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Antibiotics Resistance Pattern and Molecular Detection of ESBL Genes in *E. coli* from both Surface and Underground Water used for Domestic Purposes in Selected Locations in Ibadan, Oyo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Water quality and human health have been strongly related to each other as water acts as a medium for the transmission of antibiotic-resistant microorganisms particularly Extended Spectrum Beta-lactamase (ESBL)-producing Escherichia coli. This study aims at assessing physicochemical parameters, antibiotic susceptibility patterns, and molecular detection of the ESBL gene in isolated *E. coli* from surface and underground water sources in three selected local government areas in Oyo State, Nigeria. Water samples were collected in some selected three local governments and analyzed for physicochemical properties, isolation and characterization of *E. coli*, antibiotic susceptibility patterns, phenotypic expression of ESBLs *E. coli,* and molecular detection of the ESBL gene using the standard method. The physicochemical analysis results showed that hardness mean value ranged from 29.467±0.233 to 2.133±0.318, acidity has the highest mean value of 7.533±0.120, alkalinity mean value range from 31.333±0.186 to 6.167±0.176, conductivity has the highest mean value of 1.774±0.002, total suspended solids (TSS) mean value range from 141.427±0.015 to 0.821±0.003, dissolved oxygen (DO) has the highest mean value of 5.840±0.089, pH mean value range from 6.460±0.54 to 3.963±0.133, and temperature has the highest mean value of 27.200±0.153. Antibiotic susceptibility pattern results reveal that all 12 (100%) ESBL *E. coli* strains exhibited resistance to nalidixic acid, ampiclox, cefotaxime, ceftriaxone, cefuroxime, and augmentin, while cefexime (91.67%), cefepime (83.33%), gentamicin (75.0%), imipenem (75.0%), levofloxacin (66.67%), ofloxacin (58.33%), and nitrofurantoin (58.33%) had the above resistance rates. Molecular analysis results revealed the presence of ESBL genes in all tested *E. coli* isolates, and the percentages of occurrence were blaNDM (83.33%), blaTEM (75%), blaCTX-M (66.67%), and blaOXA (50%). blaIMP and blaSHV. (16.67%), while blaVIM and blaKPC were not detected. The findings of this study revealed that there is a need for improved water quality monitoring and public health interventions to mitigate the risks associated with antibiotic-resistant bacteria in water sources.

Keywords: Extended-spectrum beta-lactamase; escherichia coli; cephalosporins.

1. INTRODUCTION

Water is an essential resource for human activities, and its development has taken various forms throughout history. However, with the increase in population and various types of pollution, the quality of water available for consumption has been declining [1]. As a result, humans have been seeking ways to capture and store clean water and also redirect freshwater resources in order to reduce their vulnerability to irregular river flow and unpredictable rainfall [1].

In its natural state, water contains suspended impurities, including microorganisms, as well as dissolved impurities. Additionally, human activities can introduce other pollutants into water sources. Throughout history, the relationship between water and human health has been closely intertwined [2]. Waterborne diseases, caused by pathogens, have been a major cause of human illness and death. Water serves as a medium for the transfer of these organisms to humans, highlighting the strong association between water quality and human health [3]. While water provides essential elements, pollution can render it a hazardous substance detrimental to human health [4]. The discharge of untreated or minimally treated wastewater can lead to disease outbreaks. During rainfall, microorganisms can be washed into lakes, rivers, streams, or groundwater, potentially causing waterborne diseases if consumed without treatment [3].

In developing countries such as Nigeria, the primary water-related issues faced by humanity are the insufficient quantity and poor quality of water. Due to the uneven distribution of water, it is often not available in the desired amount and quality. This problem is prevalent in most towns, cities, and rural areas, leading to an increase in waterborne diseases such as cholera, dysentery, and typhoid fever [4]. These diseases are caused

by the contamination of water through indirect or direct contact with animal or human excreta, which contain harmful microorganisms. The consumption or use of such contaminated water, especially for cooking, can result in infections or the acquisition of resistance genes from the microorganisms present in animal excreta [5].

Antibiotic resistance, particularly against third-generation cephalosporins and carbapenems, poses a significant threat to the global healthcare system. The primary mechanism of resistance that undermines the effectiveness of expanded-spectrum cephalosporins in the Enterobacteriaceae family is the production of plasmid-mediated enzymes known as extended spectrum β-lactamases (ESBLs) [6]. These enzymes deactivate the aforementioned antibiotic compounds by disrupting their β-lactamase rings. Escherichia coli *(E. coli*), a prominent member of the Enterobacteriaceae family, has been greatly affected by the emergence of ESBLs [7]. Additionally, certain strains of *E. coli* that disseminate high levels of β-lactamase due to gene mutations are considered a significant global concern [7-10]. This study aims to assess the physicochemical properties, investigate antibiotic resistance patterns, and detect ESBL genes in *E. coli* from both surface and groundwater used for domestic purposes in selected locations in Ibadan, Oyo State.

2. MATERIALS AND METHODS

2.1 Study Area Site

This water analysis was performed at the microbiology laboratory of the Department of Biological Science, Lead City University, Ibadan, Oyo State. The study encompassed three selected local government areas in Ibadan, Oyo State: Ibadan South East (Mapo), Ibadan North West (Onireke), and Oluyole Local Government.

2.2 Sample Size Determination

A total of 30 water samples were randomly collected from both boreholes and streams in the aforementioned three local government areas in Ibadan, Oyo State, for subsequent analysis.

2.3 Collection and Storage of Samples

Duplicate water samples (300 ml each) were collected in sterile bottles. The samples were kept chilled in a cooler during transport to the laboratory, where they were analyzed physicochemically and microbiologically.

2.4 Sample Preparation

2.4.1 Physicochemical analysis of the water sample

The water samples were analyzed for seven parameters: pH, conductivity, dissolved oxygen (DO), temperature, total dissolved solids (TDS), hardness, and total suspended solids (TSS), following the standard procedures recommended in the water quality monitoring guidelines by Maushkar (2007).

2.4.1.1 pH

The pH of the water samples was determined using a pH meter (model HI 98130 HANNA, Mauritius, IramacSdn. Bhd.) in accordance with the protocols established by the American Public Health Association (APHA, 2005) and the American Society for Testing and Materials (ASTM). The pH meter was calibrated with pH 4.0, 7.0, and 10.0 standard solutions before measuring the pH of each water sample.

2.4.1.2 Conductivity and turbidity

Conductivity was assessed using a conductivity meter (model HI 98130 HANNA, Mauritius, IramacSdn. Bhd.) calibrated with a standard solution. The probe was immersed in the water sample, and readings were taken. Turbidity measurements were taken using a turbidity meter (model 2100P Turbidimeter HACH, Colombia, USA, Arachem (M) Sdn. Bhd.). Samples were allowed to settle, and readings were recorded once the stability indicator disappeared.

2.4.1.3 Total suspended solid and total dissolved solid

The measurement of Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) involved filtration and gravimetric methods following standard protocols.

2.4.1.4 Total hardness and dissolved oxygen

Total hardness was determined using a titration method with an EDTA solution. The dissolved oxygen content was measured using a specific glass bottle and titrated with sodium thiosulfate.

2.5 Bacteriological Analysis of the Water Samples

Bacteriological analysis was conducted following the preparation of MacConkey Agar (MCCA), Nutrient Agar (NA), and Eosin Methylene Blue Agar (EMBA) under sterilized conditions. To ensure sterility, the membrane filtration machine components were autoclaved. Water samples were passed through a specialized membrane to capture microorganisms. The filters were then transferred to selective media and incubated. For sub-culturing, nutrient agar was prepared, and colonies from the EMB plate were streaked on it and incubated. Presumptive *E. coli* isolates displaying specific characteristics on EMB agar were subjected to Gram staining and various biochemical tests—citrate utilization, indole production, motility, and triple sugar ion tests—to determine their identity and characteristics.

