(RESEARCH ARTICLE)

Co-occurrence of antibiotics and chlorine resistance in *enterobacteriaceae* isolated from groundwater sources in Ado Ekiti

AJAYI F. A [1, *], ENIOLA K.I.T [2] and BALOGUN O. B [2]

[1] Department of Science Laboratory Technology, Microbiology Unit, Federal Polytechnic, P.M.B. 5351, Ado-Ekiti, Ekiti State Nigeria.
[2] Environmental and Public Health Research Laboratory, Department of Biological Sciences, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State.

Abstract

Groundwater, which is heavily depended on for water needs in Sub-Saharan Africa including Nigeria, is increasingly at risk of contamination from agricultural, industrial, and sewage effluent. This poses a major threat to public health. The study investigates the co-occurrence of antibiotics and chlorine resistance in Enterobacteriaceae isolated from groundwater sources. Ten samples of groundwater were collected from each of our different locations in Ado-Ekiti (Ureje, Olokemeji, Covenant, and Dalimore). Physicochemical characterization of the water samples, isolation and molecular characterization of Enterobacteriaceae, as well as antibiotics and chlorine susceptibilities of the isolated were determined using standard methods. Results showed that electrical conductivity ranged from 152.1 µS/cm to 852.8 µS/cm, pH values varied between 5.59 and 8.7, Turbidity ranged from 2.54 to 4.66 NTU. Iron concentrations were as high as 0.63 mg/L and lead levels were up to 0.06 mg/L, surpassing WHO standards. *Enterococcus faecalis*, *Klebsiella sp.*, *Proteus mirabilis* and *Salmonella sp* were the predominant isolates. Three of the isolates: *Salmonella enterica* (resistant to all 10 antibiotics MAR index= 1), *Klebsiella* sp (resistant to 9 antibiotics MAR index= 0.9), and *Enterococcus faecalis* (resistant to 4 antibiotics MAR index= 0.4) were multidrug resistant, while *Proteus mirabilis* was resistant to ciprofloxacin only. The isolates showed susceptibility to chlorine as from 0.40 mg/mL, *Enterococcus faecalis* was the most susceptible to chlorine, while *Klebsiella pneumoniae* was the most resistant (16.50 mm at 1.00 mg/mL). These findings highlight a critical public health concern, as the dual resistance of groundwater-associated Enterobacteriaceae undermines the goal of conventional water treatment protocols, therefore enhanced monitoring and improved disinfection strategies are urgently recommended to safeguard community health.

Keywords: Antibiotics; Chlorine; *Enterobacteriaceae*; Groundwater; Occurrence; Resistance

1. Introduction

On a global scale, around 2.5 billion people rely on groundwater for their drinking water (Ouellette *et al.*, 2019). Because of its apparent cleanliness, it frequently receives little treatment, making it more susceptible to infection by opportunistic, Gram-negative bacteria like Salmonella and *E. coli* as well as pathogens like Enterobacteriaceae. These bacteria are remarkably adaptive to environmental stressors, becoming resistant to disinfectants such as chlorine and antibiotics (Chen *et al.*, 2020; Binnie *et al.*, 2021).

Agricultural runoff, industrial effluents, and poor sanitation facilities are some of the main causes of water contamination worldwide (EPA, 2020). Poverty, dense settlements, and inadequate sanitation make the problem worse

* Corresponding author: AJAYI F. A Email: arisefunmiajayi@gmail.com

in underdeveloped nations (WHO, 2017). This is especially noticeable in areas of Nigeria like Ekiti State, where a lack of sanitary facilities and informal settlements greatly contaminate the groundwater.

In Ekiti, surface water, dams, and raw groundwater are regularly contaminated by untreated sewage, animal waste, and agricultural leftovers (Raji and Abdulkadir, 2020). Pathogenic bacteria, viruses, and protozoa may be present in these waters. According to Omole *et al.* (2015), the presence of E. coli in particular is a reliable signal of fecal contamination and the possible presence of additional dangerous pathogens.

