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Molecular Identification, Phylogenetic Analysis, and Antibiotic Susceptibility Profiles of Bacteria Isolated from Borehole Water across Seasonal Variations

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Abstract

Groundwater serves as a major source of potable water in many developing regions; however, its microbiological quality remains a public health concern. This study evaluated the bacterial diversity, seasonal variation, molecular identification, phylogenetic relationships, and antibiotic susceptibility profiles of bacteria isolated from borehole water sources. Total bacterial counts were determined for both rainy and dry seasons, revealing values ranging from 3.65–3.92 Log CFU/mL and 3.54–3.83 Log CFU/mL, respectively, with slightly higher counts observed during the rainy season. Bacterial isolates were identified using 16S rRNA gene sequencing and analyzed through BLASTn, which confirmed high similarity (97.88 – 99.86 %) with known species including *Escherichia coli*, *Klebsiella aerogenes*, *Salmonella enterica*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*. Phylogenetic analysis using the Neighbor-Joining method revealed clear clustering of isolates with reference strains, supported by high bootstrap values. Antibiotic susceptibility testing showed that most isolates were sensitive to fluoroquinolones, with ciprofloxacin demonstrating the highest efficacy. The findings highlight the presence of potentially pathogenic bacteria in borehole water and underscore the importance of continuous monitoring and appropriate water treatment strategies to safeguard public health.

Keywords: Antiobiotic, susceptibility, Ground water, Public health, Seasonal variation, Water monitoring.

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1. Introduction

Access to safe and potable water remains a fundamental public health priority worldwide, particularly in developing countries where groundwater sources such as boreholes are widely used for domestic purposes. Although groundwater is often considered microbiologically safer than surface water, it is not completely free from contamination, as pathogens can enter through seepage, poor sanitation, and environmental runoff (Egurefa *et al*. 2020a; 2020b; World Health Organization, 2022; Okolo *et al.*, 2025). Consequently, the consumption of contaminated water has been linked to a wide range of waterborne diseases, posing significant health risks to communities (Centers for Disease Control and Prevention, 2022; Okpalaunegbu *et al.*, 2025; Obiefuna *et al.* 2025; Obiefuna *et al.* 2026).

Microbial contamination of drinking water is commonly assessed using indicator organisms such as *Escherichia coli*, which signifies fecal contamination and the possible presence of enteric pathogens (Cabral, 2010; Uba, 2019a; 2019b; Uba *et al.*, 2019a; 2019b). In addition, environmental and opportunistic pathogens such as *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Salmonella enterica*, and *Proteus mirabilis* have been frequently isolated from water sources and are known to cause infections, especially in immunocompromised individuals (Ashbolt, 2015; Anichebe *et al*., 2019; Okoye *et al.* 2020a; 2020b; 2020c). The presence of these organisms in groundwater highlights the need for continuous monitoring and improved water management practices (Uba, 2019c; Uba *et al*. 2019c; Uba *et al*. 2024; Mere *et al.* 2025; Enemchukwu *et al*. 2026a; 2026b).

Advances in molecular biology have significantly enhanced the identification and characterization of microorganisms in environmental samples. The use of 16S rRNA gene sequencing has become a gold standard for bacterial identification due to its high accuracy and reliability (Janda & Abbott, 2007; Uba *et al*. 2016; Nkamigbo *et al*. 2020a; 2020b; Njoku *et al*. 2019a; 2019b)). Sequence comparison using bioinformatics tools such as BLAST enables the determination of genetic similarity between unknown isolates and reference organisms in databases, while phylogenetic analysis provides insights into evolutionary relationships and taxonomic classification (Srinivasan *et al*., 2015; Uba *et al.*, 2017; Okoye *et al*., 2026). These molecular approaches offer greater precision than traditional culture-based and biochemical methods (Uba e*t al.*, 2021a; 2021b; Dokubo *et al*., 2022a; 2022b; Anidu *et al*., 2023; Obiefoka *et al*., 2023; Ubajekwe *et al*., 2025; Uba *et al*., 2025).

