 37°C for 24h in a test-tube containing 9 mL of nutrient broth and 1 mL of acridine orange (1 g/1000 mL). After incubation, the broth culture was shaken and loopful subcultured onto MHA plates, and antibiotic sensitivity testing was carried out as previously described (CLSI, 2020). The pre-curing and post-curing antibiograms of the isolates were observed and compared. Loss of ARM indicated plasmid-mediated resistance, while expression of ARM indicated chromosome-mediated resistance.

### Extraction of bacteria plasmid DNA

The DNA of bacteria was extracted from liquid culture medium (1.5 mL) grown at 37°C for 24h. Each bacterial cell was harvested by centrifugation at 15,000 g for 1 min, and the supernatant was discarded. The cell pellet was resuspended in 300  $\mu$ L of resuspension solution, and lysozyme solution (2  $\mu$ L) was added and mixed well, and the tube was incubated at 37°C for 60 min. The tube was centrifuged at 15,000 g for 1 min. and the supernatant was discarded and the cell pellet resuspended in cell lysis solution (300  $\mu$ L). RNase A solution (1.5  $\mu$ L) was added and vortexed, incubated at 37°C for 30 min. cooled on ice for 1 min. and protein precipitation solution (100  $\mu$ L) was added and vortexed for 30 seconds. This was followed by centrifugation at 15,000 g for 5 min. and the supernatant was transferred to a 1.5 mL microcentrifuge tube containing 300  $\mu$ L isopropanol. The sample was mixed gently for 1 min., and centrifuged at 15,000 g for 1 min. the supernatant was discarded, 500  $\mu$ L washing buffer was added and inverted several times in the tube to wash the DNA pellet. The tube was centrifuged at 15,000 g for 1 min. and the ethanol was discarded and the DNA pellet dried at room temperature for 15 min. Finally, 50  $\mu$ L of DNA hydration solution was added to the dried DNA pellet and incubated at 65°C for 60 min. The extracted plasmid DNA (pDNA) samples were stored at -20°C.

### Statistical analysis

The Statistical Package for Social Sciences (IBM SPSS, Window Software Version 22.0, Armonk, NY: IBM Corp.) was used to analyze the data. Pearson's correlation between bacterial counts and physicochemical parameters of water samples.

## Results

The mean Total Heterotrophic Bacterial Counts (THBC), Total Coliform Counts (TCC), and Faecal Coliform Counts (FCC) of the stream and well water samples collected from 10 different sampling sites are presented in Table 1. The bacterial counts from the stream water samples varied with sites, as the lowest THBC of  $1.1 \pm 0.1 \times 10^6$  CFU mL<sup>-1</sup> was obtained in SW3 and the highest THBC of  $4.3 \pm 0.3 \times 10^6$  CFU mL<sup>-1</sup> was obtained in SW2. The TCC ranged from  $6.6 \pm 0.1 \times 10^4$  CFU mL<sup>-1</sup> as obtained in SW3 to  $84.3 \pm 0.3 \times 10^6$  CFU mL<sup>-1</sup> in SW2, while the FCC of the stream water samples was  $\leq 3.5 \pm 0.1 \times 10^4$  CFU mL<sup>-1</sup> (Table 1). The well water samples had THBC and

TCC ranging between  $1.1 \pm 0.2 \times 10^5$  and  $7.8 \pm 0.2 \times 10^5$  (CFU mL<sup>-1</sup>),  $2.8 \pm 0.0 \times 10^5$  and  $2.1 \pm 0.1 \times 10^4$  (CFU mL<sup>-1</sup>), respectively. Sample WWS had FCC was  $3.2 \pm 0.2 \times 10^3$  CFU mL<sup>-1</sup>. Twenty-six bacterial isolates, belonging to six genera (*Staphylococcus*, *Pseudomonas*, *Escherichia*, *Enterobacter*, *Klebsiella*, and *Shigella*) were isolated from the stream and well water samples (Table 1). *E. coli* and *S. aureus* had the highest occurrence of 23.1% each, followed by *E. aerogenes* and *K. pneumoniae* with 19.2% each, *P. aeruginosa* had 11.5%, while *Shigella* spp. had the lowest occurrence of 3.8%. The morphological and biochemical characteristics (catalase, coagulase, Vogues Proskauer, methyl red, indole, urease, motility, citrate, hydrogen sulphide, oxidase, optochin, spore, fructose, mannitol, maltose, galactose, lactose, glucose, and sucrose) of bacterial isolates are presented in Table 2. The PCR amplification of the 16sRNA of selected isolates is shown in Figure 2.