In water treatment, chlorine is still one of the most popular and reasonably priced disinfectants, particularly in environments with limited resources. According to Bartram and Cotruvo (2019), it has potent bactericidal qualities and gives enduring defense against recontamination. Chlorination does have certain drawbacks, though, most notably its inefficiency against some viruses and protozoa and the possibility of producing harmful byproducts like trihalomethanes (THMs) (Dawit *et al.*, 2017).

Microbial resistance to chlorine has become a serious worry, which is alarming. Due in large part to defense mechanisms such glutathione synthesis and oxidative stress response pathways, studies have demonstrated that E. coli strains may withstand exposure to chlorine at concentrations higher than permissible limits (Chesney *et al.*, 2016; Ibekwe *et al.*, 2023). This resistance increases the hazards to public health and compromises the effectiveness of disinfection procedures.

The emergence of antibiotic resistance exacerbates the issue even more. Many multidrug-resistant bacteria currently flourish in natural reservoirs like groundwater as a result of the indiscriminate and excessive use of antibiotics in medicine and agriculture (Da Silva *et al.*, 2017; Yoon *et al.*, 2018). There have been more and more reports of the co-resistance phenomenon, which occurs when bacteria show resistance to both antibiotics and chlorine, especially in Enterobacteriaceae (Gu *et al.*, 2019; Liu *et al.*, 2021).

Because it seriously undermines traditional water treatment methods and raises the possibility of outbreaks of hard-to-treat waterborne illnesses, this dual resistance poses a substantial issue. Because of its inadequate infrastructure and monitoring capabilities, Ekiti State is especially vulnerable, much like many other poor nations.

Current research emphasizes how critical it is to comprehend the genetic foundation of co-resistance and how urgent it is to implement stronger monitoring and sanitation measures (Ajayi and Akonai, 2023; Mead *et al.*, 2019). To protect water quality and public health, both internationally and locally in places like Ekiti, a comprehensive strategy that incorporates enhanced treatment technology, decreased antibiotic abuse, and focused monitoring is essential.

Growing densities in residential areas combined with issues with sewer management are becoming a major issue. Drinking water contamination is increasing as a result of improper handling of household and animal waste, as well as waste water from unplanned urbanization. Devastating outbreaks could result from the rising prevalence of antibiotic and chlorine resistance.

Because it seriously undermines traditional water treatment methods and raises the possibility of outbreaks of hard-to-treat waterborne illnesses, this dual resistance poses a substantial issue. Rapid unplanned urbanization and inadequate sewer infrastructure have raised the risk of water contamination and increased environmental microbial pressure in Ado-Ekiti, the capital of Ekiti State. Due to the lack of a sophisticated sewage system, the region is vulnerable to the development of gastrointestinal diseases, particularly strains of E. coli that are resistant to antibiotics (CDC, 2019).

According to recent studies, gastrointestinal disorders associated with contaminated water sources are becoming more common in Ekiti villages, especially during the rainy season. Public health experts have seen a rise in the prevalence of waterborne illnesses such cholera, typhoid, and acute diarrheal infections in Ado-Ekiti and the surrounding areas. These illnesses are frequently connected to untreated or inadequately treated surface and groundwater (WHO, 2017). Effective disease management and outbreak prevention are directly threatened by the known increase in antibiotic- and chlorine-resistant bacteria in local water supplies, which exacerbates this public health risk.

In light of these difficulties, a more thorough examination of the prevalence and resistance patterns of waterborne pathogens in Ekiti's groundwater is required in order to guide better sanitation regulations and therapeutic approaches. The region can only reduce the threats presented by co-resistant Enterobacteriaceae and protect the health of its people by means of focused treatments and localized surveillance. It is therefore the aim of this study is to determine co-

occurrence of antibiotics and chlorine resistance in Enterobacteriaceae strains isolated from groundwater sources in Ado-Ekiti.

2. Materials and methods

2.1. Study Area

The study was conducted in Ado-Ekiti, the capital of Ekiti State, Nigeria, located between 5°N and 7°E. Ado-Ekiti is a major administrative, educational, and commercial center. The population is estimated between 424,000 and 500,000, predominantly composed of the Ekiti subgroup of the Yoruba ethnic group. English and Ekiti Yoruba are the primary languages.