Seasonal variation is another critical factor influencing the microbiological quality of groundwater. Increased rainfall during the wet season enhances surface runoff and infiltration, facilitating the transport of microorganisms into groundwater systems. As a result, higher bacterial loads are often reported during the rainy season compared to the dry season (Fierer & Jackson, 2006; Uba *et al*. 2020c; 2020d; 2020e; 2020f; 2020g; 2020h; Dokubo *et al*., 2024). Understanding these seasonal dynamics is essential for effective water quality assessment and management (Dokubo and Uba, 2023; Uba and Obiefuna, 2023).

Furthermore, the emergence and spread of antimicrobial resistance among environmental bacteria have become a global concern (Ubani *et al*., 2024a; 2024b; 2025; Ekwenze *et al*., 2025). Water sources can act as reservoirs for resistant bacteria and resistance genes, which may be transferred to human pathogens (Kümmerer, 2009; Uba *et al*., 2020a; 2020b; Alisa *et al*., 2020; Anukam *et al*., 2020a; 2020b; Umeh *et al.*, 2020; 2021). Therefore, antibiotic susceptibility testing is crucial for evaluating the resistance patterns of bacterial isolates and determining the effectiveness of commonly used antimicrobial agents. Standardized guidelines provided by the Clinical and Laboratory Standards Institute (2022) ensure reliable interpretation of susceptibility results.

In light of these concerns, this study aims to evaluate the microbiological quality of borehole water by determining total bacterial counts across seasons, identifying bacterial isolates using molecular techniques, analyzing their phylogenetic relationships, and assessing their antibiotic susceptibility profiles. The findings of this study will contribute to improved understanding of groundwater safety and provide essential data for public health interventions.

2. Materials and Methods

2.1 Study Area

This study was carried out in Agbor. Agbor is the headquarters of Ika South Local Government Area of Delta State in the South South region of Nigeria. Agbor has a population put at162,594 according to National Census Commission (2006) and a land mass of 685km^2. The geographical coordinates of Agbor are 6"254° N latitude, 6"194° E longitude using WGS84 is standard and 427 ft elevation. The topography within two miles of Agbor contains only variations in the elevation with a maximum elevation of 367ft and an average elevation above sea level of 504ft. Agbor lies within the equatorial climate with two distinct seasons, the wet (April to September) and dry (October to March), high humidity of between 24°Cto 27°C, which supports the rainforest vegetation. The discovery and exploration of oil in commercial quantity in Ekuku Agbor and environs have earned Agbor the status of oil producing city within the Niger Delta region of Nigeria. Agbor has the blessing of abundant natural water bodies. The people of Agbor are known for commerce and agricultural activities. Agbor hosts amenities such as University of Delta, Delta College of Nursing, 181 Army Amphibious battalion, General Hospital, Area Command of Nigeria Police Force, and the adorable Dein Royal Palace. The Dein of Agbor is the traditional ruler of Agbor Kingdom with over seventy communities (Olobanyi *et al*., 2004).

A drive around the borehole locations was undertaken to enable proper capture of the accurate borehole points using the handheld Global Positioning System. The coordinates of the borehole locations are as follows: Oghonim 5"678° N latitude, 8"706° E longitude; Aziken 5"687° N latitude, 8"699° E longitude; Aliagwu 5"719° N latitude, 8"697° E longitude; Dein 5"820° N latitude, 8"523° E longitude; Golden Cocktail 5"687° N latitude, 8"700° E longitude; Police Area Command 5"683° N latitude, 8"700° E longitude; Ewuru 5"687° N latitude, 8"699° E longitude; Jim Ovia 5"659° N latitude, 8"709° E longitude; Omumu 5"638° N latitude, 8"725° E longitude; Uvbe Oza 5"654° N latitude, 8"760° E longitude; Idumukwu 5"679° N latitude, 8"739° E longitude; General Hospital 5"704° N latitude, 8"694° E longitude; Lucky Irabor 5"708° N latitude, 8"691° E longitude; Jodes 5"788° N latitude, 8"709° E longitude; Agholor 5"719° N latitude, 8"698° E longitude. The coordinate positions were acquired and inputted on the Google map. On-screen vectorization of features like towns, roads and boundaries were directly observed and recorded. The features from the Google map were zoomed to a very high resolution where all the features became very clear as represented in the samples map presented in this work. The mapping was carried out in collaboration with Geo-trust Project Services Ltd, a surveying firm in Agbor.