**Table 1.** Mean bacterial counts and bacterial isolates from stream and well water samples.

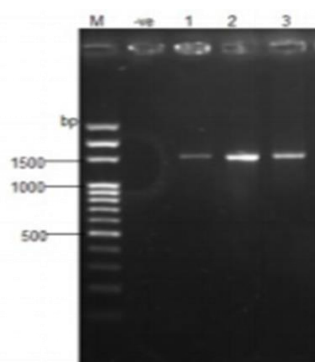
Sampling Site	Sample		Mean Counts (CFU mL <sup>-1</sup> )			Bacterial Isolated
	Source	Code	THBC	TCC	FCC	
Omoologbe	Stream	SW1	$2.3 \pm 0.2 \times 10^6$	$8.9 \pm 0.0 \times 10^5$	$2.3 \pm 0.2 \times 10^4$	<i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumoniae</i>
Iritu	Stream	SW2	$4.3 \pm 0.3 \times 10^6$	$1.0 \pm 0.1 \times 10^5$	$3.0 \pm 0.0 \times 10^3$	<i>Shigella</i> sp., <i>E. coli</i> , <i>K. pneumoniae</i>
Agbede	Stream	SW3	$1.1 \pm 0.1 \times 10^6$	$6.6 \pm 0.1 \times 10^4$	$2.0 \pm 0.1 \times 10^3$	<i>Pseudomonas</i> sp., <i>E. coli</i> , <i>K. pneumoniae</i> ,
Ilagbo	Stream	SW4	$1.3 \pm 0.2 \times 10^6$	$2.7 \pm 0.3 \times 10^5$	$1.1 \pm 0.1 \times 10^4$	<i>Pseudomonas</i> sp., <i>E. aerogenes</i> , <i>E. coli</i> ,
Imila	Stream	SW5	$3.3 \pm 0.3 \times 10^6$	$1.2 \pm 0.1 \times 10^5$	$3.5 \pm 0.1 \times 10^4$	<i>S. aureus</i> , <i>E. coli</i> , <i>E. aerogenes</i>
Okeowa	Well	WW1	$7.8 \pm 0.2 \times 10^5$	$2.1 \pm 0.1 \times 10^4$	NG	<i>S. aureus</i> , <i>E. aerogenes</i>
Iloro	Well	WW2	$1.1 \pm 0.2 \times 10^5$	$2.8 \pm 0.0 \times 10^3$	NG	<i>S. aureus</i> , <i>E. aerogenes</i> .
Upebi	Well	WW3	$1.7 \pm 0.1 \times 10^5$	$3.2 \pm 0.1 \times 10^3$	NG	<i>Pseudomonas</i> sp., <i>K. pneumoniae</i>
Ere	Well	WW4	$1.4 \pm 0.2 \times 10^5$	$4.1 \pm 0.2 \times 10^3$	NG	<i>S. aureus</i> , <i>K. pneumoniae</i> ,
Ilumope	Well	WW5	$2.8 \pm 0.0 \times 10^5$	$7.2 \pm 0.2 \times 10^3$	$3.2 \pm 0.2 \times 10^3$	<i>E. aerogenes</i> , <i>E. coli</i> , <i>S. aureus</i>

Keys: TBHC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; FCC: Faecal Coliform Counts; CFU: Colony Forming Units; NG: No growth.

**Table 2.** Morphological and biochemical characteristics of bacterial isolates from stream and well water samples.

Gram Reaction	Coagulase	Catalase	Starch hydrolysis	Voges Proskauer	Methyl Red	Indole	Motility	Citrate Utilization	Hydrogen Sulphide	Oxidase	Opsochin Disc	Sugar Fermentation Test								Probable Bacteria
												Fructose	Xylose	Raffinose	Mannose	Maltose	Galactose	Lactose	Glucose	
+ cocci	+	+	-	+	+	-	-	+	-	+	nd	-	-	-	+	+	+	+	+	<i>Staphylococcus aureus</i>
- rod	-	+	-	+	-	-	-	+	-	-	nd	+	+	+	+	+	+	+	+	<i>Klebsiella pneumoniae</i>
- rod	-	+	-	+	-	-	+	+	-	-	nd	+	+	+	+	+	+	+	+	<i>Enterobacter aerogenes</i>
- rod	-	+	-	-	-	+	+	+	-	+	nd	-	-	-	+	-	-	-	-	<i>Pseudomonas aeruginosa</i>
- rod	-	+	-	-	+	+	-	-	-	-	nd	-	+	-	+	+	+	+	+	<i>Escherichia coli</i>
- rod	-	+	-	-	+	+	-	-	-	-	nd	+	-	-	+	-	+	+	-	<i>Shigella</i> spp

Keys: nd: Not determined; + Positive; - Negative.



**Figure 2.** PCR amplification of the 16S rRNA of selected isolates. M: Marker 1: *Enterobacter aerogenes* (EeWW1); 2: *Pseudomonas aeruginosa* (PaSW); and 3: *Staphylococcus aureus* (SaWW1).

The physico-chemical parameters of stream and well water samples during the study period are shown in Table 3. The quality of the water samples observed during this study was compared with World Health Organization and Environmental Protection Agency acceptable levels in the guidelines for drinking water reported. The mean temperature of the stream water during the study period ranged from  $26.2 \pm 0.4^\circ\text{C}$  to  $27.0 \pm 0.1^\circ\text{C}$ , while the mean temperature of the well water ranged from between  $26.0 \pm 0.3^\circ\text{C}$  and  $26.4 \pm 0.4^\circ\text{C}$ . The

highest mean pH concentration was observed in stream water sample SW3 ( $6.9 \pm 0.1$ ), while the well water WW5 had the lowest mean pH concentration of  $6.4 \pm 0.1$ . The conductivity and turbidity of stream and well water samples was  $\leq 0.02 \mu\text{S cm}^{-1}$  and  $\leq 0.02 \text{ NTU}$ , respectively. The dissolved oxygen ( $\text{mg L}^{-1}$ ) of the stream and well water ranged from  $14.9 \pm 0.2$  to  $24.0 \pm 1.0$  and these values were higher than  $\leq 5$  recommended by WHO and EPA. The range of mean total hardness ( $\text{mg L}^{-1}$ ) of the well water ( $34.9 \pm 0.2$  and  $40.1 \pm 0.8$ ) was higher than the range of mean total hardness of the well water ( $26.3 \pm 0.3$  and  $31.2 \pm 0.8$ ). The mean sulphate levels of stream and well water were  $\leq 11.0 \pm 1.0 \text{ mg L}^{-1}$ , while the mean magnesium levels ranged from  $52.0 \pm 1.7 \text{ mg L}^{-1}$  as observed in stream water SW3 to  $67.0 \pm 1.5 \text{ mg L}^{-1}$  as observed in well water WW3 (Table 3).