2.6 Identification of ESBL *E. coli* Isolates Phenotypically

ESBL-producing *E. coli* isolates were identified using the double disk synergy test (DDST) with specific antibiotic disks.

2.7 Antibiotic Resistance Profile

The antibiotic resistance profile of phenotypically confirmed ESBL *E. coli* isolates was determined using the disc diffusion method, testing their susceptibility to 13 different antibiotics.
A standardized inoculum was prepared; direct broth suspension was made from a discrete colony selected from an 18-24hour culture using sterile peptone water. The suspension was adjusted to match the 0.5 McFarland standard for the study. The dried surface of a Mueller-Hinton agar plate was then inoculated by pouring the standardized inoculum onto the plate and rotating it to ensure even distribution. Excess inoculum was removed by decanting it into a bowl containing disinfectant liquid. The surface of the inoculated plates was allowed to dry before applying the drug-impregnated disks

The antibiotic disks were aseptically dispensed onto the surface of the inoculated agar plate, and each disk was pressed down to ensure complete contact with the agar surface. The plates were then inverted and incubated at 35°C for 18-24hours, and the diameter of the zone of inhibition was measured and recorded. The results of the susceptibility testing were interpreted using the standard interpretative charts provided by (CLSI) 2016.

2.8 DNA Extraction of ESBL Gene in Isolated *E.coli*

Single colonies grown on medium were transferred to 1.5ml of liquid medium and cultures were grown on a shaker for 48 hours at 28ºC. Following this, cultures were centrifuged at 4600g for 5 minutes and the resulting pellets were resuspended in 520µl of TE buffer (10mM TrisHCl, 1mM EDTA, pH 8.0). Subsequently, 15µl of 20% SDS and 3µl of Proteinase K (20 mg/ml) were added and the mixture was incubated for 1 hour at 37ºC. Next, 100µl of 5M NaCl and 80µL of a 10% CTAB solution in 0.7M NaCl were added and the suspension was vortexed. The mixture was then incubated for 10 minutes at 65ºC and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 minutes and centrifugation at 7200g for 20 minutes. The aqueous phase was transferred to a new tube and isopropanol (1:0.6) was added to precipitate DNA at –20ºC for 16 hours. DNA was collected by centrifugation at 13000g for 10 minutes, washed with 500µl of 70% ethanol, air-dried at room temperature for approximately 3 hours and finally dissolved in 50µl of TE buffer.

2.9 Molecular Identification of β-lactamase Coding Genes in Selected *E. coli* Using Polymerase Chain Reaction (PCR)

PCR reactions were conducted using specific reagents and primer sets to identify β-lactamase-coding genes in selected *E. coli* isolates. The reaction cocktail utilized for all PCR reactions per primer set included the following reagent volumes in micro liters: 2.5 of 5X PCR SYBR green buffer, 0.75 of MgCl2, 0.25 of 10pM DNTP, 0.25 of 10pM of each forward and backward primer, and 0.06 of 8000U of taq DNA polymerase. The mixture was then made up to 10.5 with sterile distilled water, to which 2 µl of template was added. A buffer control was also included to eliminate any possibility of false amplification. The primer sequence and PCR profile used in amplifying each fragment are presented in the Table 1 below. PCR was conducted in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

Table 1. Primer Use for Detection of Extended Spectrum Beta-lactamase

Multiplex	Gene	Primer	Primer sequence 5'3'	Profile
Multiplex1	Bla VIM 502	VIM F	GGTGTTTGGTCGCATATCGCAA	An initial denaturing 5 min at 94ºC, then 35 cycles of 94ºC for 30 s, 50ºC for 40 s 72ºC for 40 s and terminate at 72ºC for 10 min
		VIM R	ATTCAGCCAGATCGGCATCGGC	
	blaNDM 624	NDMF	GGTTTGGCGATCTGGTTTTC	
		NDM R	CGGAATGGCTCATCACGATC	
Multiplex2	Bla IMP 568	IMP F	TCGTTTGAAGAAGTTAACG	An initial denaturing 5 min at 94ºC, then 35 cycles of 94ºC for 30s, 47ºC for 40 s and 72ºC for 30s. and terminate at 72ºC for 10 mins
		IMP R	ATGTAAGTTTCAAGAGTGATGC	
	BlaSHV319	SHVF	GCCTTGACCGCTGGGAAAC	
		SHVR	GGCGTATCCCGCAGATAAAT	
	bla OXA 190	OXA R	TTCTGTTGTTTGGGTTTCGC	
		OXA R	ACGCAGGAATTGAATTTGTTC	
Multiplex3	BlaTem 258	Tem F	GTCGCCGCATACACTATTCTCA	An initial denaturing 5 min at 94ºC, then 35 cycles of 94ºC for 30 s, 49ºC for 40s72ºC for 35 s and terminate at 72ºC for 10 min
		Tem R	CGCTCGTCGTTTGGTATGG	
	bla KPC 496	KPC F	CATTCAAGGGCTTTCTTGCTGC	
		KPCR	ACGACGGCATAGTCATTTGC	
singleplex	CTXM 593	CTXMF	ATGTGCAGYACCAGTAARGTKATGGC	An initial denaturing 5 min at 94ºC, then 35 cycles of 94ºC for 30 s, 60ºC for 40 s 72ºC for 35 s and terminate at 72ºC for 10 min

3. RESULTS

3.1 Results of Physicochemical Analysis

Tables 2-5 contains data regarding the physicochemical properties of water samples collected from various sources in different locations in Oyo State.

3.2 Antibiotic resistance patterns and phenotypic expression of ESBL of the isolated *E. coli*

Nineteen *E. coli* strains were isolated and identified, but only 13 of them tested positive for the phenotypic confirmation of Extended-Spectrum Beta-Lactamase (ESBL) using the double disc synergy test method, while the remaining 7 yielded negative results (refer to Table 7).

Further examination of the β-lactam family revealed that all *E. coli* strains producing ESBLs displayed resistance to penicillin derivatives, including Augmentin (30 µg) and Ampiclox (10 µg), as well as the first-generation fluoroquinolone Nalidixic Acid (30 µg; refer to Fig. 1). Additionally, they exhibited resistance to all third-generation Cephalosporins, except Cefexime (5 µg), which had a resistance rate of 91.67%. In contrast, the second generation of Fluoroquinolones and Nitrofurantoin (300 µg) showed the lowest resistance rates among ESBL *E. coli* isolates, ranging from 58.33% to 66.67%. Moreover, 75% of ESBL *E. coli* strains exhibited resistance to Imipenem (10 µg) and Gentamicin (10 µg) (refer to Fig. 2).

3.3 Molecular Identification of β-lactamase Coding Genes in Selected *E. coli*

ESBL gene analysis using multiplex PCR was conducted on *E. coli* isolates, employing agarose gel electrophoresis (Figs. 2-5). Specific bands at different sizes indicated the presence of ESBL genes. For instance, a 624bp band indicated the NDM gene, 502bp for VIM, 319bp for SHV, and 190bp for OXA. Similarly, 560bp represented the IMP gene, 256bp for TEM, 600bp for CTX-M and 496bp for KPC. Results showed that all *E. coli* isolates tested carried ESBL genes. The most common was blaNDM, detected in 83.33% of isolates, followed by blaTEM in 75%. Additionally, blaCTX-M and blaOXA were found in 66.67% and 50% of samples, while blaIMP and blaSHV each had a 16.67% prevalence. Notably, blaVIM and blaKPC were absent. In summary, all *E. coli* isolates were positive for ESBL genes, as shown in Fig. 6.

