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

Abstract

Water quality and human health have been strongly related to each other as water act as a medium for the transmission of antibiotic resistance microorganism particularly Extended Spectrum Beta-lactamase (ESBL) producing Escherichia coli. This study aim at assessing, physiochemical parameters, antibiotics susceptibility patterns and molecular detection of ESBLs gene in isolated *E. coli*from surface and underground water sources in some selected three local government areas in Oyo State, Nigeria. Water samples were collected in some selected three local government and were analysed for physiochemical properties, isolation and characterization of *E. coli*, antibiotics susceptibility pattern, phenotypic expression of ESBLs *E. coli* and molecular detection of ESBLs gene using standard method. The physiochemical analysis results shown that hardness mean value rangefrom 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. Antibiotics susceptibility pattern results reveal that all the 12 (100%) ESBL*E. coli*strain 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 result revealed that presence of ESBL genes in all tested *E. coli* isolates and the percentage of occurrence were blaNDM (83.33%),blaTEM (75%), blaCTX-M (66.67%),blaOXA (50%). blaIMP and blaSHV. (16.67%) while blaVIM and blaKPC were not detected. The finding of this study revealed that there is 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

Comment [VC1]: This study aims at assessing

Comment [VC2]: 'in three selected' - to avoid some repeatedly in the whole document

Comment [VC3]: The phrase "physiochemical properties" should be "physicochemical properties" throughout the document

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1.0 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, have 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]. 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.

2.0 Materials and Method

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 analysed and physiochemically andmicrobiologically.

2.4 Sample Preparation

2.4.1 Physiochemical 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.

2.4.1.3Total Suspended Solid and Total Dissolve Solid

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

2.4.1.4Total Hardness and Dissolved Oxygen

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

2.5 Bacteriological Analysis of the Water Samples

Bacteriological analysis was conducted following the preparation ofMacConkey 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 disk diffusion method, testing their susceptibility to 13 different antibiotics.

2.8 Detection of ESBL Encoding Gene Using Multiplex PCR

DNA was extracted from *E. coli* isolates, and multiplex PCR was employed to detect ESBL encoding genes.

2.9 Molecular Identification 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.

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	ATGTAAGTTTCAAGAGT	

			GATGC	
	BlaSHV319	SHVF	GCCTTGACCGCTGGGAAAC	
		SHVR	GGCGTATCCCGCAGATAAAT	
	bla OXA 190	OXAR	TTCTGTTGTTTGGGTTTCGC	
		OXAR	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

3.0 Results

3.1 Results of Physicochemical Analysis

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

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

Data are represented mean ± SE.

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

LAW: Local Government 1 Well Water
LAS: Local Government 1 Stream Water
LAB: Local Government 1 Borehole Water
E.C. Electric Conductivity
TDS: Total Dissolve Solid
TSS: Total Suspended Solid
TEMP: Temperature

Comment [VC8]: Solids

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±1.866d	18.100±0.611a	2.200±0.058e	2.133±0.318e	10.000±0.577c	15.767±0.623ab	15.300±0.351b	100-250
Acidity	1.567±0.033b	3.500±0.493b	2.600±0.379b	2.433±0.504b	1.933±0.481b	2.233±0.338b	5.300±1.102a	-
Alkalinity	9.300±0.058f	9.600±0.058g	14.967±0.177e	15.167±0.033d	21.100±0.058b	29.300±0.058a	17.467±0.033c	<200
E. C.	0.184±0.000g	1.170±0.047b	0.261±0.016f	0.355±0.016e	0.555±0.021d	0.912±0.012c	1.551±0.011a	<1000
TDS	127.253±0.127d	132.300±0.150b	116.393±0.197g	122.387±0.193f	128.047±0.023c	124.140±0.570e	141.327±0.163a	<500
TSS	1.166±0.007a	1.160±0.000a	1.170±0.001a	1.196±0.000a	1.175±0.000a	1.308±0.143a	1.159±0.002a	<500
DO	3.170±0.068c	3.363±0.179b	3.477±0.130b	3.160±0.091c	3.170±0.070c	3.257±0.094c	4.057±0.098a	5.0–7.0
pH	5.367±0.176c	5.580±0.180b	5.397±0.154c	5.397±0.154c	5.523±0.161b	5.647±0.120b	5.837±0.109a	6.5 – 8.5
TEMP	27.200±0.153a	27.200±0.153a	27.200±0.153a	27.200±0.153a	27.200±0.153a	27.200±0.153a	27.200±0.153a	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. Electric Conductivity
TDS: Total Dissolve Solid
TSS: Total Suspended Solid
TEMP: Temperature