**Table 3.** Physicochemical analysis of stream and water samples.

Parameters	Stream Water ( $\text{mm} \pm \text{S.D}$ )					Well Water ( $\text{mm} \pm \text{S.D}$ )					Standard	
	SW1	SW2	SW3	SW4	SW5	WW1	WW2	WW3	WW4	WW5	WHO	EPA
Colour	-	-	+	-	-	-	-	-	-	-	-	-
Odour	-	-	-	-	-	-	-	-	-	-	-	-
Temperature ( $^{\circ}\text{C}$ )	$26.2 \pm 0.4$	$26.2 \pm 0.5$	$26.9 \pm 0.1$	$26.3 \pm 0.4$	$27.0 \pm 0.1$	$26.4 \pm 0.4$	$26.1 \pm 0.3$	$26.3 \pm 0.3$	$26.2 \pm 0.4$	$26.0 \pm 0.3$	NS	NS
pH	$6.5 \pm 0.0$	$6.7 \pm 0.1$	$6.9 \pm 0.1$	$6.4 \pm 0.2$	$5.9 \pm 0.2$	$6.6 \pm 0.1$	$6.5 \pm 0.1$	$6.8 \pm 0.1$	$6.6 \pm 0.0$	$6.4 \pm 0.1$	$6.5 - 8.5$	$6.5 - 8.5$
Conductivity ( $\mu\text{S cm}^{-1}$ )	$0.02 \pm 0.0$	$0.01 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$	$0.01 \pm 0.0$	$0.02 \pm 0.0$	$0.01 \pm 0.0$	$0.02 \pm 0.0$	$\leq 1000$	NS
Turbidity (NTU)	$0.02 \pm 0.0$	$0.01 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$	$0.02 \pm 0.0$	$0.01 \pm 0.0$	$0.02 \pm 0.0$	$0.01 \pm 0.0$	$0.02 \pm 0.0$	$0.04 \pm 0.0$	6	0-5
Dissolved Oxygen	$18.3 \pm 2.5$	$23.3 \pm 2.1$	$23.0 \pm 0.9$	$23.2 \pm 1.2$	$24.0 \pm 1.0$	$16.2 \pm 1.4$	$15.3 \pm 0.3$	$14.9 \pm 0.2$	$18.1 \pm 0.5$	$17.4 \pm 0.6$	$< 5$	3-5
Total hardness ( $\text{mg L}^{-1}$ )	$26.3 \pm 0.3$	$28.7 \pm 0.2$	$30.9 \pm 0.1$	$29.6 \pm 0.8$	$31.2 \pm 0.8$	$35.6 \pm 0.8$	$34.9 \pm 0.2$	$38.7 \pm 0.2$	$38.9 \pm 0.4$	$40.1 \pm 0.8$	500	500
Sulphate ( $\text{mg L}^{-1}$ )	$10.0 \pm 0.9$	$10.0 \pm 0.0$	$10.0 \pm 1.0$	$10.0 \pm 1.7$	$10.3 \pm 0.6$	$11.0 \pm 0.9$	$11.0 \pm 0.7$	$11.0 \pm 0.1$	$11.0 \pm 0.6$	$11.0 \pm 1.0$	250	NS
Magnesium ( $\text{mg L}^{-1}$ )	$53.0 \pm 1.6$	$55.0 \pm 2.6$	$52.0 \pm 1.7$	$56.0 \pm 2.0$	$54.0 \pm 1.0$	$65.0 \pm 1.0$	$64.0 \pm 1.7$	$67.0 \pm 1.5$	$66.0 \pm 0.0$	$67.0 \pm 0.5$	50	50

Keys: WHO: World health Organization; EPA: Environmental Protection Agency; SW1: Omologbe Stream; SW2: Irutu Stream; SW3: Agbede Stream; SW4: Ilagbo Stream; SW5: Imila Stream; WW1: Okeowa Well; WW2: Iloro Well; WW3: Upebi Well; WW4: Ere Well; WW5: Ilumope Well; NS: No Standard. +: Coloured; Odourless/Colourless.

Of the 22 bacterial isolates subjected to antibiotic sensitivity testing, 18 (81.8%) were multidrug resistant, while 4 (18.9%) exhibited non-multidrug resistance. The resistance rate of Amoxicillin in *E. coli* was high, while  $\geq 66.7$  *S. aureus* were showed sensitivity to Ampicloxacin, Rimfapicin, Amoxicillin, Levofloxacin, Erythromycin, and Streptomycin; 45.5% of bacterial isolates were resistant to Streptomycin and Gentamycin each, and 68.1% of isolates were resistant to Chloramphenicol. All (100%) *P. aeruginosa* were resistant to Chloramphenicol, Sparfloxacin and Pefloxacin, while all the *K. pneumoniae* were sensitive to Augmentin. The results showed a high percentage ( $\geq 71.4\%$ ) of sensitivity to Cotrimoxazole among *E. coli* and *Shigella* spp. (Table 4). The resistance of *E. aerogenes* ranged from 33.3% for Streptomycin, Chloramphenicol, and Pefloxacin to 66.7% for Cotrimoxazole and Augmentin.