Table 2. Physicochemical characteristics of water samples collected from streams, wells and boreholes in different locations of local government a compared with who recommended limits

Parameters	Location of Water Sources								WHO
	LAB7	LAB10	LAS1	LAS5	LAS9	LAW2	LAW3	LAW8	
Hardness	7.467±0.133	8.000±0.000	5.200±0.173	10.933±0.318	8.467±0.296	4.133±0.133	6.100±0.100	8.767±0.088	100-250
Acidity	1.700±0.252	3.067±0.067	1.967±0.033	2.400±0.252	2.000±0.000	2.933±0.233	2.900±0.100a	3.200±0.493	-
Alkalinity	28.133±0.067	6.167±0.176	11.267±0.467	19.700±0.100	15.100±0.265	14.500±0.300	7.067±0.186	7.500±0.289	<200
E. C.	0.580±0.015	0.615±0.006	0.249±0.002	0.607±0.003	0.447±0.014	0.308±0.003	0.242±0.004	0.523±0.006	<1000
TDS	124.170±0.119	118.120±0.092	122.087±0.059	128.233±0.145	122.113±0.094	128.233±0.187	132.160±0.122	142.043±0.030	<500
TSS	0.821±0.003	0.871±0.030	0.906±0.059	0.953±0.082	0.851±0.016	0.892±0.059	0.911±0.046	0.895±0.047	<500
DO	4.130±0.091	5.127±0.096	4.230±0.038	5.063±0.272	4.253±0.136	2.930±0.036	3.250±0.032	5.840±0.089	5.0–7.0
pH	5.843±0.085	5.657±0.127	5.590±0.238	5.397±0.150	5.610±0.220	5.823±0.114	5.533±0.176	5.123±0.098	6.5 – 8.5
TEMP	24.500±0.289	24.500±0.289	24.500±0.289	24.500±0.289	24.500±0.289	24.500±0.289	24.500±0.289	24.500±0.289	28 – 30

Data are represented mean ± standard error (SE).
LAW: Local Government 1 Well Water
LAS: Local Government 1 Stream Water
LAB: Local Government 1 Borehole Water
E.C. Electrical Conductivity
TDS: Total Dissolve Solids
TSS: Total Suspended Solids
TEMP: Temperature

Table 3. Physicochemical characteristics of water samples collected from streams, wells and boreholes in different locations of local government b compared with who recommended limits

Parameters	Location of Water Sources							WHO
	LBB1	LBB8	LBS2	LBS3	LBS5	LBW6	LBW7	
Hardness	5.333±1.866	18.100±0.611	2.200±0.058	2.133±0.318	10.000±0.577	15.767±0.623	15.300±0.351	100-250
Acidity	1.567±0.033	3.500±0.493	2.600±0.379	2.433±0.504	1.933±0.481	2.233±0.338	5.300±1.102	-
Alkalinity	9.300±0.058	9.600±0.058	14.967±0.177	15.167±0.033	21.100±0.058	29.300±0.058	17.467±0.033	<200
E. C.	0.184±0.000	1.170±0.047	0.261±0.016	0.355±0.016	0.555±0.021	0.912±0.012	1.551±0.011	<1000
TDS	127.253±0.127	132.300±0.150	116.393±0.197	122.387±0.193	128.047±0.023	124.140±0.570	141.327±0.163	<500
TSS	1.166±0.007	1.160±0.000	1.170±0.001	1.196±0.000	1.175±0.000	1.308±0.143	1.159±0.002	<500
DO	3.170±0.068	3.363±0.179	3.477±0.130	3.160±0.091	3.170±0.070	3.257±0.094	4.057±0.098	5.0–7.0
pH	5.367±0.176	5.580±0.180	5.397±0.154	5.397±0.154	5.523±0.161	5.647±0.120	5.837±0.109	6.5 – 8.5
TEMP	27.200±0.153	27.200±0.153	27.200±0.153	27.200±0.153	27.200±0.153	27.200±0.153	27.200±0.153	28 – 30

Data are represented mean ± SE.
LBW: Local Government 2 Well Water
LBS: Local Government 2 Stream Water
LBB: Local Government 2 Borehole Water
E.C. Electrical Conductivity
TDS: Total Dissolve Solids
TSS: Total Suspended Solids
TEMP: Temperature

Table 4. Physicochemical characteristics of water samples collected from streams, well and borehole in different locations of local government c compared with who recommended limits

Parameters	Location of Water Sources									WHO
	LCB1	LCB3	LCS2	LCW4	LCW5	LCW6	LCW7	LCW8	LCW9	
Hardness	14.900±0.058	9.700±0.0577	6.000±0.100	11.067±0.033	26.033±0.033	22.100±0.058	16.167±0.088	29.467±0.233	26.967±0.033	100-250
Acidity	6.800±0.252	4.120±0.340c	3.800±0.351	5.433±0.296	6.760±0.444	7.533±0.120	4.700±0.600	4.800±0.153	7.020±0.080	-
Alkalinity	25.467±0.120	17.533±0.260	9.367±0.176	13.900±0.200	21.300±0.058	21.000±0.231	14.167±0.524	8.967±0.233	31.333±0.186	<200
E. C.	0.477±0.011	0.363±0.016	0.202±0.0006	0.342±0.003	1.409±0.006	1.334±0.002	0.628±0.109	1.774±0.002	1.082±0.002	<1000
TDS	1.123±0.001	1.133±0.006	1.134±0.002	1.143±0.007	1.165±0.008	1.160±0.005	1.131±0.010	1.131±0.005	1.161±0.000	<500
TSS	127.383±0.009	132.557±0.009	141.427±0.015	123.697±0.009	121.643±0.015	121.613±0.030	128.133±0.009	116.640±0.044	123.750±0.032	<500
DO	4.633±0.176	4.087±0.127	3.567±0.203	3.440±0.197	3.933±0.035	3.990±0.106	3.567±0.203	3.860±0.131	3.587±0.221	5.0–7.0
pH	6.140±0.140	6.350±0.139	6.460±0.154	6.277±0.153	6.157±0.087	3.963±0.133	6.147±0.148	6.023±0.115	6.090±0.133	6.5–8.5
TEMP	26.633±0.273	26.633±0.273	26.633±0.273	26.633±0.273	26.633±0.273	26.633±0.273	26.633±0.273	26.633±0.273	26.633±0.273	28–30

Data are represented mean ± SE.
LCW: Local Government 3 Well Water
LCS: Local Government 3 Stream Water
LCB: Local Government 3 Borehole Water
E.C. Electrical Conductivity
TDS: Total Dissolve Solids
TSS: Total Suspended Solids
TEMP: Temperature

Table 5. Mean variations in water quality parameters by the locations of sampling

	LGA		LGB		LGC	
Parameters	Mean Square	**P Value**	Mean Square	**P Value**	Mean Square	**P Value**
Hardness (mg/L)	14.17**	<0.0001	135.63**	<0.0001	212.68**	<0.0001
Acidity	1.00**	0.002	4.75**	0.01	5.78**	<0.0001
Alkalinity (mg/L)	168.01**	<0.0001	144.62**	<0.0001	165.43**	<0.0001
E. C. (µS/cm)	0.08**	<0.0001	0.79**	<0.0001	0.96**	<0.0001
TDS (mg/L)	167.44**	<0.0001	187.66**	<0.0001	0.001**	<0.0001
TSS (mg/L)	0.01ns	0.098	0.01ns	0.478	158.53**	<0.0001
DO (mg/L)	2.84**	<0.0001	0.31**	<0.0001	3.85**	<0.0001
pH	0.16**	<0.0001	5.54**	<0.0001	1.73**	<0.0001

**indicates significant level at P ≤0.05*
*** indicates significant level at P ≤0.01*
P Value- Probability Levels
ns- Non-significant
E.C. Electrical Conductivity
TDS: Total Dissolve Solids
TSS: Total Suspended Solids
DO: Dissolved Oxygen
Local government A: LGA
Local government B: LGB
Local government C: LGC