2.2. Sampling

Well water samples were randomly collected from four sites in Ado-Ekiti (Covenant, Dallimore, Ureje, and Olokemeji). Samples were placed in sterile, labeled containers and transported on ice to the Microbiology Laboratory, Department of Science Laboratory Technology, Federal Polytechnic, Ado-Ekiti.

2.3. Physicochemical Analysis of Water Samples

Standard analytical procedures were employed to assess pH, total solids (TS), total dissolved solids (TDS), electrical conductivity (EC), turbidity, and heavy metals.

2.4. Total Bacterial Count

Serially diluted samples were plated on MacConkey agar using the pour plate method and incubated at 37°C for 24 hours. Pure colonies were subcultured for identification.

2.5. Bacterial Identification

Biochemical tests conducted include indole production, hydrogen sulfide production, and triple sugar iron (TSI) test for sugar fermentation and gas production.

2.6. Antibiotic Susceptibility Testing

Performed using the disc diffusion method on nutrient agar. Plates were incubated at 37°C for 24 hours. Zones of inhibition were recorded.

2.7. Chlorine Susceptibility Testing

Kirby-Bauer method was used to assess resistance to chlorine concentrations (0.1–0.4 mg/L). Tap water served as control. Zones of inhibition were measured.

2.8. Molecular Characterization of Resistant Enterobacteriaceae

- Plasmid curing: Twelve colonies from each isolate were cultured in LB medium and incubated at 37°C. Post-curing, strains were tested for changes in resistance profiles.
- Detection of resistance genes: DNA was extracted using PrepMan Ultra reagent. The lysate was boiled, centrifuged, and the supernatant used for PCR.
- PCR assay: PCR was performed using a Bio-Rad MJ MINI thermal cycler. Extracted DNA served as template for resistance gene amplification.

2.9. Statistical Analysis

Means and standard deviations were computed. Spearman's correlation was used to assess the relationship between antibiotic and chlorine resistance profiles using Microsoft Excel.

3. Results

Table 1 details the physicochemical parameters of well water samples from four locations in Ado-Ekiti; Covenant, Ureje, Dallimore, and Olokemeji; and compares them against WHO drinking water standards. Electrical conductivity in all locations exceeded the WHO limit of 250 μS/cm, especially in Olokemeji (852.8 μS/cm) and Ureje (796.9 μS/cm),

indicating elevated dissolved ion content. pH values ranged from 5.59 to 8.7, with Ureje below the acceptable WHO minimum (6.5), suggesting acidity. Temperature values (23.88°C–27.23°C) were above the WHO recommended range (2.8–13°C), which may promote microbial growth. Iron concentrations exceeded the WHO limit (0.3 mg/L) in three of the four sites, particularly in Covenant (0.63 mg/L). Lead was above the permissible level (0.01 mg/L) in Ureje (0.06 mg/L) and Covenant (0.03 mg/L), posing serious toxicological concerns. Total dissolved solids (TDS) exceeded the WHO threshold (500 ppm) in Olokemeji, suggesting high mineral content. Parameters like turbidity, alkalinity, and hardness remained within acceptable limits.

Table 2 presents the colonial morphology, Gram staining results, and biochemical test profiles of bacteria isolated from well water samples in Ado-Ekiti Metropolis. The isolates exhibited diverse morphologies and biochemical characteristics, allowing for preliminary identification based on standard microbiological methods. The identified organisms include *Proteus* spp., *Enterococcus* spp., *Salmonella* spp., and *Klebsiella* spp. Most of the isolates were Gram-negative rods, except *Enterococcus spp.*, which appeared as Gram-positive cocci. Morphological distinctions such as colony color (light pink for *Proteus*, black for *Salmonella*, and golden yellow for *Enterococcus*) supported differentiation.

Figure 1 shows the distribution of bacterial counts (CFU/100ml) in well water samples collected from four different areas in Ado-Ekiti. The boxplot highlights higher median values in Ureje and Covenant, suggesting greater microbial contamination in those locations compared to Dallimore and Olokemeji.