**Table 4.** Antibiotic susceptibility profiles of bacteria from stream and well water samples.

Isolates Code	Antibiotic Susceptibility Profile																INF
	ST	SXT	NB	CH	SP	E	CPX	AM	LEV	AU	APX	CN	PEX	RD	OFX	AMX	
EcSW1	S	S	-	R	S	-	S	R	-	R	-	R	S	-	S	-	MDR
EcSW2	R	S	-	S	S	-	S	S	-	R	-	R	S	-	R	-	MDR
EcSW3	R	S	-	S	S	-	S	R	-	S	-	S	S	-	S	-	NMDR
EcSW4	S	R	-	R	R	-	R	R	-	R	-	R	S	-	S	-	MDR
EcWW1	S	S	-	S	R	-	R	S	-	S	-	S	R	-	R	-	MDR
EcWW2	S	R	-	R	S	-	S	S	-	S	-	R	R	-	S	-	MDR
EcWW3	R	S	-	R	S	-	S	R	-	S	-	S	S	-	S	-	MDR
EeSW1	S	R	-	S	S	-	S	S	-	R	-	S	R	-	R	-	MDR
EeWW1	R	R	-	R	S	-	S	R	-	R	-	S	S	-	S	-	MDR
EeWW2	S	S	-	S	S	-	S	S	-	S	-	S	S	-	R	-	NMDR
KpSW1	S	S	-	R	R	-	R	S	-	S	-	R	S	-	R	-	MDR
KpSW2	S	R	-	R	R	-	R	R	-	S	-	S	S	-	S	-	MDR
KpSW3	R	R	-	S	S	-	S	S	-	S	-	S	S	-	S	-	NMDR
KpWW1	R	R	-	R	S	-	S	S	-	S	-	S	R	-	S	-	MDR
SsSW	R	S	-	R	S	-	S	S	-	S	-	R	S	-	R	-	MDR
PsSW	S	S	-	R	R	-	R	R	-	R	-	R	R	-	S	-	MDR
PaWW1	R	S	-	R	R	-	R	S	-	S	-	S	R	-	S	-	MDR
PaWW2	R	R	-	R	R	-	S	S	-	R	-	R	R	-	S	-	MDR
PaWW3	S	S	-	R	R	-	R	S	-	S	-	R	R	-	R	-	MDR
SaSW	S	-	S	R	-	S	-	-	S	-	S	S	-	S	-	S	NMDR
SaWW1	R	-	S	R	-	R	-	-	S	-	S	R	-	S	-	S	MDR
SaWW2	S	-	R	S	-	S	-	-	S	-	R	S	-	R	-	R	MDR

Keys: *E. coli* (EcSW1, EcSW2, EcSW3, EcSW4, EcWW1, EcWW2, EcWW3); *E. aerogenes* (EeSW1, EeWW1, EeWW2); *K. pneumoniae* (KpSW1, KpSW2, KpSW3, KpWW1); *Shigella* spp SsSW; *P. aeruginosa* (PaSW, PaWW1, PaWW2, PaWW3); *S. aureus* (SaSW, SaWW1, SaWW2); MDR: Multidrug Resistant; NMDR: Non-Multidrug Resistant; S: Sensitive, R: Resistant.

Table 5 shows the pre-curing and post-curing antibiotic resistance profiles of bacterial isolates from stream and well water samples. The results showed that *E. coli* EcSW3, *E. aerogenes*.

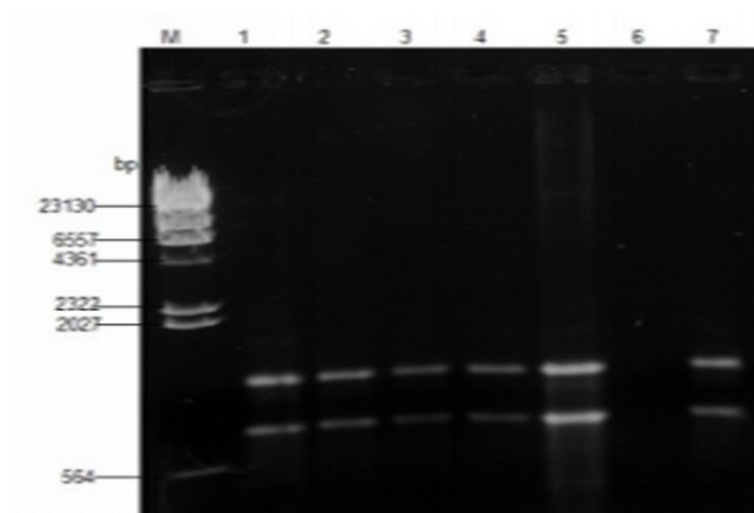
EeWW2, *K. pneumoniae* KpSW3 and *S. aureus* SaSW had their entire antibiotic resistant markers presumptively located on the plasmids. Ten isolates had all their antibiotic resistant markers located on the chromosomes, while eight isolates (*E. coli* EcSW2, EcSW4, EcWW1 and EcWW3; *K. pneumoniae* KpSW1; *Shigella* spp SsSW; *P. aeruginosa* PaWW1 and PaWW3; *S. aureus* SaWW1) had their antibiotic resistant markers residing both on the self-replicative extra-chromosomal plasmids and the chromosomes (Table 5).

**Table 5.** Pre and post-curing of plasmids of bacterial isolates from stream and well water samples.

Isolate Code	Pre-Curing Antibigram	Post-Curing Antibigram
EcSW1	AM/AU/CN/CH	AM/CN
EcSW2	ST/AU/CN/OFX	ST/AU/CN/OFX
EcSW3	ST/AM	-
EcSW4	ST/SXT/CH/SP/CPX/AM/AU/CN	ST/SXT/CH/SP/CPX/AM/AU/CN
EcWW1	SP/CPX/PEX/OFX	SP/CPX/PEX/OFX
EcWW2	CN/SXT/PEX/CH	CN/SXT/PEX/CH
EcWW3	ST/AM/CH/C	ST/AM/CH/C
EpSW1	SXT/AU/PEX/OFX	SXT/AU/PEX/OFX
EpWW1	ST/SXT/AM/CH/AU	ST/SXT/AM
EpWW2	OFX	-
KsSW1	CH/SP/CN/OFX/CPX	CH/SP/CN/OFX/CPX
KsSW2	SXT/CH/SP/CPX/AM	SXT/CH
KsSW3	ST/SXT	-
KsWW1	ST/SXT/CH/PEX	ST//PEX
SsSW	ST/CH/CN/CH/OFX	ST/CH/CN/CH/OFX
PsSW	CH/SP/CPX/AM/AU/CN/PEX	SP/CPX/AM/CN/PEX
PsWW1	ST/CH/SP/CPX/PEX	ST/CH/SP/CPX/PEX
PsWW2	ST/SXT/CH/SP/AU/CN/PEX	ST/SXT/SP/CN/PEX
PsWW3	CH /SP/CPX/CN/PEX/OFX	CH /SP/CPX/CN/PEX/OFX
SaSW	CH	-
SaWW1	ST/CH/E/CN	ST/CH/E/CN
SaWW2	NB/APX/RD/AMX	RD/AMX

Keys: *E. coli* (EcSW1, EcSW2, EcSW3, EcSW4, EcWW1, EcWW2, EcWW3); *E. aerogenes* (EeSW1, EeWW1, EeWW2); *K. pneumoniae* (KpSW1, KpSW2, KpSW3, KpWW1); *Shigella* spp SsSW; *P. aeruginosa* (PaSW, PaWW1, PaWW2, PaWW3); *S. aureus* (SaSW, SaWW1, SaWW2).