2.2 Research Center

The physicochemical analysis was done at Projects Development Institute (PRODA) Enugu, bacteriological analyses were done in Applied Microbiology and Brewing Laboratory Nnamdi Azikiwe University, Awka, while molecular characterization was done at International Institute of Tropical Agriculture, Ibadan Headquarters, Nigeria.

2.3 Survey of Sanitary Conditions within the Sampling Area

The questions were adopted from Sharon *et al.* (2008) and structured in accordance with objectives of this research. Vital information about the boreholes were noted during a walk across the locations of the boreholes before sampling.

Proximity of the boreholes to septic tanks, height of well slab/apron, well depth, and concrete linings for the wells were obtained by oral interview and visual analysis.

2.4 Sample Collection

All the sampled points were selected randomly within Agbor, Delta State. Sixty (60) samples were aseptically collected from 15 different boreholes between June and July, 2022 (rainy season); January and February, 2023 (dry season) (Alfred *et al*. 2023; 2025). The selected borehole waters were those used for drinking and for other domestic purposes. These water samples were collected in the morning period (7am – 9am) (Uba *et al.*, 2026a; 2026b; 2026c). During the rainy season, fifteen samples were collected in June, analyzed and recorded, this was repeated in July and average of the values was taken, making it a total of thirty samples (Orji and Oghonim, 2023; Oghonim *et al.* 2023; 2026). This was also done in the dry season period to give a total of sixty samples for both seasons. Samples for the bacteriological analysis were aseptically collected in sterilized one-litre plastic container (which was sterilized by rinsing with 70 % ethanol and then with sterile water and thereafter with the respective water sample, three times before collection). The water was left to rush for 2 min (This allows the nozzle of the tap to be flushed and any stagnant water in the service pipe to be discharged). A roll of cotton-wool soaked in ethanol and ignited to decontaminate the faucet of the borehole before collection. The collected samples were kept at 4 °C in the ice box packed with ice and transported to the laboratory for analysis within 2 hrs as described by Okafor *et al.*, (2023), Ele *et al*. (2025), Uba aand Okonkwo *et al*. (2025) and Okwonkwo *et al.* (2026). These boreholes were: Oghonim, Aziken, Agholor, Aliagwu, Dein, Golden Cocktail, Police, Ewuru, Jim Ovia, Omumu, Uvbe Ozanogo, Idumukwu, Central Hospital, Lucky Irabor.

2.5 Bacteriological Analysis

Bacterial isolation was carried out as described by Ibo *et al*. (2020); Uba and Udaba (2026) and Dokubo and Uba (2026). The borehole water samples were homogenized by shaking them for 25 times, beside a Bunsen burner. The total viable count (total plate count) was determined using the pour plate technique, cultured in triplicates. One (1) millilitre of the samples was aseptically transferred into the Petri plates. The plates were labelled before inoculation and the culture medium was Nutrient Agar. The medium was prepared according to the manufacturer's instruction and sterilized by autoclaving at 121 ^{0}C for 15 min at 15 psi and then allowed to cool to 45 ^{0}C before dispensing 20 mL into sterile Petri-dishes and allowed to solidify, inverted to prevent condensation droppings from the lid into the agar and incubated in the incubator at 37 ^{0}C for 24 hrs. A control was equally prepared without adding the sample. The bacterial colonies ranging from 30 to 300 were counted and expressed in logarithm of colony forming unit per mL (CFU/mL). The growth colonies were counted and sub-cultured on a freshly prepared nutrient agar to obtain pure cultures. The pure cultures were subcultured in slant and preserved in a refrigerator (^{0}C) for characterization and identification (Idu *et al*., 2026a; 2026b; Ibe *et al*. 2023, Chukwura *et al*. 2025).