Table 3 presents the distribution and relative abundance of four major bacterial species; Enterococcus faecalis, Klebsiella spp., Proteus mirabilis, and Salmonella spp.; isolated from well water samples collected across different areas in Ado-Ekiti metropolis (Covenant, Dallimore, Ureje, and Olokemeji). Among the 90 total isolates recorded, *Enterococcus faecalis* was the most prevalent, accounting for 45.5% of total isolates, and was consistently found across all sampling locations, with the highest incidence in Olokemeji (OKJ1). *Salmonella* spp. ranked second in prevalence at 17.8%, with moderate presence in several locations including Covenant and Ureje. *Klebsiella* spp. represented 14.4% of isolates, particularly prominent in URJ1 and OKJ1. *Proteus mirabilis* was the least frequent, constituting 5.6% of the total isolates, and was sporadically detected in only a few samples such as DAL1 and URJ2.

Table 4 presents the zones of inhibition (mm) of standard antibiotics tested against four bacterial isolates. Enterococcus faecalis displayed the highest susceptibility, particularly to Ciprofloxacin (22 mm), Amoxicillin (20 mm), and Sparfloxacin (18 mm). Proteus mirabilis also showed good response to multiple antibiotics including Sparfloxacin, Tarivid, and Pefloxacin (20 mm each). *Klebsiella* spp. exhibited generally low sensitivity, with all inhibition zones at 10–14 mm, suggesting reduced susceptibility or resistance to most antibiotics tested. *Salmonella* spp. had moderate sensitivity, with notable activity against Ciprofloxacin and Tarivid (20 mm).

Table 5 presents the zones of inhibition (in mm) observed when varying concentrations of chlorine (0.20 to 1.00 mg/mL) were applied to four bacterial isolates from well water samples in Ado-Ekiti. The table evaluates the bactericidal effectiveness of chlorine across different microbial species. Enterococcus faecalis showed the greatest susceptibility to chlorine, with inhibition zones ranging from 8.45 mm to 25.00 mm, and a high average response (16.69 mm). This suggests it is consistently vulnerable to chlorination. Klebsiella pneumoniae and Proteus mirabilis displayed moderate and variable responses, with average inhibition zones of 8.11 mm and 6.82 mm respectively. Their response patterns fluctuated with concentration, indicating partial resistance or tolerance at some levels. Salmonella enterica showed inconsistent and relatively low inhibition, with a mean zone of 7.32 mm and poor inhibition at the highest and lowest concentrations, suggesting potential resistance or adaptation.

Plate 1 illustrates the agarose gel electrophoresis results confirming successful amplification of the 16S rRNA gene region from selected bacterial isolates. Distinct DNA bands are visible for *Enterococcus feacalis (*A), *Proteus mirabilis (B), Salmonella enterica (*SS), *Pseudomonas aeruginosa (*PO1), and *Klebsiella pneumoniae (*KO1)

Table 1 Physicochemical characteristics of selected water samples

Parameters	Covenant	Ureje	Dallimore	Olokemeji	WHO standard
Electrical conductivity (µS/cm)	593.4	796.9	152.1	852.8	250
Turbidity (NTU)	3.561	4.64	2.543	4.66	5 – 50
pH	8.3	5.59	7.9	8.7	6.5 – 8.5
Temperature (°C)	27.23	23.89	26.46	23.88	2.8 – 13

Alkalinity	78.743	82.093	64.707	87.1	120
Total hardness	52.66	56.149	41.81	62.754	300
Total suspended solids (ppm)	10.521	12.164	1.78	8.088	Nl
Total dissolved solids (ppm)	279.9	405.0	97.2	517.2	500
Iron (mg/L)	0.63	0.60	0.06	0.44	0.3
Lead (mg/L)	0.03	0.06	0.00	0.01 m	0.01
Copper (mg/L)	0.18	0.31	Nd	0.24	0.5

Nd = Not detected; Nl = Not listed

Table 2 Colonial morphology and biochemical characterization of bacteria isolated from well water in Ado-Ekiti Metropolis