Table 6. Rates of antimicrobial resistance among *E. coli* isolates

Classes of antibiotic	Name of antibiotic	ESBL (n = 12)			Non-ESBL (n = 7)		
		R	S	I	R	S	I
Cephalosporins (3rd Generation)	Cefotaxime 25 µg	12	0	0	2	3	2
	Cefexime 5 µg	11	0	1	2	3	2
	Ceftriaxone 45 µg	12	0	0	2	5	0
	Cefuroxime 30 µg	12	0	0	6	1	0
Cephalosporins (4th Generation)	Cefepime 30 µg	10	2	0	1	6	0
Penicillin derivatives	Ampiclox 10 µg	12	0	0	6	1	0
	Augmentin 30 µg	12	0	0	7	0	0
Fluoroquinolones (1st Generation)	Nalidixic Acid 30 µg	12	0	0	7	0	0
Fluoroquinolones (2nd Generation)	Levofloxacin 5 µg	8	4	0	4	2	1
	Ofloxacin 5 µg	7	5	0	4	3	0
Carbapenems	Imipenem 10 µg	9	2	1	6	0	1
Nitrofuran	Nitrofurantoin 300 µg	7	1	3	3	2	2
Amimoglycosides	Gentamicin 10 µg	9	3	0	4	3	0

S: SENSITIVE {Zone of inhibition ≥19mm}
I: INTERMEDIATE {Zone of inhibition 14-18mm}
R: RESISTANCE {Zone of inhibition <13}
ESBL: Extended Spectrum Beta Lactamase

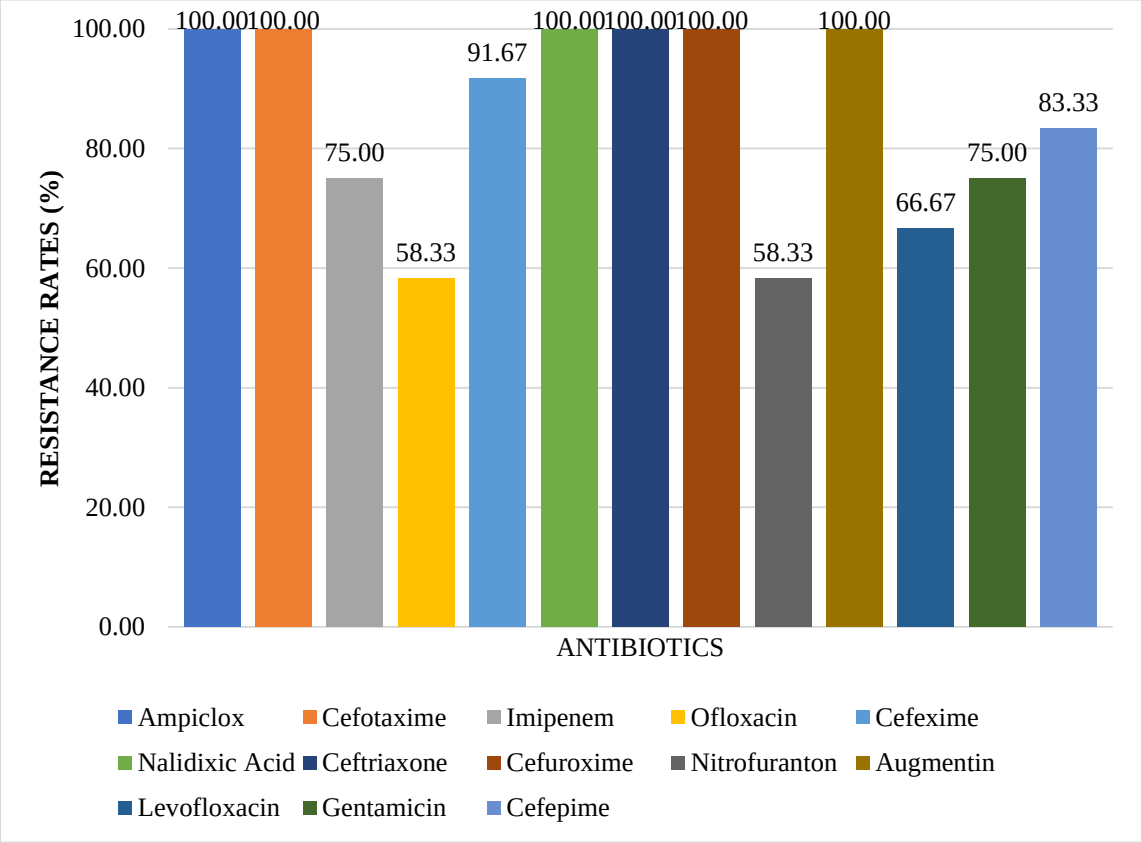

Fig. 1. Antibiotic resistance pattern of ESBL *E. coli* isolates against 13 different antibiotics

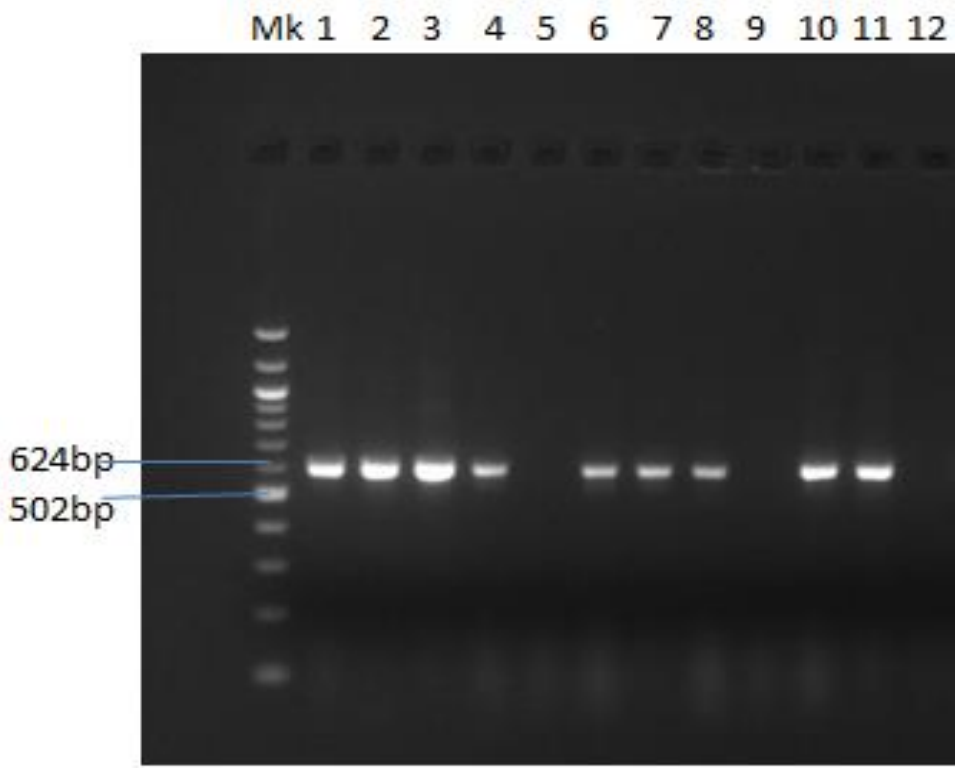

Fig. 2. Agarose gel electrophoresis of the Multiplex PCR products of ESBL gene NDM (624bp) and VIM (502bp) amplified from *E. coli* isolates

Key: Level 1-12 E. coli Isolates MK-Marker (Ladder)