2.6 Characterization and identification of the bacterial isolates

2.6.1 Cultural characteristics

The cultural characteristics of the respective isolates were examined and recorded (Uba and Chukwura, 2016; Okafor *et al*. 2021a; 2021b; Ofunwa *et al*. 2024).

2.6.2 Gram-staining and microscopic examination

This was done according to the procedure described by Cheesbrough (2010).

2.6.3 Biochemical tests

The following biochemical tests such as catalase, coagulase, citrate utilization, oxidase, urease, indole, motility, Voges-proskauer, methyl red and sugar fermentation were carried out as described by Cheesbrough (2010), Uba *et al*. (2018a), (2018b), Okeke *et al*. (2025a) and (2025b).

2.6.4 Molecular characterization

Molecular characterization such as DNA extraction, agarose gel electrophoresis, polymerase chain reaction, amplification conditions for the PCR, gene sequencing, blasting and phylogenetic tree construction were carried out on the seven selected bacterial strains as described by Uba *et al*. (2018c); (2019a); (2019d) and (2019e) .

2.7 Antibiotics Susceptibility Assay

The method of NCCLS using Kirby-Bauer disc diffusion technique was employed in this study as follows:

2.7.1 Preparation of 0.5 McFarland Standard and Standardization of Inoculum

This is a barium sulphate standard against which the turbidity of the test isolates can be compared. Then, 1 % w/v solution of barium chloride solution was prepared by dissolving 0.5 g of dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 mL of sterile water. Thereafter, 1 % v/v solution of sulphuric acid solution (H_2SO_4) was prepared by adding 1 mL of concentrated sulphuric acid into 99 mL of sterile water, mixed by swirling. MacFarland standard was prepared by adding 0.6 mL of 1 % w/v solution of barium chloride solution to 99.4 mL of 1 % v/v solution of sulphuric acid solution to obtain a turbidity standard, which is equivalent to 1.5×10^8 cells per mL. A small portion (3 mL) of the resultant turbid solution was transferred into sterile test-tube and plugged with cotton wool.

2.7.2 Antibiotic assay

Using a sterile wire loop, 3 well-isolated colonies of each of the test organisms was emulsified in 3 mL of normal saline, which was also plugged with cotton wool. With the aid of a sheet of paper, and in a good light, the turbidity of the suspension was matched with the turbidity of the standard, and each of the standardized inocula kept for use but not later than 15 min. If the suspension is not turbid enough, more bacteria can be added but if too turbid, it can be diluted with more normal saline which is the diluent. Muller-Hinton agar was prepared according to manufacturer's instruction. It was allowed to cool to about 45 ºC before dispensing into the labelled Petri-plates and allowed to gel. The standardized inocula were seeded onto the gelled medium using swab sticks. The commercially prepared antibiotic discs (Levofloxacin (10µg), Ofloxacin (30µg), Ciprofloxacin (10µg), Pefloxacin (10µg)) were placed atop the medium using sterile forceps and incubated at 37 ^{0}C for 24 hrs. Zones of inhibition were measured using meter rule. Interpretation of the zone sizes was done by referring to the standard tables according to NCCLS guidelines and for consideration whether the organism is susceptible, intermediate or resistant to that particular antibiotic as described by NCCLS (2002).

2.8 Data Analysis

Data analysis was done using a two-way analysis of variance (ANOVA) to determine the disparity in the bacteriological parameter during both seasons. Values less than 0.05 were considered significant at 95 % confidence intervals (Uba *et al.*, 2020h; Afulukwe *et al.,* 2025; 2026).