Gram staining	Shape	Colour	Oxidase	Motility	Urease	Indole	Coagulase	Catalase	Citrate	Glucose	Mannitol	Maltose	Lactose	**Probable organism**
–ve	Rod	Light pink	–ve	+ve	+ve	–ve	–ve	+ve	+ve	+ve	–ve	–ve	–ve	*Proteus spp*
+ve	Cocci	Black	–ve	–ve	–ve	–ve	+ve	–ve	–ve	+ve	+ve	+ve	+ve	*Enterococcus* spp.
–ve	Rod	Black	–ve	+ve	–ve	–ve	–ve	+ve	–ve	+ve	+ve	+ve	–ve	*Salmonella* spp.
–ve	Rod	Pink	–ve	–ve	+ve	–ve	–ve	+ve	+ve	+ve	+ve	+ve	+ve	*Klebsiella* spp.
+ve	Cocci	Golden yellow	–ve	–ve	–ve	–ve	–ve	+ve	–ve	+ve	+ve	+ve	+ve	*Enterococcus* spp.

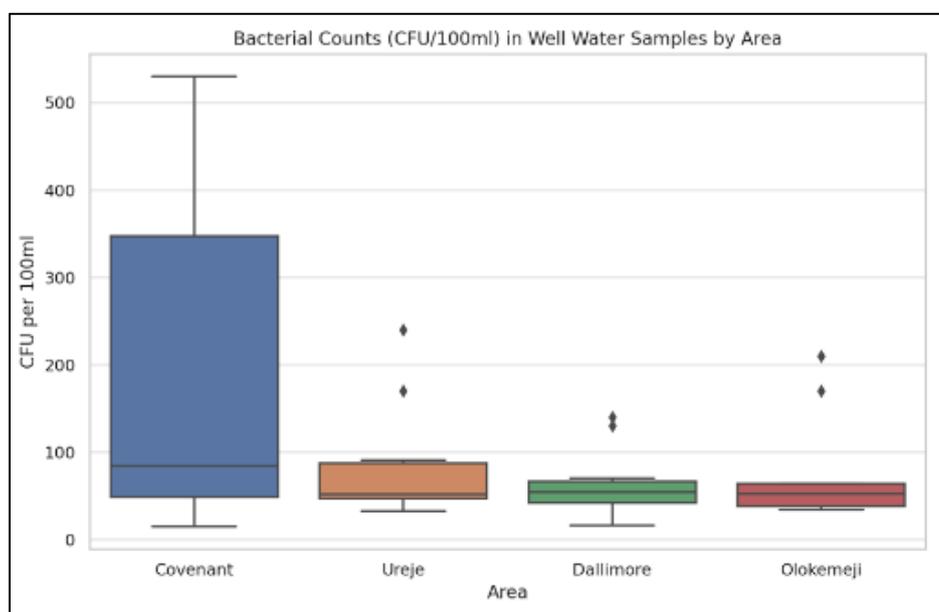**Keys:** (+ve) - positive, (-ve) - negative

Figure 1 Bacterial Counts in Well Water

Table 3 Distribution of bacteria isolates from well water samples

Sample Code	*Enterococcus faecalis* (%)	*Klebsiella* spp. (%)	*Proteus mirabilis* (%)	*Salmonella* spp. (%)	Total Isolates
COV1	5 (58.1)	2 (15.4)	0 (0.0)	4 (25.0)	11
COV2	0 (0.0)	2 (15.4)	0 (0.0)	0 (0.0)	7
COV3	2 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)	2
DAL1	4 (10.8)	0 (0.0)	3 (60.0)	0 (0.0)	7
DAL2	5 (13.5)	0 (0.0)	0 (0.0)	2 (12.5)	7
URJ1	3 (8.1)	5 (38.5)	0 (0.0)	0 (0.0)	12
URJ2	5 (13.5)	0 (0.0)	2 (40.0)	3 (18.8)	13
OKJ1	11 (74.3)	4 (30.8)	0 (0.0)	5 (31.3)	20
OKJ2	2 (5.4)	0 (0.0)	0 (0.0)	2 (12.5)	4
OKJ3	4 (10.8)	0 (0.0)	0 (0.0)	0 (0.0)	7

COV – Samples from Covenant area; DAL – Samples from Dallimore area; URJ – Samples from Ureje area; OKJ – Samples from Olokemeji area

Table 4 Antibiotic susceptibility pattern of bacterial isolates from well-water in Ado-Ekiti metropolis