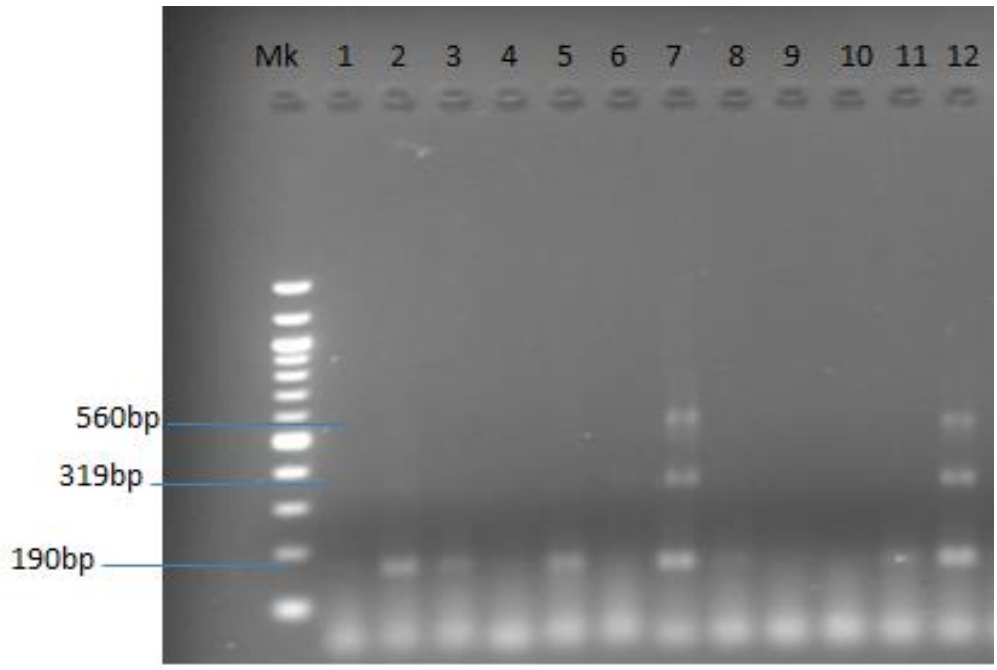

Fig. 3. Agarose gel electrophoresis of the Multiplex PCR products of ESBL gene SHV (319bp), OXA (190bp) and IMP (560bp) amplified from *E. coli* isolates

Key: Level 1-12 E. coli Isolates MK-Marker (Ladder)

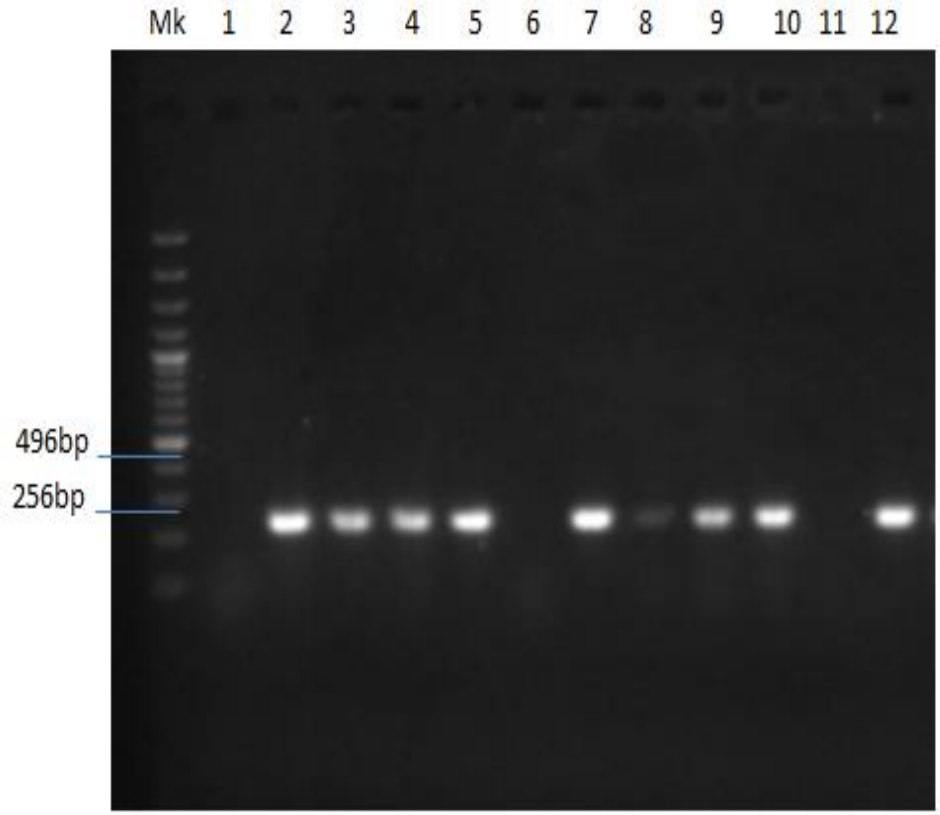

Fig. 4. Agarose gel electrophoresis of the multiplex PCR products of ESBL gene KPC (496bp) and TEM (256bp) amplified from *E. coli* isolates.
Key: Level 1-12 E. coli Isolates Mk-Marker (Ladder)

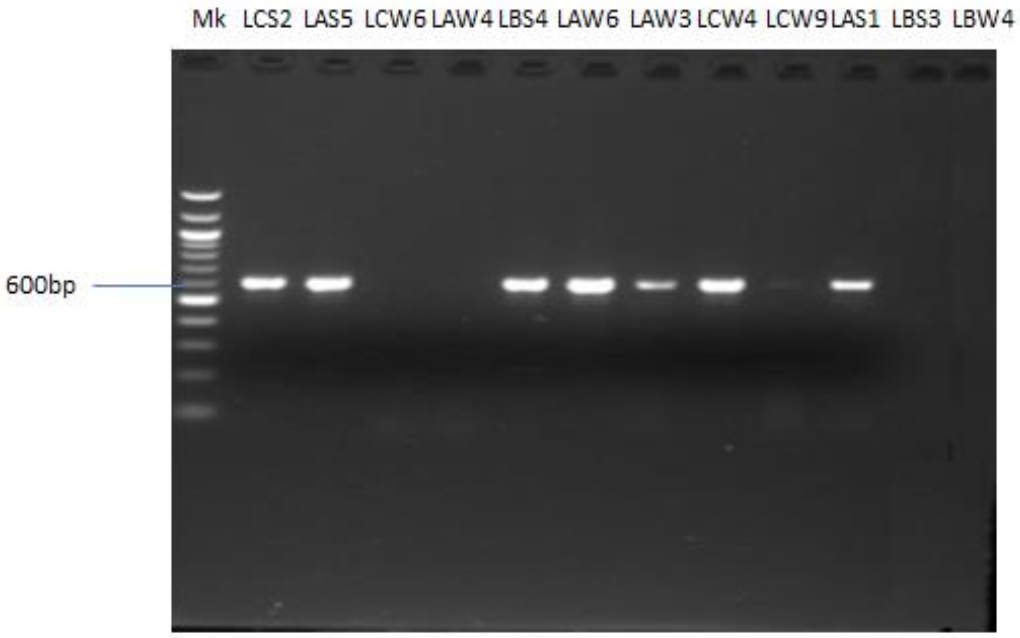

Fig. 5. Agarose gel electrophoresis of the PCR products of ESBL gene CTX-M (600bp) amplified from *E. coli* isolates
Mk-Marker (Ladder)

Table 7. Result summary of β-lactamase coding genes in selected *E. coli*

SAMPLE ID	VIM	NDM	IMP	KPC	SHV	TEM	CTX	OXA
1	-	+	-	-	-	-	+	-
2	-	+	-	-	-	+	+	+
3	-	+	-	-	-	+	-	+
4	-	+	-	-	-	+	-	-
5	-	-	-	-	-	-	+	+
6	-	+	-	-	-	+	+	-
7	-	+	+	-	+	+	+	+
8	-	+	-	-	-	+	+	-
9	-	-	-	-	-	+	+	-
10	-	+	-	-	-	+	+	-
11	-	+	-	-	-	-	-	+
12	-	+	+	-	+	+	-	+