3. Results and Discussion

The results presented in Table 1 showed the logarithmic total bacterial counts (Log CFU/mL) of borehole water samples across different locations during the rainy and dry seasons. Overall, the data indicated that bacterial counts were consistently higher during the rainy season compared to the dry season in all sampled locations. This seasonal variation suggests that rainfall likely contributes to increased microbial contamination, possibly due to surface runoff, leaching of contaminants, and infiltration of polluted water into boreholes. Among the locations, Omumu (3.92 log CFU/mL) recorded the highest bacterial load during the rainy season, followed closely by Golden Cocktail (3.88 log CFU/mL) and Jodes (3.86 log CFU/mL). In contrast, Oghonim (3.65 log CFU/mL) and Central Hospital (3.67 log CFU/mL) showed relatively lower counts, indicating better microbial quality in these areas. During the dry season, a general decline in bacterial counts was observed across all sites. However, some locations such as Jim Ovia (3.81 log CFU/mL) and Omumu (3.83 log CFU/mL) still maintained relatively high counts, suggesting persistent contamination sources independent of seasonal effects. The variation between locations may be attributed to factors such as borehole construction and maintenance practices, proximity to contamination sources (e.g., septic tanks, refuse dumps) and hydrogeological characteristics of the area. Although the WHO (2006) standard was not explicitly provided in the table, the observed bacterial counts suggest potential microbial contamination, as potable water is expected to have minimal or no detectable bacterial load, particularly for indicator organisms. This result agreed with the findings of Nwachukwu *et al*. (2013) that there are high counts of bacteria pathogens in most borehole water in some parts of Nigeria.

Table 1: Logarithm count of total bacterial population of borehole water samples for rainy and dry season

Borehole location	LogCFU/mL	
	Rainy season	Dry season
Oghonim	3.65	3.61
Aziken	3.78	3.74
Agholor	3.69	3.63
Aliagwu	3.74	3.70
Dein	3.77	3.69
Golden Cocktail	3.88	3.77
Police	3.79	3.76
Ewuru	3.77	3.67
Jim Ovia	3.85	3.81
Omumu	3.92	3.83
Uvbe Ozanogogo	3.81	3.68
Idumukwu	3.80	3.69
Central Hospital	3.67	3.64
Lucky Irabor	3.83	3.67
Jodes	3.86	3.54
WHO (2006)	-	-

The result of the bacteriological characteristics showed that Gram negative bacteria were dominant in the studied borehole water samples. The bacterial identification revealed the presence of seven isolates: *Escherichia coli*, *Klebsiella aerogenes*, *Salmonella enterica*, *Proteus mirabilis* strain LYRY45, *Proteus mirabilis* stain AMJ230, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, respectively (Table 2). The BLASTn analysis of the 16S rRNA gene sequences revealed that all isolates showed high similarity (97.88–99.86 %) with known bacterial species in the NCBI database (Table 3). Isolate A exhibited 99.86 % similarity with *Escherichia coli*, while isolate B showed 99.21 % similarity with *Klebsiella aerogenes*. Similarly, isolates D and E were closely related to *Proteus mirabilis*, with percentage identities above 98 %. The *Pseudomonas* isolates F and G also demonstrated high homology with *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, respectively. These findings confirm the taxonomic identities of the isolates and suggest a high degree of genetic relatedness with previously characterized strains. Oghonim (2023) reported the isolation of these bacterial strains from selected borehole water samples in Agbor, Delta State, Nigeria.

Table 2: Morphological and biochemical characteristics of the selected bacterial strains from borehole water samples

Strain code	Colony morphology	Gram reactions	Microscopy	Motility	Catalase	Citrate	Oxidase	Coagulase
A	Green metallic sheen on EMB	-	Straight rods	Motile	+	+	-	-
B	Mucoid pink in MA	+	Rods	Non-motile	+	+	-	-
C	Translucent smooth black small round colonies on SSA	-	Rods	Motile	+	+	-	-
D	Colourless mucoid colonies	-	Rods	Motile	+	+	-	-
E	Colourless mucoid colonies	-	Rods	Motile	+	+	-	-
F	Circular lemon green on CA	-	Rods	Motile	+	+	+	-
G	Circular lemon green on CA	-	Rods	Motile	+	+	+	-

Key: + = Positive; - = Negative EMB = Eosine methylene blue agar; CA = Cetrimide agar; SSA = Salmonella-Shigella agar; MA = MacConkey agar