The plasmid profiles of selected isolates from streams and well water samples are shown in Figure 3. The isolates had two plasmids each with molecular sizes < 2.027 Kbp.



**Figure 3.** Plasmid profiles of isolates from streams and well water samples. M: Hind III Marker; Line 1: *E. coli* (EcSW1); Line 2: *E. aerogenes* (EeWW1); Line 3: *K. pneumoniae* (KpWW1); Line 4: *P. aeruginosa* (PaSW); Line 5: *P. aeruginosa* (PaWW2); Line 6: *K. pneumoniae* (KpSW1) without plasmid; Line 7: *S. aureus* (SaWW2).

In stream water sample SW1, there was a strong positive correlation between THBC and conductivity ( $r^2 = 0.9982$ ), magnesium ( $r^2 = 0.7206$ ), while in stream water sample SW2, TCC exhibited a very strong negative correlation with pH and dissolved oxygen with a correlation coefficient ( $r^2$ ) of -0.9967 and -0.9707,



respectively ( $p < 0.05$ ) (Table 6). In stream water sample SW3, FCC was observed to have a positive correlation with pH ( $r^2 = 0.5484$ ), total hardness ( $r^2 = 0.9644$ ) and sulphate ( $r = 0.4997$ ). In stream water sample SW4, a highly positive relationship was exhibited between THBC and dissolved oxygen ( $r^2 = 0.9781$ ) at  $p < 0.05$  (Table 7). The Pearson's correlations between bacterial counts and physicochemical parameters of stream water sample SW5 and well water samples WW1 are presented in Table 8. TCC was found to have a very strong negative relationship with conductivity ( $r^2 = -0.9449$ ), turbidity ( $r^2 = -0.8669$ ), total hardness ( $-0.9209$ ), sulphate ( $-0.8265$ ), and magnesium ( $-0.8803$ ) in well water WW2 at the 0.05 level. In well water WW3, the THBC and FCC showed a strong negative relationship with total hardness ( $r^2 = -0.8996$ ), while TCC exhibited a weak positive relationship with pH (Table 9). The Pearson's correlations between bacterial counts and physicochemical parameters of stream water sample WW4 and well water samples WW5 are presented in Table 10.

**Table 6.** Pearson's correlation matrix between bacterial counts and physicochemical parameters of water samples.

	THBC	TCC	FCC	Temp	pH	CDTVT	TUBDT	DO	THDN	SO <sub>4</sub> <sup>2-</sup>	Mg
SW1: Omoologbe Stream											
THBC	1										
TCC	-0.7559	1									
FCC	0.9819	-0.6186	1								
Temp	-0.5005	0.9449	-0.3273	1							
pH	-0.5023	-0.1889	-0.6547	-0.5086	1						
CDTVT	0.9982	-0.7559	0.9820	-0.5011	-0.5095	1					
TUBDT	-0.9979	0.9595	-0.9870	0.5211	0.5023	-0.8999	1				
DO	-0.9934	0.8260	-0.9538	0.5960	0.3974	-0.9934	0.9934	1			
THDN	0.8660	-0.9820	0.7559	-0.8660	0.0110	0.6688	-0.9266	-0.9177	1		
SO <sub>4</sub> <sup>2-</sup>	-0.9819	0.8660	-0.9286	0.6547	0.3273	-0.9820	0.9819	0.9972	0.9449	1	
Mg	0.7206	0.9908	0.8386	0.2402	0.9608	0.7206	0.7206	0.6363	0.2774	0.5766	1
SW2: Iritu Stream											
THBC	1										
TCC	0.1890	1									
FCC	-0.3273	0.8660	1								
Temp	-0.5636	-0.9177	-0.5960	1							
pH	-0.2691	-0.9967	-0.8220	0.9472	1						
CDTVT	0.9991	0.9959	0.9178	-0.8778	-0.7859	1					
TUBDT	0.1908	0.7998	0.9575	-0.9078	-0.9850	0.9986	1				
DO	-0.4193	-0.9707	-0.7206	0.9862	0.9872	-0.9567	-0.9446	1			
THDN	-0.7382	0.5229	0.8790	-0.1411	-0.4511	0.5981	0.5981	-0.3028	1		
SO <sub>4</sub> <sup>2-</sup>	-0.3273	0.8660	0.9989	-0.5960	-0.8220	0.7073	0.9078	-0.7206	0.8790	1	
Mg	0.8660	0.6547	0.8890	-0.9011	-0.7146	0.5833	0.5830	-0.8171	-0.3020	0.1889	1

Keys: TBHC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Counts; CDTV: Conductivity; TUBDT: Turbidity; DO: Dissolved Oxygen; THDN: Total hardness; SO<sub>4</sub><sup>2-</sup>: Sulphate; Mg: Magnesium.