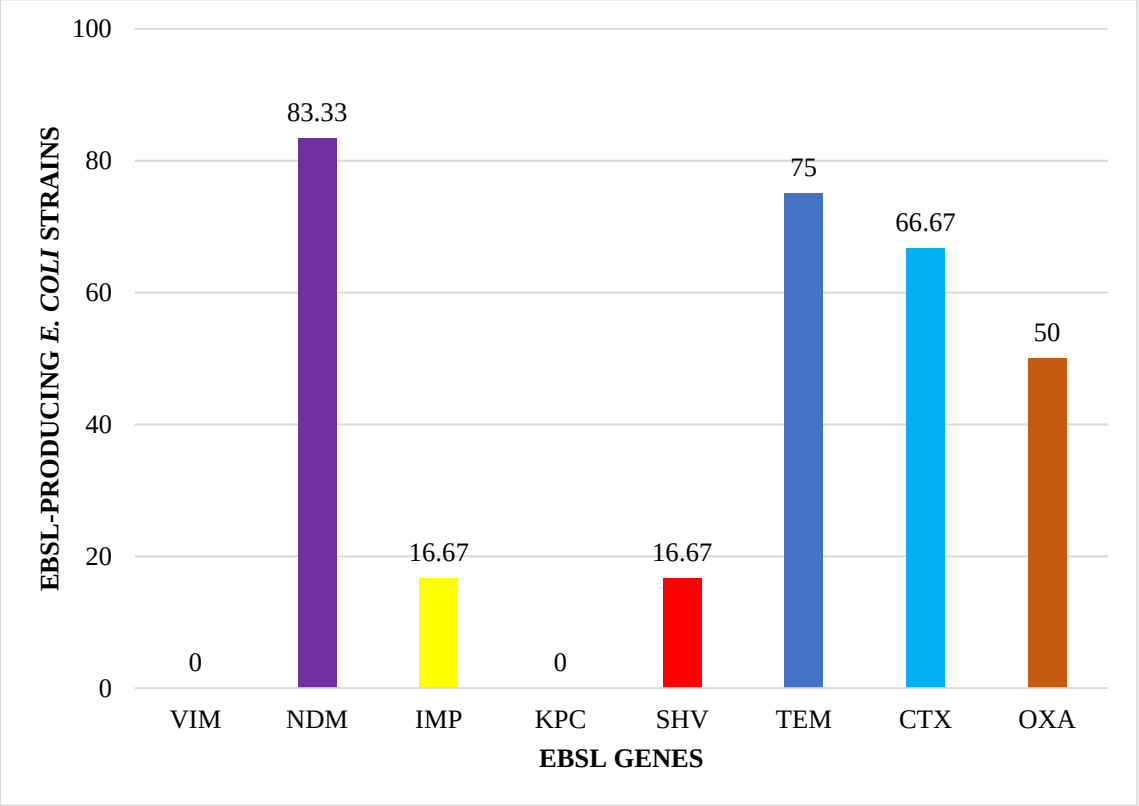

Fig. 6. Agarose gel electrophoresis of the PCR products of ESBL gene CTX-M amplified from *E. coli* isolates

4. DISCUSSION

The physicochemical properties of water are indicators of the safety of domestic water; dysregulation in some of these parameters can pose a health risk. The rise and dissemination of antibiotic resistance in bacterial pathogens, especially Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli*, present a notable worldwide health challenge. In the quest to protect public health and gain insights into the intricacies of antibiotic resistance, scientific investigations have delved into the resistance profiles and molecular identification of ESBL genes within *E. coli* [11].

Water hardness refers to the concentration of dissolved minerals, primarily calcium and magnesium ions, in water [12]. The mean hardness values for all the water sampled in

different locations fall within the acceptable limit for water meant for domestic use of the World Health Organization (WHO) for hardness of 100–250 mg/L. The result of this study is in line with the findings of previous research that reported an average total hardness of 132.5 ± 47.41 mg/L in water samples from locations in Nigeria [13].

Water alkalinity is a measure of the capacity of water to resist changes in pH when acids are added to it, and it primarily reflects the presence of dissolved alkaline compounds, such as bicarbonates, carbonates, and hydroxides [14]. This study found that the average alkalinity levels in water samples taken from different locations were consistently low, falling within the acceptable limit of WHO standards for alkalinity of 200 mg/L. The result of this study is not in line with the findings of previous research that reported noticeably higher alkalinity readings ranging from 140 mg/L to 368 mg/L for a total of 736 water samples taken from three local governments (abakaliki, ebonyi, and ikwo) in ebonyi. of 276.50 mg/L [15]. Industrial activity and changes in weather patterns may be to blame for the lower alkalinity seen in the research region. Water with high alkalinity levels has the ability to corrode metal pipes, reducing the usable diameter of the pipes [12]. Drinking water with a high alkalinity content might lead to health problems such as digestive disorders like cramps, abdominal discomfort, and diarrhea [16].

Water electric conductivity, often referred to as the electrical conductivity (EC) of water, is a measure of the ability of water to conduct electrical current [17]. In natural water bodies, the phenomenon known as electrical conductivity (EC) is influenced by a number of variables, such as the presence of salts, their behavior, valence, total concentration, and temperature [18]. The conductivity values in all of the tested places in this investigation were found to be noticeably low, falling within the acceptable limit of the WHO standard for electrical conductivity of 1000 S/m (Table 4). The result of this study is in line with the findings of previous research that reported that water samples taken in Ebonyi State had an average conductivity value of 2.30 ± 0.40 [19].

Total Dissolved Solids (TDS) in water refers to the measurement of all inorganic and organic substances present in a liquid solution that have dissolved in it [15]. If the TDS level in a water sample is less than 500 mg/L, it is regarded as being of excellent quality. The water becomes unsafe to drink when the TDS level reaches around 1000 mg/L or above [20]. The average TDS value across all water samples in this research is less than 500 mg/L, which falls within the acceptable limit of the WHO's standard for TDS (Table 4). The result of this study is in line with the findings of previous research in Adamawa State, Nigeria, which were lower than those from the present study but fell within the acceptable limit of the WHO standard for two river sources [21]. High TDS concentrations may change the composition of water and increase salt, changing the clarity, color, and taste of the water, and this may indicate that the water contains potentially dangerous minerals and microbes [20].

Total Suspended Solids (TSS) in water refers to the measurement of solid particles and materials that are suspended in water, and it typically includes various fine particles such as silt, clay, organic matter, and other contaminants that are not dissolved but remain suspended in the water [22]. The mean TSS levels found in all water samples from every research location fall within the acceptable limit of the WHO standard for TSS at 500 mg/L.

Dissolved oxygen (DO) refers to the amount of oxygen present in water [18]. The level of dissolved oxygen in aquatic habitats is a key indicator of how much organic waste is being broken down by aerobic and anaerobic organisms [23]. Only 3 out of the 24 research locations (12.5%) had mean values of dissolved oxygen (DO) within acceptable limits of WHO standards for (DO) 5.0–7.0 mg/L (Table 4). The result of this study is in line with the findings of previous research that reported a greater concentration of dissolved oxygen at 9.08 mg/L [24] and 6.67 mg/L [22].

The pH of water is a measure of its acidity, or alkalinity. It quantifies the concentration of hydrogen ions (H+) in the water, which determines whether the water is acidic (pH less than 7), neutral (pH 7), or alkaline (pH greater than 7) [25]. The pH readings in all research locations fall below the acceptable limit of the WHO standard—pH 6.5–8.5. Plant decomposition, industrial pollution, or acid rain are all possible reasons for the acidic pH values seen in all the water sources [25].

Water temperature refers to the measurement of the warmth or coldness of water, typically expressed in degrees Celsius (°C) or Fahrenheit (°F) [26]. A key physicochemical element often used to determine whether water is fit for human consumption is temperature [26]. All sampling locations had temperatures fall within the

acceptable limit of WHO standards (20°C–30°C) for domestic water use. The result of this study is in line with the findings of previous research that was carried out in Ebonyi State and Adamawa State [19,22]. Increased temperatures over the WHO-acceptable limits may have negative impacts, including preventing oxygen dissolution, speeding up chemical processes, and generating thermal pollution, without necessarily indicating the presence of contaminants [27].

The results of the biochemical tests have provided valuable insights into the composition of the bacterial isolates, including 19 *E. coli* isolates from 30 water samples. The result of this study is in line with the findings of previous research that reported a significant presence of *E. coli* in water samples. Their explanation for this phenomenon was grounded in the higher occurrence of thermotolerant (fecal) coliform in temperate environments, contrasting with the infrequent occurrence of *E. coli* [28].