Table 2 Continued

Isolates	Indole	Urease	Methyl red	Voges Proskauer	H_2S	Sucrose	Glucose	Maltose	Lactose	Identity
A	+	-	+	-	-	A	A/G	A/G	A/G	*Escherichia coli*
B	-	+	-	+	-	A/G	A/G	A/G	A/G	*Klebsiella aerogenes*
C	-	-	+	-	+	-	A	A	-	*Salmonella enterica*
D	-	+	+	-	+	-	A/G	-	-	*Proteus mirabilis* strain LYRY45
E	-	+	+	-	+	-	A/G	-	-	*Proteus mirabilis* strain AMJ230
F	-	-	-	-	-	-	-	-	-	*Pseudomonas aeruginosa*
G	-	+	-	-	+	-	-	-	-	*Pseudomonas fluorescens*

Key: A= Acid, A/G = Acid and Gas; - = No acid and gas 72

Table 3: Blasting profile of the selected bacterial strains based on 16S rRNA gene analysis

Isolate Code	Identified Organism	Closest NCBI Match	Accession Number	Query Coverage (%)	Percent Identity (%)	E-value	Bit Score
A	*Escherichia coli* strain Zina17	*Escherichia coli* strain K-12	NR_024570.1	100	99.86	0.0	2450
B	*Klebsiella aerogenes* strain D72_SO3R	*Klebsiella aerogenes* strain KCTC 2190	NR_118568.1	99	99.21	0.0	2380
C	*Salmonella enterica* strain 2011K-1440	*Salmonella enterica* subsp. enterica	NR_121761.1	100	99.74	0.0	2435
D	*Proteus mirabilis* strain LYRY45	*Proteus mirabilis* strain ATCC 29906	NR_114479.1	98	98.65	0.0	2290
E	*Proteus mirabilis* strain AMJ230	*Proteus mirabilis* strain ATCC 29906	NR_114479.1	99	98.92	0.0	2315
F	*Pseudomonas aeruginosa* strain ARa	*Pseudomonas aeruginosa* strain DSM 50071	NR_117678.1	100	99.63	0.0	2410
G	*Pseudomonas fluorescens* strain 3	*Pseudomonas fluorescens* strain IAM 12022	NR_114042.1	97	97.88	0.0	2205

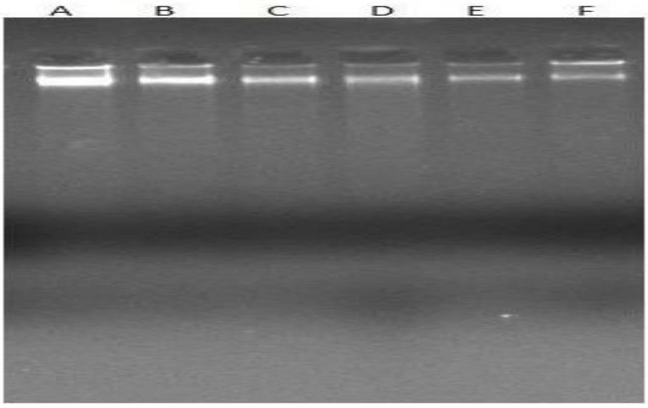

Figure 1: Electrophoregram of the DNA extracted from the bacterial strains

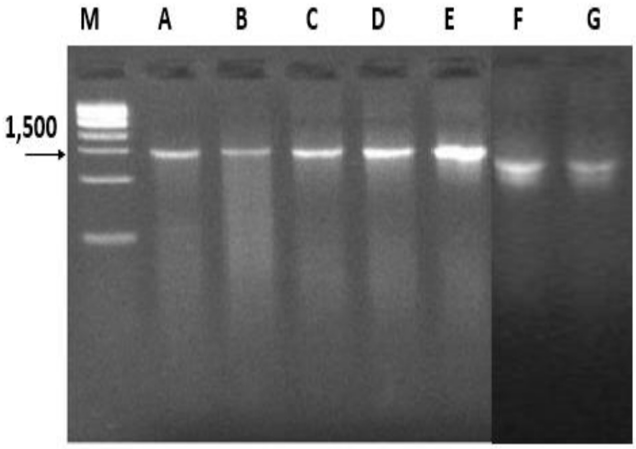

Figure 2: Electrophoregram of the 16SrRNA gene at about 1,500 bp
Key: M is a 1 Kbp DNA ladder