**Table 7.** Pearson's correlation matrix between bacterial counts and physicochemical parameters of water samples.

	THBC	TCC	FCC	Temp	pH	CDTVT	TUBDT	DO	THDN	SO <sub>4</sub> <sup>2-</sup>	Mg
SW3: Agbade Stream											
THBC	1										
TCC	-0.8225	1									
FCC	0.8219	-0.9966	1								
Temp	-0.8432	0.9972	-0.8995	1							
pH	0.6054	-0.0444	0.5484	-0.0843	1						
CDTVT	-0.9042	0.5123	-0.5504	0.5011	-0.8874	1					
TUBDT	-0.4468	0.6768	-0.8798	0.9176	0.4415	0.0219	1				
DO	-0.8409	0.9994	-0.7994	0.9994	-0.0783	0.5291	0.8599	1			
THDN	0.6408	-0.9639	0.9644	-0.9639	-0.2231	-0.2515	-0.9731	-0.9543	1		
SO <sub>4</sub> <sup>2-</sup>	0.9042	-0.5001	0.4997	-0.5015	0.8874	-0.9997	-0.0219	-0.5291	0.2515	1	
Mg	0.5451	0.0293	-0.0293	0.0293	0.9738	-0.8518	0.5064	-0.0046	-0.2943	0.8509	1
SW4: Ilagbo Stream											
THBC	1										
TCC	-0.4701	1									
FCC	0.4998	-0.9994	1								
Temp	-0.4539	0.9998	-0.9986	1							
pH	0.4995	0.5294	-0.5008	0.5447	1						
CDTVT	-0.5002	0.9994	-0.9952	0.9986	0.5005	1					
TUBDT	-0.4007	0.9978	-0.9938	0.9783	0.5931	0.9938	1				
DO	0.9781	-0.2755	0.3083	-0.2581	0.6697	-0.3083	-0.2007	1			



THDN	-0.7156	-0.2803	0.2472	-0.2976	-0.9627	-0.2472	-0.3533	-0.8456	1		
SO <sub>4</sub> <sup>2-</sup>	0.7364	0.2511	-0.2178	0.2686	0.9541	0.2177	0.3248	0.8614	-0.9995	1	
Mg	0.6762	-0.9681	0.9761	-0.9634	-0.2999	-0.9761	-0.9459	0.5076	0.0308	-0.0105	1

Keys: TBHC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Counts; CDTVT: Conductivity; TUBDT: Turbidity; DO: Dissolved Oxygen; THDN: Total hardness; SO<sub>4</sub><sup>2-</sup>: Sulphate; Mg: Magnesium

**Table 8.** Pearson's correlation matrix between bacterial counts and physicochemical parameters of water samples.

	TBHC	TCC	FCC	TEMP	pH	CDTVT	TUB	DO	THDN	SO <sub>4</sub>	MG
SW5: Imila Stream											
TBHC	1										
TCC	0.8515	1									
FCC	0.5345	-0.0284	1								
TEMP	0.4997	0.8799	-0.5000	1							
pH	0.9608	0.6726	0.7206	0.2402	1						
CDTVT	0.0822	-0.4526	0.9042	-0.8225	0.3554	1					
TUB	0.1889	0.6758	-0.7559	0.9449	-0.0909	-0.9631	1				
DO	0.0821	-0.4526	0.9042	-0.8219	0.3554	0.9989	-0.9631	1			
THDN	-0.9974	-0.8871	-0.4364	-0.5611	-0.9383	-0.0102	-0.2592	-0.0102	1		
SO <sub>4</sub>	-0.9387	-0.9801	-0.1707	-0.7679	-0.8062	0.2665	-0.5168	0.2665	0.9611	1	
Mg	-0.3642	0.1782	-0.9887	0.6244	-0.6082	-0.9581	0.8457	-0.9581	0.2963	0.0207	1
WW1: Okeowa Well											
TBHC	1										
TCC	-0.8803	1									
FCC	-0.7898	1	1								
TEMP	-0.9896	0.8038	0.8029	1							
pH	-0.5523	0.0908	0.0907	0.6665	1						
CDTVT	-0.0293	0.5123	0.5311	-0.1147	-0.8171	1					
TUB	-0.5825	0.0908	0.1901	0.7065	0.9989	-0.8171	1				
DO	-0.3437	-0.1429	-0.1426	0.4752	0.9727	-0.9286	0.9727	1			
THDN	0.5797	-0.8968	-0.7768	-0.4565	0.3591	-0.8315	0.3591	0.5659	1		
SO <sub>4</sub>	0.9995	-0.8943	-0.8902	-0.9848	-0.5268	-0.0596	-0.5268	-0.3151	0.6041	1	
Mg	0.8511	-0.5508	-0.5065	-0.9177	-0.9078	0.5000	-0.9078	-0.7857	0.0653	0.8347	1

Keys: TBHC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Counts; CDTVT: Conductivity; TUBDT: Turbidity; DO: Dissolved Oxygen; THDN: Total hardness; SO<sub>4</sub><sup>2-</sup>: Sulphate; Mg: Magnesium.