Comment [VC9]: Electrical

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

LCW: Local Government 3 Well Water
LCS: Local Government 3 Stream Water
LCB: Local Government 3 Borehole Water
E.C. Electric Conductivity

TDS: Total Dissolve Solid
TSS: Total Suspended Solid
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 $P \leq 0.05$
** indicates significant level at $P \leq 0.01$
P Value- Probability Levels
ns- Non-significant
E.C. Electric Conductivity
TDS: Total Dissolve Solid
TSS: Total Suspended Solid
DO: Dissolved Oxygen
pH
Local government A: LGA
Local government B: LGB
Local government C: LGC

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 Figure 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 Figure 2).

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)	Cefotaxime25 µg	12	0	0	2	3	2
	Cefexime5 µg	11	0	1	2	3	2
	Ceftriaxone45 µg	12	0	0	2	5	0
	Cefuroxime30 µg	12	0	0	6	1	0
Cephalosporins (4th Generation)	Cefepime 30 µg	10	2	0	1	6	0
Penicillin derivatives	Ampiclox10 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)	Levofloxacin5 µg	8	4	0	4	2	1
	Ofloxacin 5 µg	7	5	0	4	3	0
Carbapenems	Imipenem10 µg	9	2	1	6	0	1
Nitrofuran	Nitrofuranton 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

Comment [VC10]: Nitrofurantoin

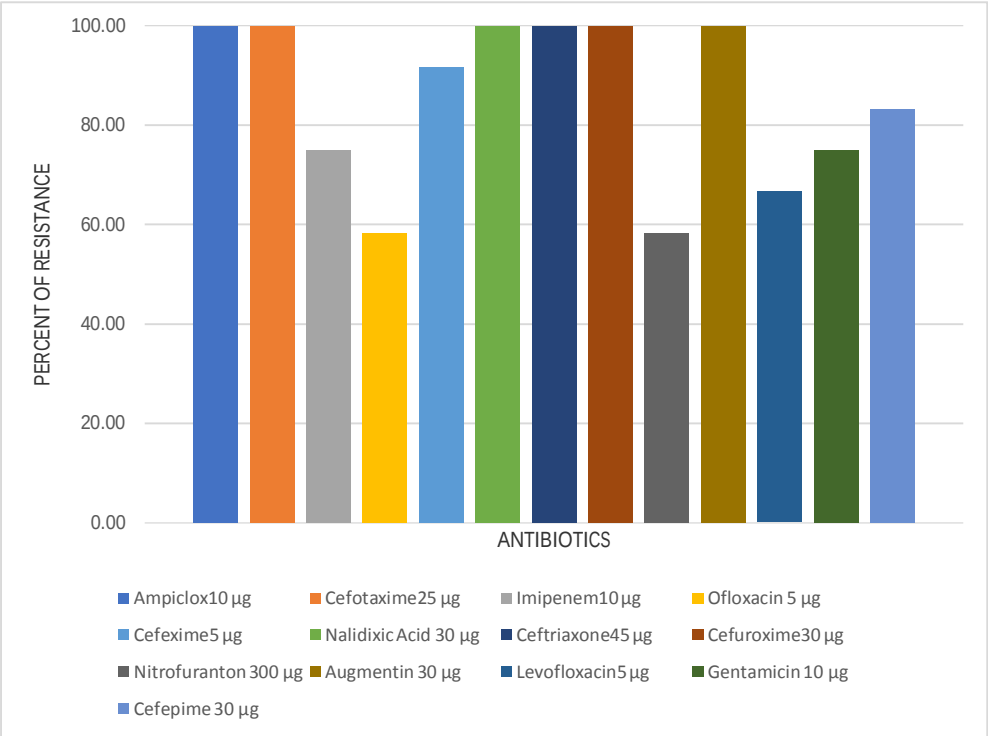

Figure 1: Antibiotic resistance pattern of ESBL *E. coli*isolates against 13 different antibiotics

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

ESBL gene analysis using Multiplex PCR was conducted on *E. coli* isolates, employing agarose gel electrophoresis (Plate 1-4). Specific bands at different sizes indicated the presence of ESBL genes. For instance, a 624 bp band indicated the NDM gene, 502 bp for VIM, 319 bp for SHV, and 190 bp for OXA. Similarly, 560 bp represented the IMP gene, 256 bp for TEM, and 496 bp for KPC. Results showed that all *E. coli* isolates tested carried ESBL genes. 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 Figure 2.