The resistance patterns within the β-lactam family indicated a consistent trend. All *E. coli* strains producing ESBLs exhibited resistance to penicillin derivatives such as augumentin and ampiclox, as well as the first-generation fluoroquinolone, nalidixic acid, highlighting the widespread resistance to these antibiotics within the sample population. The result of this study is in line with the findings of previous research that reported that 68.2% of 110 *E. coli* isolates showed fluoroquinolone resistance [29]. Notably, penicillin-based antibiotics, such as augumentin (which contains amoxicillin) and ampiclox (which contains ampicilin), form a crucial part of contemporary medicine. However, the enzymatic activity of ESBLs severely reduces their efficacy against the *E. coli* that produces ESBLs [30]. Due to this hydrolysis, these antibiotics are no longer effective against ESBL-producing *E. coli*. Interestingly, the second generation of fluoroquinolones and nitrofurantoin showed relatively lower antibiotic resistance pattern rates among the ESBL *E. coli* isolates, ranging from 58.33% to 66.67%. The result of this study is in line with findings from previous research that reported increased *E. coli* resistance to ampicillin and piperacillin, as well as decreased resistance to meropenem, amikacin, and nitrofurantoin [31]. Moreover, the finding that a high percentage of ESBL *E. coli* strains were resistant to imipenem 75.0% and gentamicin 75.0% suggests limitations in the effectiveness of these antibiotics against ESBL-producing *E. coli*. The result of this study is not in line with the findings of previous research that reported a low rate of resistance to imipenem for *E. coli* [31]. Furthermore, the third generation of cephalosporins, e.g., cefotaxime at 100%, ceftriaxone at 100%, and cefuroxime at 100%, cefexime at 91.67%, and cefepime at 83.33%, exhibited substantially high antibiotic resistance pattern rates against ESBL-producing *E. coli* strains, emphasizing the challenges in using these antibiotics to treat infections caused by these strains.

The findings from this study provide valuable insights into the prevalence and distribution of ESBL genes in *E. coli* isolates. It is evident from these results that ESBL genes are widespread among the *E. coli* isolates under investigation, with all isolates testing positive for at least one ESBL gene. Remarkably, this study did not detect the presence of blaVIM and blaKPC genes in any of the ESBL *E. coli* isolates studied. The result of this study is not in line with the findings of previous research that reported blaKPC-2 (26.67%) and blaVIM-1 (25%%) genes in *E. coli* isolates [32]. The most predominant ESBL-encoding gene identified in the study is blaNDM, which was present in 10 out of 12 isolates (83.33%). This is in line with previous research that has also reported a high prevalence of blaNDM in *E. coli* strains, indicating its significance as a major contributor to ESBL production [33]. Additionally, our study revealed a substantial presence of the blaTEM, blaCTX-M, and blaOXAgenes in 9 out of 12 isolates (75%), 66.67% (8 out of 12), and 50% (6 out of 12) of the ESBL *E. coli* isolates, respectively. The result of this study is in line with findings from previous research that have highlighted the widespread distribution of blaTEM and blaCTX-M in ESBL-producing *E. coli* [33,34]. This occurs because plasmids bearing blaCTX-M genes are known to also include additional genes that confer resistance to a variety of antibiotics. Furthermore, the co-selection that may occur when many resistance genes are present on a single replicon may contribute to the extensive dispersion [35]. In this study, it was observed that there are lower prevalence rates for blaIMP and blaSHV, with each gene detected in 16.67% of the isolates. While these genes are less common in the result of this study, they still contribute to the overall diversity of ESBL genes in *E. coli* isolates. The result of this study is not in line with the findings of previous research in Maiduguri that reported blaSHV (36.4%) as the predominant gene, followed by blaTEM (31.4%) and blaCTX-M (27.3%) [36]. All these studies confirmed that gene predominance varied between regions and locations and, to a large

extent, determined the resistance profiles of the organisms in the Oyo state. It was also noticed in this study that some of the *E. coli* isolates possessed multiple EBSL genes, as had been established in some earlier studies [36,37].

The high rate of ESBL-producing *E. coli* found in water samples from various locations in Oyo State could be attributed to several factors. One significant factor contributing to the presence of ESBL-producing *E. coli* in water sources is environmental contamination. Water bodies may become susceptible to contamination through various means, including the runoff from agricultural areas where antibiotics are extensively employed in livestock farming [38]. Additionally, due to the prevalence of ESBL *E. coli* in food-producing animals, specifically chickens and young cattle, it is possible that animal feces could play a part in the spread of these bacteria [39]. This research found a significant presence of ESBL *E. coli* in surface and ground water samples. The result of this study is in line with the findings of previous research conducted in Pakistan (57%) and India (64%) [40,41].

5. CONCLUSION

This study investigated antibiotic resistance patterns and the molecular detection of Escherichia coli (*E. coli*) that produce extended-spectrum beta-lactamase (ESBL) in residential water sources in specific regions of Oyo State, Nigeria. The majority of these water samples had low pH and dissolved oxygen (DO) values. Furthermore, the presence of ESBL-producing *E. coli* in these water samples underlines the potential health risks associated with the transmission of antibiotic-resistant organisms through domestic water usage. Analysis of the antimicrobial resistance profiles of ESBL-producing *E. coli* isolates revealed significant resistance to commonly used antibiotics. Molecular analysis unveiled the distribution of ESBL genes in the *E. coli* isolates, with blaNDM being the most prevalent.

Implementing water treatment measures and quality monitoring programs is critical to ensuring access to clean and safe drinking water. Urgent antibiotic stewardship initiatives, promoting responsible antibiotic use in healthcare and agriculture, are necessary to combat antibiotic resistance caused by environmental contamination. Regular monitoring of water sources for ESBL-producing *E. coli* and other antibiotic-resistant bacteria is essential for detecting emerging threats to public health and guiding targeted interventions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

This research work was in no way generated by an artificial intelligence language model or tool. It is the original work of the above named authors.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Aboh F, Giwa J, Giwa A. Microbiological assessment of well waters in Samaru, Zaria, Kaduna state, Nigeria. Ann Afr Med. 2015;14(1):32–38.
2. Abigail AO, Adediran KO, Adebambo MA, Abeo AF, Gambo ED, Ntasin PY, & Ibrahim AM. Bacteriological evaluation of surface and groundwater used for domestic purposes in Ibadan. Asian J Res Infect Dis. 2024;15(4):14-23.
3. Beshiru A, Okareh OT, Chigor VN, Igbinosa EO. Assessment of water quality of rivers that serve as water sources for drinking and domestic functions in rural and pre-urban communities in Edo North, Nigeria. Environ Monit Assess. 2018; 190(7).
4. Dahunsi SO, Owamah HI, Ayandiran TA, Oranusi SU. Drinking water quality and public health of selected towns in South Western Nigeria. Water Qual Exp Health. 2014;6(3):143–153.
5. Owamah HI. A comprehensive assessment of groundwater quality for drinking purpose in a Nigerian rural Niger delta community. Groundwater Sustain Dev. 2019;10(7).
6. Martinson JNV, Walk ST. Escherichia coli residency in the gut of healthy human adults. EcoSalPlus. 2020;9(1).
7. Hollinshead WD, Rodriguez S, Martin HG, Wang G, Baidoo EE, Sale KL. Pfk mutants. Biotechnol Biofuels. 2016;9(1):212.
8. Elutade OO, Emmanuel VT, Akinola OT. Occurrence and Antibiotic Profile of Some Enteric Bacteria in Retailed Sachet Water Sold in Oluponna, Osun State, Nigeria. J. Sci. Res. Rep. [Internet]. 2019 ;21(6):1-8.