Bacterial isolates	Standard Antibiotics									
	Zones of inhibition (mm)									
	CN	AU	AM	CPX	SP	CH	SXT	S	OFX	PEF
Klebsiella spp.	10.00	10.00	10.00	20.00	14.00	10.00	10.00	18.00	10.00	10.00
P. mirabilis	13.00	15.00	15.00	15.00	20.00	10.00	10.00	20.00	20.00	20.00
Salmonella	10.00	10.00	15.00	20.00	13.00	10.00	10.00	10.00	20.00	15.00
E. feacalis	16.00	10.00	20.00	22.00	18.00	10.00	10.00	10.00	10.00	15.00

CN – Gentamycin; AU – Augmentin; AM – Amoxacillin; CPX – Ciprofloxacin; SP – Sparfloxacin; CH – Chloramphenicol; SXT – Septrin; S – Streptomycin; OFX – Tarivid; PEF – Pefloxacin

Table 5 Chlorine susceptibility pattern of bacterial isolates from well-water in Ado-Ekiti metropolis

Bacterial isolates	Diameters of zones of inhibition (mm)				
	Concentrations (mg/mL)				
	0.20	0.40	0.60	0.80	1.00
Klebsiella pneumoniae	11.00	0.85	6.40	5.80	16.50
Proteus mirabilis	5.35	11.5	0.75	10.00	6.50
Salmonella enterica	0.90	14.00	11.00	10.00	0.70
Enterococcus feacalis	8.45	18.00	15.50	16.50	25.00
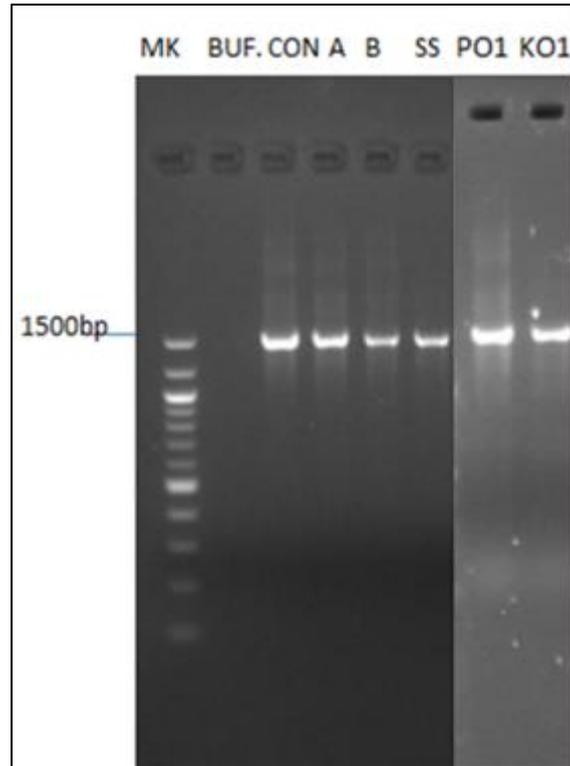
KEY: A = Enterococcus faecalis; B = Proteus mirabilis; SS = Salmonella enterica; PO1 = Pseudomonas aeruginosa; KO1 = Klebsiella pneumoniae

Figure 2 Agarose gel showing the positive amplification of the 16S regions amplified from the selected bacteria samples

4. Discussion

The physicochemical analysis of well water samples from four locations in Ado-Ekiti (Olokemeji, Covenant, Ureje, and Dallimore) showed a number of differences from the World Health Organization's (WHO) guidelines for drinks. All locations except Dallimore had electrical conductivity levels above the suggested threshold of 250 μS/cm. Particularly high values of 852.8 μS/cm and 796.9 μS/cm, respectively, were obtained at Olokemeji and Ureje, suggesting higher quantities of dissolved ionic compounds. With a pH of 5.59, which is quite acidic and below the WHO standard of 6.5, Ureje's results varied greatly. Such acidity, which increases the solubility of hazardous metals and increases the danger of corrosion to water infrastructure, can be caused by organic acid contamination or natural geological reasons.