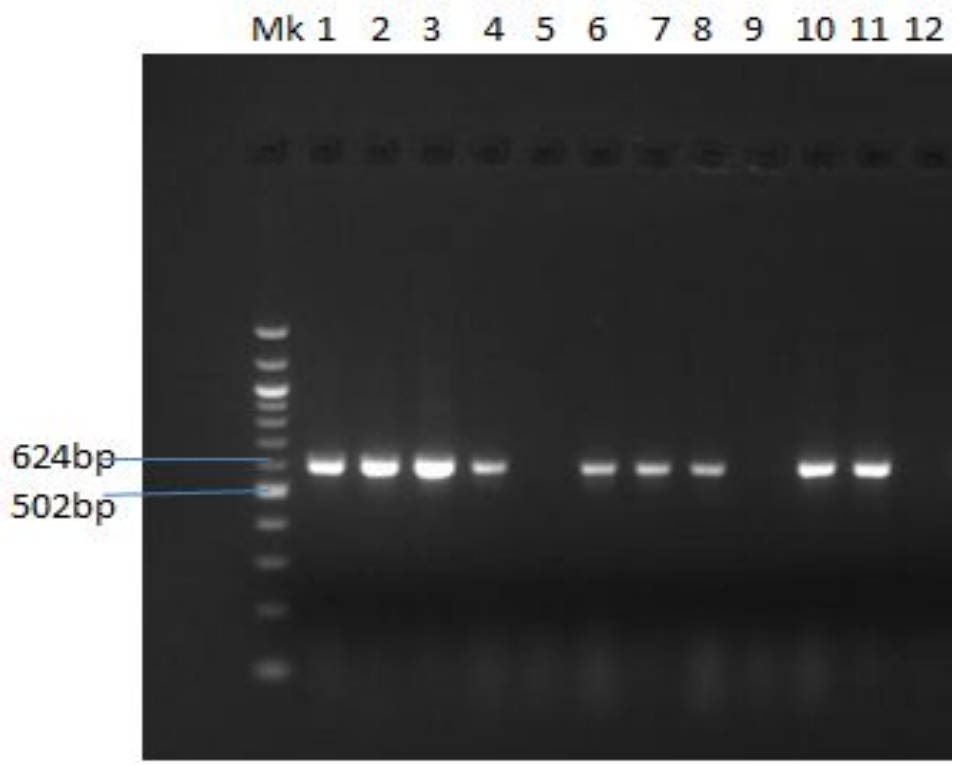

Plate 1: Mk: Marker, C Agarose gel electrophoresis of the Multiplex PCR products of ESBL gene NDM and VIM amplified from *E. coli*isolates

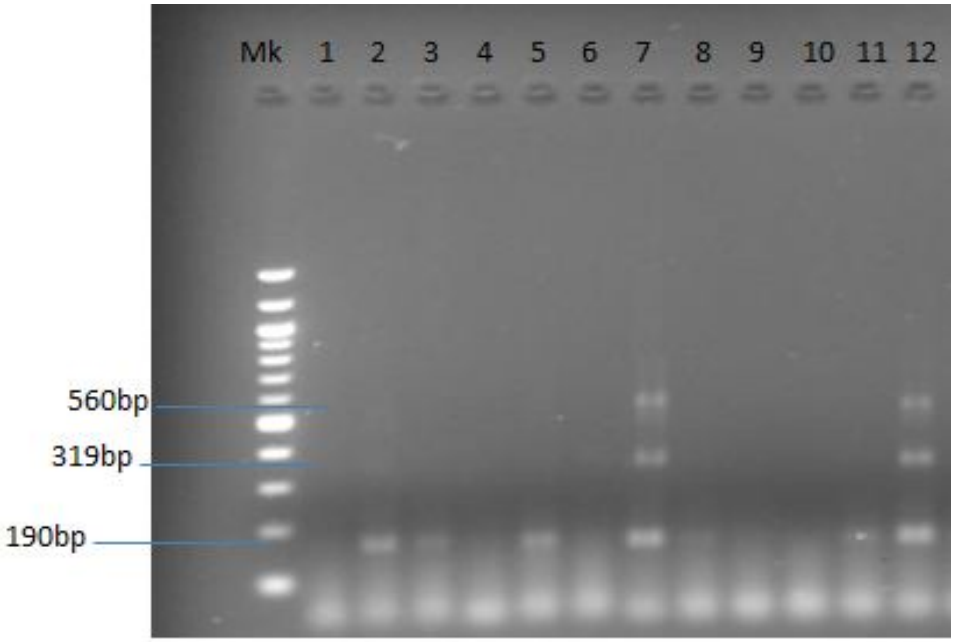

Plate 2: Mk: Marker, C Agarose gel electrophoresis of the Multiplex PCR products of ESBL gene SHV, OXA and IMP amplified from *E. coli*isolates.