Phylogenetic analysis was carried out in order to determine the likely ecological origin of the bacterial strains from the different sampling site. The phylogenetic relationships among the bacterial isolates were inferred based on 16S rRNA gene sequences, using the Neighbor-Joining (NJ) method with bootstrap analysis (1,000 replicates) as shown in Figure 3. The resulting dendrogram revealed clear clustering patterns consistent with BLASTn identification. All isolates grouped into distinct clades corresponding to their respective genera, indicating strong evolutionary relatedness with reference strains obtained from the NCBI database. Isolate A (Zina17) clustered tightly with reference strains of *Escherichia coli*, forming a well-supported clade with high bootstrap values (> 95 %), confirming its taxonomic placement within the genus *Escherichia*. Similarly, isolate B (D72_SO3R) grouped within the *Klebsiella aerogenes* cluster, showing close evolutionary proximity to type strains, which supports its identification. The isolate C (2011K-1440) formed a distinct branch within the *Salmonella enterica* lineage, clustering closely with other *Salmonella enterica* subspecies. The high bootstrap support observed in this cluster suggests strong confidence in the evolutionary relationship and species-level identification. Both isolates D (LYRY45) and E (AMJ230) were positioned within the same clade as *Proteus mirabilis* reference strains. Their close clustering indicates minimal genetic divergence, suggesting that they may share a recent common ancestor or belong to closely related strains of the same species. The *Pseudomonas* isolates displayed genus-specific clustering patterns. Isolate F (ARa) grouped strongly with *Pseudomonas aeruginosa*, while isolate G (strain 3) clustered with *Pseudomonas fluorescens*. Although both belong to the same genus, they formed separate sub-clades, reflecting species-level divergence within the genus *Pseudomonas*. The phylogenetic tree or clustering pattern observed in the dendrogram corroborates BLAST results, strengthening identification accuracy. The low genetic distances suggest high sequence conservation among isolates and reference strains as described by Gupta *et al*. (2018) and Oghonim and Onuorah (2025).