Available:https://journaljsrr.com/index.php/JSRR/article/view/927

9. A-A ON, EAC. Antibiotics and Antifungal Resistance Patterns of Microbial Isolates from Dish Washing Sponges in the University of Port-Harcourt, Nigeria. AJOB [Internet]. 2023;19(1):45-52.
   Available:https://journalajob.com/index.php/AJOB/article/view/358
10. Tahri L, Hafiane FZ, Fekhaoui M. Prevalence and antibiotic resistance of the Escherichia coli in the groundwater (Tadla-Morocco). Groundwater for Sustainable Development. 2021 May 1;13:100572
11. Ahsan A, Rehman TAU, Irshad H, Shahzad MA, Siddique A, Jamil A, Ali A. Antibiotic resistance pattern and molecular detection of ESBL-associated genes in *E. coli* from surface and wastewater of Islamabad capital territory, Pakistan. J Water Health. 2022;20(4):601–609.
12. Akhtar N, Syakir Ishak MI, Bhawani SA, Umar K. Various natural and anthropogenic factors responsible for water quality degradation: a review. Water. 2021;13(19):2660.
13. Adejumo RO, Adagunodo TA, Bility H, Lukman AF, Isibor PO. Physicochemical constituents of groundwater and its quality in crystalline bedrock, Nigeria. Int J Civ Eng Sci Technol. 2018;9(8):887-903.
14. Moussavi SP, Kadier A, Singh R, Rostami R, Ghanbari F, Zaidi NS, Phalakornkule C, Asaithambi P, Aryanti PTP, Nugroho FA. Analyses of sustainable indicators of water resources for redesigning the health promoting water delivery networks: a case study in Sahneh, Iran. Case Stud Chem Environ Eng. 2023;7:100346.
15. Iroha IR, Ude IU, Okoronkwo C, Ovia K, Okafor COO, Akuma SO. Comparative assessment of physicochemical characteristics, metal levels and anion contents of water from different aquatic environments in Ebonyi state. Biomed J Sci & Tech Res. 2020;29(5):22834-22865.
16. Nwali BU, Okaka NC, Ogbanshi ME, & Idenyi JN. Physicochemical water analysis of Ikwo-Ihie River in Ivo Local Government Area and Ope-Ekwe River in Izzi Local Government Area in Ebonyi State, Nigeria. Adv Biol Res. 2016;10(2):82-85.
17. Moussavi SP, Kadier A, Singh R, Rostami R, Ghanbari F, Zaidi NS, Phalakornkule C, Asaithambi P, Aryanti PTP, & Nugroho FA. Analyses of sustainable indicators of water resources for redesigning the health promoting water delivery networks: A case study in Sahneh, Iran. Case Stud Chem Environ Eng. 2023;7:100346.
18. Bajgai J, Kim CS, Rahman H, Jeong ES, Jang HY, Kim KE, Choi J, Cho IY, Lee KJ, & Lee M. Effects of alkaline-reduced water on gastrointestinal diseases. Processes. 2022;10(1):87.
19. Adjovu GE, Stephen H, & Ahmad S. A machine learning approach for the estimation of total dissolved solids concentration in Lake Mead using electrical conductivity and temperature. Water. 2023;15(13):2439.
20. World Health Organization. Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda.
    Available:https://www.who.int/publications/i/item/9789240045064
    Accessed on 10/May/2024
21. Omer NH. Water quality parameters. Water Quality - Science, Assessments and Policy. 2019.
22. Tula M, Enabulele O, Ophori E, Aziegbemhin A. A comprehensive study on the physicochemical characteristics of two water sources from the Adamawa North senatorial zone, Nigeria. Public Health Toxicol. 2022;2(4):1–13.
23. Burmamu BR, Law PL, Madaki SY, & Aliyu A. Environmental burden of surface and groundwater quality: how big are the contaminants in Girei Town, Nigeria? Int J Eng Sci Res Technol. 2014;3(3):1-7.
24. Kolawole OM, Afolayan O. Assessment of groundwater quality in Ilorin, North Central Nigeria. Arid Zone J Eng Technol Environ. 2017;13(1):111-126.
25. Some S, Mondal R, Mitra D, Jain D, Verma D, & Das S. Microbial pollution of water with special reference to coliform bacteria and their nexus with environment. Energy Nexus. 2021;1:100008.
26. Reuben RC, Gyar SD, Aliyu Y. Physicochemical and microbiological parameters of water from rivers in Keffi, Central Nigeria. Microbiol Res J Int. 2018;24(3):1-12.
27. Shrestha AK, Basnet NB. The correlation and regression analysis of physicochemical parameters of river water

for the evaluation of percentage contribution to electrical conductivity. J Chem. 2018;8:6-9.

28. Ram A, Tiwari SK, Pandey HK, Chaurasia AK, Singh S, Singh YV. Groundwater quality assessment using water quality index (WQI) under GIS framework. Appl Water Sci. 2021;11(2).
29. Chotinantakul K, Chusri P, Okada S. Detection and characterization of ESBL-producing Escherichia coli and additional co-existence with mcr genes from river water in northern Thailand. PeerJ. 2022; 10:e14408.
30. Kakoullis L, Papachristodoulou E, Chra P, Panos G. Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. Antibiotics. 2021;10 (4):415.
31. Dembélé R, Konaté A, Traoré O, Kaboré WAD, Soulama I, Kagambèga A, Traoré AS, Guessennd NK, Aidara-Kane A, Gassama-Sow A, Barro N. Extended spectrum beta-lactamase and fluoroquinolone resistance genes among Escherichia coli and Salmonella isolates from children with diarrhea, Burkina Faso. BMC Pediatrics. 2020;20(1).
32. Iskandar K, Murugaiyan J, Hammoudi Halat D, Hage SE, Chibabhai V, Adukkadukkam S, Roques C, Molinier L, Salameh P, Van Dongen M. Antibiotic discovery and resistance: The chase and the race. Antibiotics. 2022;11(2): 182.
33. Zeng Q, Xiao S, Gu F, He W, Xie Q, Yu F, Han L. Antimicrobial resistance and molecular epidemiology of uropathogenic Escherichia coli isolated from female patients in Shanghai, China. Front Cell Infect Microbiol. 2021;11.
34. Ahmed SS, Shariq A, Alsalloom AA, Babikir IH, Alhomoud BN. and their antimicrobial resistance patterns: Relationship with urinary tract infections. Int J Health Sci. 2019;13:48–55.
35. Koshesh M, Mansouri S, Hashemizadeh Z, Kalantar-Neyestanaki D. Identifcation of extended-spectrum β-lactamase genes and AmpC-β -lactamase in clinical isolates of Escherichia coli recovered from patients with urinary tract infections in Kerman, Iran. Arch Pediatr Infect Dis. 2017;5: e37968.
36. Bilal H, Rehman TU, Khan MA, Hameed F, Jian ZG, Han J, Yang X. Molecular epidemiology of mcr-1, blaKPC-2, and blaNDM-1 harboring clinically isolated Escherichia coli from Pakistan. Infect Drug Resist. 2021;14:1467–1479.
37. Aklilu E, Harun A, Kaur K. Molecular characterization of blaNDM, blaOXA-48, mcr-1 and blaTEM-52 positive and concurrently carbapenem and colistin resistant and extended spectrum beta-lactamase producing Escherichia coli in chicken in Malaysia. BMC Vet Res. 2022;18(1).
38. Ma J, Wu S, Shekhar NVR, Biswas S, Sahu AK. Determination of physicochemical parameters and levels of heavy metals in food waste water with environmental effects. Bioinorg Chem Appl. 2020;1–9.
39. Chu EW, Karr JR. Environmental impact: concept, consequences, measurement. Reference Module in Life Sciences. B978-0-12. 2017;809633-8.02380-3.
40. Kithar R, Majeed A, Talib A, Jaayid, & Al-Khaeun SNA. Isolation and identification of some types bacteria from shrimp (Metapenaeus affinis) and detection of histamine producing from its Metapenaeus affinis. Basrah J Agric Sci. 2016;29(1):35-56.
41. Larson A, Hartinger SM, Riveros M, Salmon-Mulanovich G, Hattendorf J, Verastegui H, Huaylinos ML, Mäusezahl D. Antibiotic-resistant Escherichia coli in drinking water samples from rural Andean households in Cajamarca, Peru. Am J Trop Med Hyg. 2019;100(6):1363–1368.

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