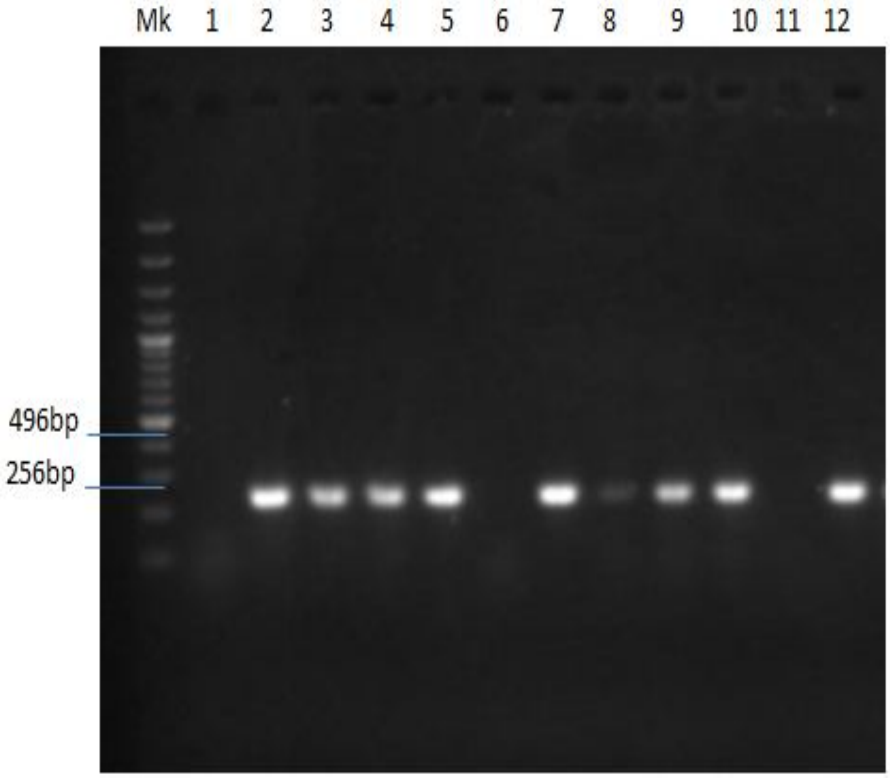

Plate 3: Mk: Marker, C Agarose gel electrophoresis of the Multiplex PCR products of ESBL gene KPC and TEM amplified from *E. coli*isolates.

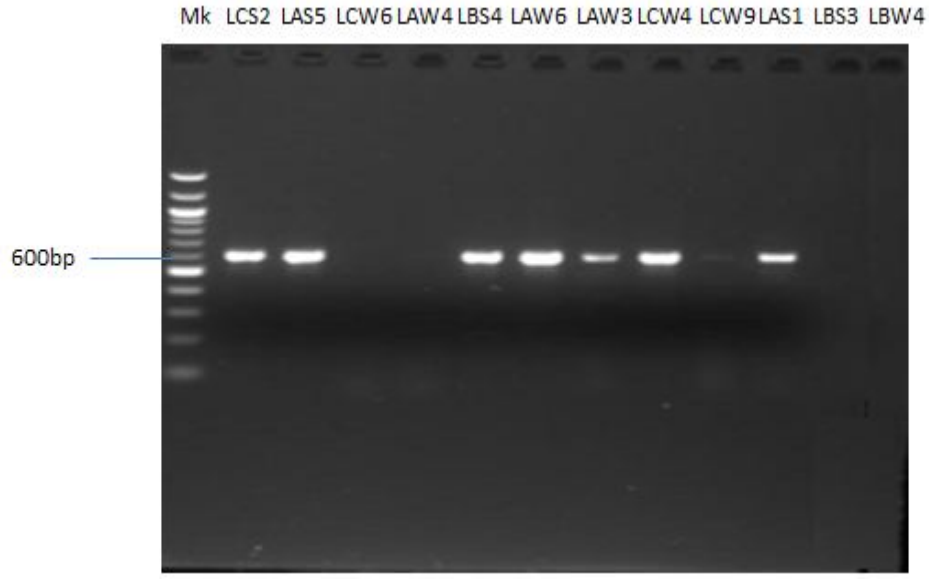

Plate 4: Mk: Marker, C Agarose gel electrophoresis of the PCR products of ESBL gene CTX-M amplified from *E. coli*isolates.