Temperature readings in all samples were between 23.88°C and 27.23°C, which is significantly higher than the WHO's recommended range of 2.8–13°C. Water safety may be negatively impacted by high water temperatures since they can encourage microbial growth and lower dissolved oxygen. In Covenant, Ureje, and Olokemeji, iron levels were higher above the WHO upper limit of 0.3 mg/L; in Covenant, they were as high as 0.63 mg/L. Similarly, toxicological concerns were raised when lead readings in Covenant (0.03 mg/L) and Ureje (0.06 mg/L) exceeded the WHO recommendation of 0.01 mg/L. Similar physicochemical anomalies were found in hand-dug wells in southwestern Nigeria by Ibekwe *et al.* (2023), who attributed these findings to leaching from rusty pipes, inadequate sanitation, and close proximity to trash dumps. Turbidity, hardness, and alkalinity all stayed within acceptable bounds, but Olokemeji's high total dissolved solids (TDS) of 517.2 ppm points to possible mineral pollution from the geologic substratum or human activity.

Numerous opportunistic and harmful bacterial species, such as *Salmonella* spp., *Proteus mirabilis*, *Klebsiella* spp., and *Enterococcus faecalis*, were found by microbiological examination. Gram staining, biochemical traits, and colony morphology were used to identify them. Except for *Enterococcus* species, which manifested as Gram-positive cocci, the majority of isolates were Gram-negative rods. According to the distribution data, *Enterococcus faecalis* was the most prevalent species, making up 45.5% of all isolates and being present at every sample site. This organism is a major sign of fecal contamination and has been linked to endocarditis, bacteremia, and urinary tract infections, particularly in immunocompromised people. The results corroborate those of Liu *et al.* (2021), who found that Enterococcus was

common in well water systems in Asian and African urban slums, emphasizing fecal infiltration as a result of inadequate sanitary infrastructure.

Klebsiella species made up 14.4% of isolates and were most prevalent in Olokemeji and Ureje. The capacity of these organisms to form biofilms, which shield them from disinfectants, and their involvement in hospital-acquired illnesses are well-known. *Salmonella* spp. was moderately dispersed throughout Covenant and Ureje, accounting for 17.8% of all isolates. This pathogen's link to foodborne gastroenteritis and typhoid fever makes it a serious public health concern. *Proteus mirabilis* was isolated in a small number of samples and had the lowest prevalence (5.6%). The well water's microbial insecurity and the risk of enteric disease transmission are highlighted by the total bacterial profile, particularly for vulnerable groups that depend on untreated water for daily consumption.

Complex co-resistance patterns among the bacterial isolates were discovered using antimicrobial susceptibility testing. Significant sensitivity to Ciprofloxacin (22 mm), Amoxicillin (20 mm), and Sparfloxacin (18 mm) was demonstrated by Enterococcus faecalis. On the other hand, it demonstrated resistance to chloramphenicol, septrin, and streptomycin. This profile of partial resistance is in line with earlier research by Ibekwe *et al*. (2023), who found that environmental exposure and abuse of conventional antibiotics were causing environmental Enterococcus strains to become more resistant to them. While Proteus mirabilis responded poorly to older drugs like Septrin, it demonstrated good responsiveness to more recent fluoroquinolones like Tarivid and Pefloxacin. With inhibition zones ranging from 10 to 14 mm, Klebsiella spp. showed decreased sensitivity to almost all tested drugs, which was the most alarming finding. Emerging extended-spectrum beta-lactamase (ESBL) resistance is reflected in this pattern, which is frequently observed in hospital and environmental settings. *Salmonella* species displayed a moderate level of susceptibility, while ciprofloxacin and tarivid were very effective.

There are serious public health issues raised by these resistance patterns. The possibility of community-acquired diseases that would be challenging to treat with first-line antibiotics is suggested by the discovery of several antibiotic-resistant organisms in public water sources. Particularly in Klebsiella species, resistance to fluoroquinolones and beta-lactam antibiotics can significantly limit treatment options and raise morbidity and healthcare expenses. Such co-resistance between environmental bacteria raises the possibility that groundwater could serve as a reservoir for genes causing antibiotic resistance, hence promoting horizontal gene transfer. In their investigation of tainted aquifers in metropolitan China, Liu *et al*. (2021) emphasized comparable hazards, stressing the part environmental water sources play in the dissemination of resistance determinants.