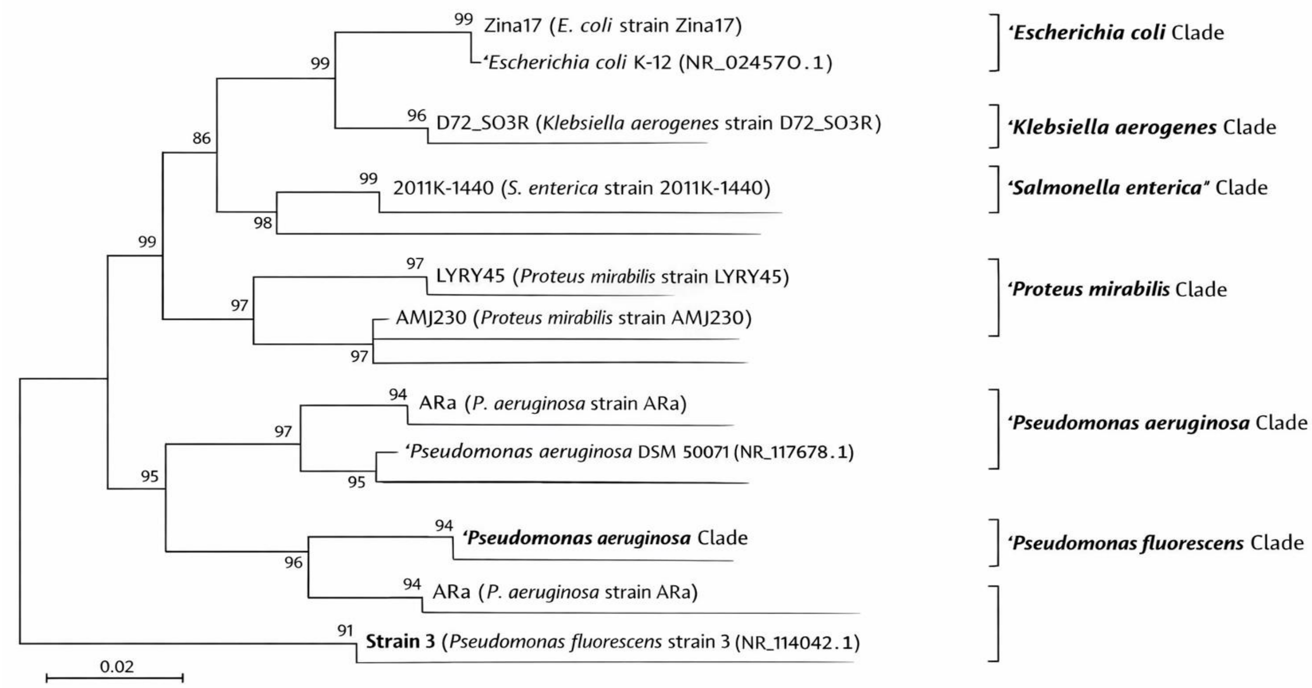

Figure 3: Phylogenetic relationship of the selected bacterial strains

Bacteria isolates were screened for their antimicrobial susceptibility pattern. All the isolated bacteria from the various borehole water samples showed varying levels of susceptibility to the five tested antibacterial agents (Levofloxacin (10 µg), ciprofloxacin (10 µg), perfloxacin (10 µg) and ofloxacin (10 µg)) (Table 4). According to NCCLS (2002) standard, the isolates showed varying degrees of sensitivity to the antibacterial agents. Interestingly, ciprofloxacin recorded the highest zones of inhibition, while ofloxacin had the least zones of inhibition. These findings were similar to that of Onuorah *et al.* (2018) who stated that 87.5 %, 75 % and 62.5 % of the bacterial isolates from water samples were sensitive to ciprofloxacin (10 µg), perfloxacin (10 µg) and ofloxacin (10 µg), respectively. The variation in susceptibility of the isolates to different antibiotics could be attributed to the difference in location of the sample sources and level of drug resistance transfer among the bacteria within the communities where the boreholes are located as reported by several researchers (Iheukwumere *et al*., 2012a; 2012b; Mundi *et al*., 2013; 2014; Okoye *et al*. 2014; Okoye *et al*., 2016a; 2016b; Anameze *et al*., 2023; Ezeamama *et al*., 2025a; 2025b; Umezulora *et al.*, 2026). The results of the antibiotic susceptibility test did not vary significantly ($p < 0.0863$) at 0.05 alpha level of significance against the isolates using two-way analysis of variance (ANOVA).

Table 4: Antibiotics susceptibility of the selected bacterial strains

Antibiotics (µg)	A	B	C	D	E	F	G
Levofloxacin (10)	25	22	21	23	19	20	24
Ofloxacin (30)	19	21	20	24	22	18	23
Ciprofloxacin (10)	28	30	24	25	20	22	26
Perfloxacin (10)	23	21	19	21	25	28	24

Source: NCCLS (2002) performance standards for antimicrobial susceptibility testing; **Key**<16: Resistance, 16-20: Intermediate, >20: Sensitive 74

4. Conclusion

This study demonstrated that borehole water sources harbor diverse bacterial populations, including potentially pathogenic species such as *Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. The observed bacterial counts, which were slightly higher during the rainy season, suggest that seasonal factors play a significant role in groundwater contamination. Molecular identification using 16S rRNA gene sequencing and BLAST analysis confirmed the taxonomic identity of the isolates with high accuracy, while phylogenetic analysis revealed strong evolutionary relationships with known reference strains. These findings validate the reliability of molecular tools in environmental microbiology studies. Antibiotic susceptibility testing indicated that most isolates were sensitive to fluoroquinolone antibiotics, particularly ciprofloxacin, although some intermediate resistance patterns were observed. This highlights the need for continuous surveillance of antimicrobial resistance in environmental bacterial populations. Overall, the presence of clinically relevant bacteria in borehole water underscores potential health risks associated with its consumption without adequate treatment. Regular monitoring, improved sanitation practices, and implementation of appropriate water treatment methods are strongly recommended to ensure water safety and protect public health.

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