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	-	+	+	-	+	+	-	+

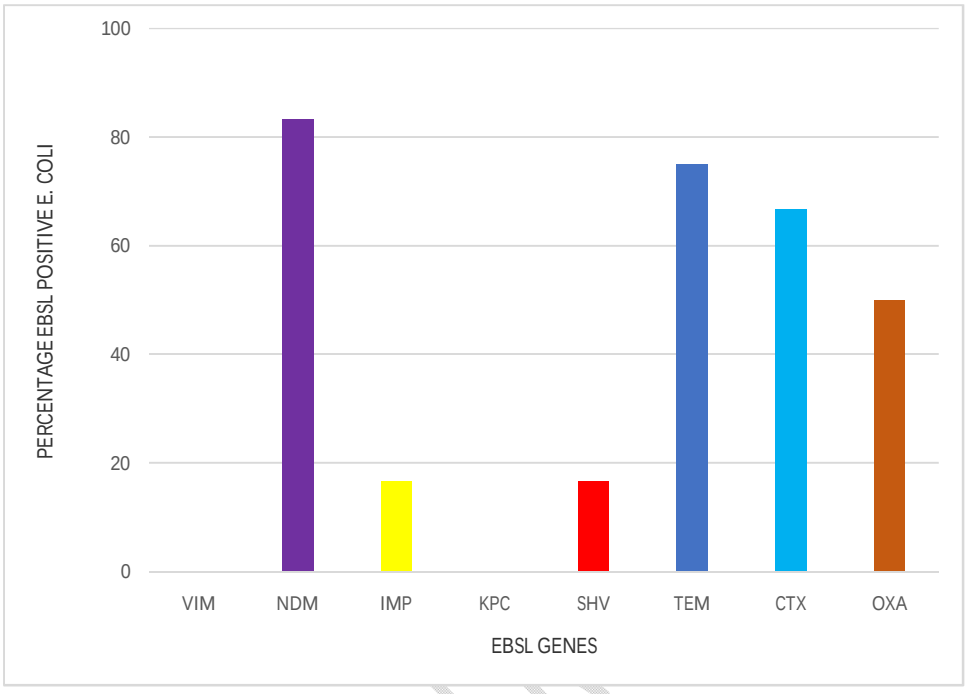

Figure 2: Mk: Marker, C Agarose gel electrophoresis of the PCR products of ESBL gene CTX-M amplified from *E. coli*isolates.

4.0 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*[8].

Water hardness refers to the concentration of dissolved minerals, primarily calcium and magnesium ions, in water [9].The mean hardness values for all the water sampled in different locations falls within acceptable limit for water meant for domestic useof the World Health Organization (WHO) for hardness 100-250 mg/L. The result of this study is in line with findings of previous research that reported an average total hardness of 132.5 ± 47.41 mg/L-1 in water samples from locations in Nigeria[10].

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[11].This study found that the average alkalinity levels in water samples taken from different locations were consistently low which fall within acceptable limit of WHO standards for alkalinity of 200 mg/L. The result of this study is not in line with findings of previous research that reported noticeably higher alkalinity readings ranging from 140mg/L to 368mg/L for a total of 736 water samples taken from three local governments (abakaliki, ebonyi and ikwo) in ebonyi. of 276.50 mg/L [12]. 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 useable diameter of the pipes[9]. Drinking water with high alkalinity content might lead to health problems such digestive disorders like cramps, abdominal discomfort, and diarrhoea[13].

Water electric conductivity, often referred to as electrical conductivity (EC) of water, is a measure of the ability of water to conduct electrical current[14]. In natural water bodies, the phenomena known as electrical conductivity (EC) is influenced by a number of variables, such as the presence of salts, their behaviour, valence, total concentration, and temperature[15]. The conductivity values in all of the tested places in this investigation were

found to be noticeably low which fall within the acceptable limit of WHO standard for electric conductivity 1000 S/m (Table 4). The result of this study is in line with findings of previous research that reported that water samples taken in Ebonyi State had an average conductivity value of 2.30 ± 0.40 [16].