**Table 9.** Pearson's correlation matrix between bacterial counts and physicochemical parameters of water samples.

	THBC	TCC	FCC	Temp	pH	CDTVT	TUBDT	DO	THDN	SO <sub>4</sub> <sup>2-</sup>	Mg
WW2: Iloro Well											
THBC	1										
TCC	0.9822	1									
FCC	0.8072	0.9042	1								
Temp	0.6187	0.7559	0.9631	1							
pH	0.2303	0.0422	-0.3886	-0.6222	1						
CDTVT	-0.8668	-0.9449	-0.9942	-0.9287	0.2872	1					
TUBDT	-0.9449	-0.8669	-0.5695	-0.3273	-0.5361	0.6547	1				
DO	0.1111	-0.1893	-0.5903	-0.7857	0.9731	0.5056	-0.3273	1			
THDN	-0.9779	-0.9209	-0.6661	-0.4409	-0.4284	0.7425	0.9924	-0.2088	1		
SO <sub>4</sub> <sup>2-</sup>	-0.7046	-0.8265	-0.9876	-0.9934	0.5284	0.9658	0.4336	0.7096	0.5409	1	
Mg	-0.7748	-0.8803	-0.9987	-0.9766	0.4368	0.9871	0.5252	0.6322	0.6257	0.9945	1
WW3: Upebi Well											
THBC	1										
TCC	0.5006	1									
FCC	0.8229	0.9042	1								
Temp	-0.8731	-0.0143	-0.4441	1							
pH	-0.8308	0.1444	-0.3866	0.9983	1						
CDTVT	0.7777	0.9333	0.9973	-0.3725	-0.3175	1					
TUBDT	-0.9997	-0.5067	-0.8226	0.8731	0.8429	-0.7777	1				
DO	0.6547	0.9827	0.9686	-0.2032	-0.1452	0.9843	-0.6547	1			
THDN	-0.9954	-0.5807	-0.8728	0.8224	0.7876	-0.8343	0.9954	-0.7241	1		
SO <sub>4</sub> <sup>2-</sup>	-0.8996	-0.5234	-0.8996	0.8731	0.8431	-0.7777	0.9979	-0.6547	0.9954	1	
Mg	-0.6547	0.3273	-0.1076	0.9401	0.9585	-0.0339	0.6547	0.1429	0.5792	0.6547	1

Keys: TBHC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Counts; CDTVT: Conductivity; TUBDT: Turbidity; DO: Dissolved Oxygen; THDN: Total hardness; SO<sub>4</sub><sup>2-</sup>: Sulphate; Mg: Magnesium.

**Table 10.** Pearson's correlation matrix between bacterial counts and physicochemical parameters of water samples.

	THBC	TCC	FCC	Temp	pH	CDTVT	TUBDT	DO	THDN	SO <sub>4</sub> <sup>2-</sup>	Mg
WW4: Ere Well											
THBC	1										
TCC	0.2099	1									
FCC	-0.8765	-0.6547	1								
Temp	0.7056	-0.5447	-0.2774	1							
pH	0.8551	-0.3373	-0.5000	0.9611	1						
CDTVT	0.8751	-0.3275	-0.5005	0.9707	0.9982	1					
TUBDT	0.2978	0.9959	-0.7206	-0.4663	-0.2402	-0.2402	1				
DO	0.9999	0.1997	-0.8714	0.7133	0.8605	0.8605	0.2878	1			
THDN	0.9907	0.0751	-0.8035	0.7954	0.9827	0.9177	0.1653	0.9921	1		
SO <sub>4</sub> <sup>2-</sup>	0.5939	0.9113	-0.9078	-0.1511	0.0908	0.0807	0.9449	0.5854	0.4792	1	
Mg	-0.8551	0.3273	0.5764	-0.9707	-0.9989	-0.9688	0.2402	-0.8605	-0.9177	-0.0811	1
WW5: Ilumope Well											
THBC	1										
TCC	-0.1076	1									
FCC	-0.4013	0.9538	1								
Temp	-0.1076	0.9989	0.9538	1							
pH	-0.8222	0.6547	0.8514	0.6546	1						
CDTVT	0.0821	0.9819	0.8811	0.9821	0.5011	1					
TUBDT	0.0819	0.8895	0.8799	0.9719	0.5401	0.9992	1				
DO	-0.9865	0.2691	0.5459	0.2691	0.9042	0.0822	0.0819	1			
THDN	-0.8949	0.5399	0.7678	0.5399	0.9897	0.3712	0.3712	0.9559	1		
SO <sub>4</sub> <sup>2-</sup>	-0.9042	-0.3273	-0.0284	-0.3273	0.4994	-0.4997	-0.5076	0.8216	0.6186	1	
Mg	-0.6611	0.8171	0.9525	0.8171	0.9707	0.6934	0.6934	0.7751	0.9265	0.2774	1

Keys: TBHC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Counts; CDTV: Conductivity; TUBDT: Turbidity; DO: Dissolved Oxygen; THDN: Total hardness; SO<sub>4</sub><sup>2-</sup>: Sulphate; Mg: Magnesium.

## Discussion

The World Health Organization recommends that water intended for human consumption be free from contamination (WHO, 2011). Water is considered a vehicle for spreading and disseminating human-associated bacteria (Faria, Vaz-Moreira, Serapicos, Nunes, & Manaia, 2009). In our study, the stream and well water had coliform count concentrations above the WHO acceptable range. The THBC of stream water in this study were higher than that of well water samples, and this substantiated the findings of Idibie, Oviojie, Isalar, and Emoghene (2018) that stream water had higher bacterial loads than well water. The stream water had higher bacterial loads than well water because of being exposed to dust, animal and human faeces, agricultural and pasture runoff, and domestic and industrial waste (Odeyemi et al., 2011; Odeyemi, Ajenifuja, & Adegboyi, 2021). Relatedly, the well water had high THBC and TCC, which might be attributed to the open hand-dug wells and this corroborated Thani, Symekher, Boga, and Oundo (2016) on a high TCC from the open well water in Mombasa County, Kenya. The location of hand-dug wells close to septic tanks or pit toilets and the poor personal hygiene of the people living around a hand-dug well could cause a high microbial load in the well water.