Enterococcus faecalis was shown to be most sensitive to rising chlorine concentrations in the chlorine susceptibility experiment, with inhibition zones spanning from 8.45 mm to 25.00 mm. This implies that, given the right circumstances, chlorination may be a useful method for rendering this bacterium inactive. *Salmonella enterica*, *Proteus mirabilis*, and *Klebsiella pneumoniae*, on the other hand, showed erratic or negligible inhibition at different chlorine doses, suggesting a possibility of chlorine tolerance or adaptation. For example, *Salmonella enterica* exhibited nearly little response at the highest and lowest dosages tested, while *Klebsiella pneumoniae* shown very little inhibition between 0.40 and 0.60 mg/mL. These patterns imply that traditional chlorine dosage might not be consistently effective against all waterborne pathogens, especially those that can form biofilms or undergo efflux-mediated detoxification. Ibekwe *et al*. (2023) observed similar results, highlighting the limited effectiveness of conventional chlorination against Gram-negative microbes that form biofilms in untreated groundwater.

The isolates' biochemical identity was validated by molecular identification using 16S rRNA sequencing and agarose gel electrophoresis. The taxonomic accuracy was confirmed by the presence of clear bands that corresponded to the amplified 16S regions for *Salmonella enterica*, *Proteus mirabilis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. High similarity with known strains was demonstrated by the phylogenetic trees derived from these genomes, confirming the traditional identification and exposing evolutionary grouping within the corresponding taxa. This molecular validation strengthens the study's resilience and shows how beneficial it is to combine molecular and traditional methods in environmental microbiology.

The study had many limitations in spite of the noteworthy results. Since water samples were only taken from four Ado-Ekiti metropolitan districts, the sample size was somewhat small. The findings' applicability to other areas of the state or region is hampered by their restricted spatial coverage. Furthermore, the study was cross-sectional and failed to take seasonal fluctuations into consideration, which may have an impact on the physicochemical and microbiological quality of the water. This analysis was unable to account for the potential for increased pollution caused by rainfall, flooding, and runoff during wet seasons. Additionally, only 16S rRNA sequencing was used for the molecular identification. More information about the existence of resistance genes and virulence factors might be possible with whole-genome

sequencing. The source or channel of pollution, such as the closeness of pit latrines, trash dumps, or animal enclosures to the wells, was also not evaluated in the study.

The study shows that physicochemical contamination and the presence of pathogenic and multidrug-resistant bacteria make well water in specific Ado-Ekiti localities unsafe for direct consumption. The prevalence of strains of Salmonella and Klebsiella that are resistant to antibiotics emphasizes the critical need for better sanitation facilities, antibiotic stewardship, and water quality monitoring. The results highlight the significance of environmental water sources in the persistence and spread of resistant diseases, supporting those of Liu *et al.* (2021) and Ibekwe *et al.* (2023). To lower exposure and related health hazards, public health officials must give priority to disinfecting and protecting groundwater as well as educating the public.

5. Conclusion

This study has shed important light on the microbiological and physicochemical characteristics of the well water in the city of Ado-Ekiti. The results show that a number of water samples had levels of lead, pH, iron, electrical conductivity, and other characteristics beyond WHO safety limits. These variations point to potential contamination from human activities like garbage disposal and infrastructure deterioration as well as geology sources. Significant microbial contamination, most likely from fecal sources, is indicated by the persistent presence of harmful bacteria such as *Salmonella* spp., *Proteus mirabilis*, *Klebsiella* spp., and *Enterococcus faecalis*. The risk of waterborne illnesses in the areas under study is further highlighted by the high prevalence of *Enterococcus faecalis* in all sample locations.

Antimicrobial susceptibility profile showed alarming resistance trends, especially in *Salmonella* and *Klebsiella* species that showed decreased sensitivity to widely used medicines. These trends raise serious public health concerns about the treatment of aquatic illnesses because they imply the presence of multidrug-resistant bacteria in the environment. The dependability of simple disinfection techniques is also called into doubt by the chlorine resistance seen in certain isolates, particularly in unofficial settings where dosage may vary. These pathogens were identified by molecular analysis using 16S rRNA sequencing, which validated the traditional microbiological methods used in the investigation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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