*Staphylococcus aureus*, *P. aeruginosa*, *E. coli*, *E. aerogenes*, *K. pneumoniae*, and *Shigella* spp. were isolated from the stream and well water in this study. The occurrences of *E. coli* and *E. aerogenes* in well water conform with Mukhopadhyay, Vishwanath, Eshwara, Shankaranarayana, and Sagir (2012), who obtained these bacteria from well water in rural and urban households in Karnataka, India. This study also substantiated the findings of Orogu, Oyeyiola, and Adebisi (2017), who isolated *P. aeruginosa* and *S. aureus* from the untreated water in Ilorin, Nigeria. The presence of coliforms and other bacteria like *Pseudomonas* and *Enterobacter* species in the water analyzed denoted water contamination as some of these species are primary bacterial indicator for faecal contamination (Odeyemi, Oluwole, & Adebayo, 2019). The presence of these bacteria in water may lead to waterborne diseases such as typhoid, dysentery and diarrhoea (WHO, 2011). These organisms may be pathogenic, and their presence can pose grave health risks to consumers in general and immune-compromised individuals in particular (Biyela, Johnson, & Carlos, 2004). *Enterobacter*, *Escherichia*, and *Shigella* species from the stream and well water in this study could be related to one or a combination of sewage effluents, such as agricultural run-off and direct faecal contamination from natural fauna (Abulreesh, 2012).

The mean pH of the stream and well water samples lies between  $5.9 \pm 0.2$  and  $6.9 \pm 0.1$ , which showed that the water samples were slightly acidic. The acidic, neutral, and alkaline pH is a characteristic of water bodies' oligotrophic, eutrophic and mesotrophic nature, respectively (Soni et al., 2013). The mean pH of stream water

(SW4, SW5) and well water (WW5) were below the permissible limit of 6.5 given by EPA (2003) and WHO (2011), indicating that these water samples are too acidic for human consumption and can lead to acidosis. Low pH values do have synergistic effects on heavy metal toxicity in water bodies (Adesakin et al., 2020). Temperature is considered one of the fundamental physico-chemical parameters used to assess water quality for human consumption. The mean temperatures ranged from  $26.0 \pm 0.3^{\circ}\text{C}$  to  $27.0 \pm 0.1^{\circ}\text{C}$ , and these values were similar to the values reported by Adesakin et al. (2020) in their study on the physico-chemical parameters of domestic water sources in Samaru, Zaria, Nigeria. On the contrary, these mean temperature values were higher than the 20 C reported in the study conducted in Bahir Dar town (Tabor, Kibret, & Abera, 2011). The DO measures the degree of pollution by organic matter, the destruction of organic substances, as well as the self-purification capacity of the water body. The lowest mean DO value obtained was  $14.9 \pm 0.2 \text{ mg L}^{-1}$ , which was above the EPA (2003) and WHO (2011) permissible limit of 5 for DO value in drinking water. In our study, all the stream and well water had a turbidity level below the 5 NTU recommended by the EPA (2003), thus indicating that the water sample was suitable for consumption with regard to turbidity levels. Studies have shown that high turbidity may be associated with higher levels of suspended organic matter (WHO, 2008), and the consumption of highly turbid water may constitute a health risk as extreme turbidity can protect disease-causing microorganisms from the effect of disinfectants and stimulate bacterial growth. The mean total hardness of the stream water ( $\leq 31.2 \pm 0.8 \text{ mg L}^{-1}$ ) and well water ( $\leq 40.1 \pm 0.8 \text{ mg L}^{-1}$ ) were considerably low compared to the WHO permissible limit for drinking water. This study revealed low mean sulphate values of stream and well water sources and were within the WHO stipulated limits of  $250 \text{ mg L}^{-1}$ . The low concentration of sulphate is attributable to the lack of anthropogenic activities that affect the concentration in water bodies (Adesakin et al., 2020).

The findings of this study revealed a considerable burden of resistance against some antibiotics such as Chloramphenicol, Streptomycin, Amoxicillin, and Gentamycin. In our study, 81.8% of isolates exhibited multidrug resistance, which was higher than the 15% MDR-phenotype reported by Delgado-Gardea et al. (2016) in their study on MDR bacteria isolated from surface water in Bassaseachic Falls National Park, Mexico. The high sensitivity of the bacteria to Levofloxacin corroborated the findings of Akinjogunla et al. (2011) in Uyo, Nigeria. The high sensitivity of *S. aureus* to Levofloxacin agrees with the results by Akanbi, Njom, Fri, Otigbu, and Clarke (2017) on the antimicrobial susceptibility of *S. aureus* isolated from recreational waters and beach sand in Eastern Cape Province, South Africa. The occurrence of Streptomycin-resistant *E. coli* and *E. aerogenes* in the water substantiated the reports of Bello, Osho, Bankole, and Bello (2013) on the antibiotic resistance profiles of *E. coli* and *E. aerogenes* isolated from well waters in Ago-Iwoye, Nigeria.

The antibiotic resistance markers (ARMs) of the bacterial isolates from the stream and well water were located on plasmids, chromosomes or both. Various agents, mainly acridine orange, inhibit plasmid replication that intercalates between DNA bases without inhibiting chromosomal replication (Akinjogunla & Enabulele, 2010). In this study, the loss of ARMs in the bacterial isolates using acridine orange agrees with the results of Yah, Eghafona, Oranusi, and Abouo (2007). The occurrence of the ARMs in chromosomes in this study also agrees with the results of Akinjogunla et al. (2017).

In our study, we observed positive and negative correlations between bacterial counts and the physicochemical parameters of the stream and well water. The occurrence of positive correlations between bacterial counts and the physicochemical parameters of the stream and well water agrees with the study of Adesakin et al. (2020) on the assessment of bacteriological quality and physico-chemical parameters of domestic water sources in the Samaru community, Nigeria.

## Conclusion

This study has shown that the stream and well water from the study areas harboured MDR bacteria with ARMs located on plasmids, chromosomes or both, indicating the possibility of horizontal transfer of antibiotic-resistant genes among the bacteria, and has equally shown the necessity to enlighten the rural populace/public on the importance of cleaning the surroundings near water sources and also implement measures that can prevent water contamination in the communities. It is therefore recommended that the members of the communities be enlightened on the dangers of drinking contaminated water and also a law must be promulgated to prevent the discharge of domestic waste, sewage, faeces, effluents, and other pollutants into these streams that serve as water source for these rural dwellers.

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