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[12]. 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[17].The average TDS value across all water samples in this research is less than 500 mg/L, which fall within acceptable limit of World Health Organisation (WHO) standard for TDS (Table 4). The result of this study is in line with findings of previous research in Adamawa State, Nigeria, which were lower than those from the present study but fall within acceptable limit of WHO standard for two river sources[18]. High TDS concentrations may change the composition of water and increase salt, changing the clarity, colour, and taste of the water and this may indicate that the water contains potentially dangerous minerals and microbes[17].

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[19]. The mean TSS levels found in all water samples from every research location fall within acceptable limit of WHO standard for TSS 500 mg/L.

Dissolved Oxygen (DO) refers to the amount of oxygen present in water [15].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[20]. Only 3 out of the 24 research locations (12.5%) had mean values of dissolved oxygen (DO) within acceptable limit of WHO standards for (DO) 5.0-7.0 mg/L (Table 4). The result of this study is in line with findings of previous research that reported a greater concentration of dissolved oxygen at 9.08mg/L [21] and 6.67 mg/L[19].

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)[22]. The pH readings in all research locations fall below the acceptable limit of World Health Organisation (WHO)'s standards—

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[22].

Water temperature refers to the measurement of the warmth or coldness of water, typically expressed in degrees Celsius (°C) or degrees Fahrenheit (°F)[23]. A key physicochemical element often used to determine whether water is fit for human consumption is temperature[23]. All sampling locations had temperatures fall within acceptable limit of WHO standard(20^oC -30^oC) for domestic water use. The result of this study is in line with findings of previous research that was carried out in Ebonyi State, and Adamawa State[16,19]. 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[24].

The results of the biochemical tests have provided valuable insights into the composition of the bacterial isolates, with 19 *E. coli* isolates from 30 water samples. The result of this study is in line with 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 thermo tolerant (faecal) coliform in temperate environments, contrasting with the infrequent occurrence of *E. coli*[25].

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 findings of previous research that reported found that 68.2% of 110 *E. coli*isolates showed fluoroquinolones resistance[26]. 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 *E. coli*that produces ESBLs[27]. 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 antibiotics 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 of previous research that reported an increased *E. coli*resistance to ampicillin and piperacillin, as well as decreased

resistance to meropenem, amikacin, and nitrofurantoin[28]. Moreover, the finding that 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 findings of previous research that reported a low rate of resistance to imipenem for *E. coli*[28].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 antibiotics 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 this 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 bla_{VIM} and bla_{KPC} genes in any of the ESBL *E. coli*isolates studied. The result of this study is not in line with findings of previous research that reported bla_{KPC-2} (26.67%) and bla_{VIM-1}(25%) genes in *E. coli*isolates[29].The most predominant ESBL-encoding gene identified in the study isbla_{NDM}, 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 bla_{NDM} in *E. coli*strains, indicating its significance as a major contributor to ESBL production[30]. Additionally, our study revealed a substantial presence of the bla_{TEM}, bla_{CTX-M} and bla_{OXA}genes in 9 out of 12 isolates (75%), 66.67% (8 out of 12) and 50% (6 out of 12) the ESBL *E. coli*isolates respectively. The result of this study is in line with findings of previous research that have highlighted the widespread distribution of bla_{TEM} and bla_{CTX-M} in ESBL-producing *E. coli*[30,31]. This occurs because plasmids bearing bla_{CTX-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[32]. In this study, it was observed that there are lower prevalence rates for bla_{IMP} and bla_{SHV}, with each gene detected in 16.67% of the isolates. While these genes are less common in the result of the 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 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%)[33]. All these studies confirmed that gene predominance varied between regions and locations and to a large extent determine 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[33,34].

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[35]. Additionally, due to the prevalence of ESBL *E. coli*in food-producing animals, specifically in chickens and young cattle, it is possible that animal faeces could play a part in the spread of these bacteria[36]. 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 findings of previous research conducted in Pakistan (57%) and India (64%)[37,38].

5.0 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 our water samples had low Ph values according to our physicochemical analysis. We also observed lower levels of dissolved oxygen (DO), which could potentially compromise water quality, despite total dissolved solids (TDS) remaining within WHO-approved limits. 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.

Our analysis of the antimicrobial resistance profiles of ESBL-producing *E. coli* isolates revealed significant resistance to commonly used antibiotics, including first-generation fluoroquinolones, cephalosporins and augmentin. Notably, resistance patterns among second generations of cephalosporins and nitrofurantoin, were relatively lower. Molecular analysis unveiled the distribution of ESBL genes in our *E. coli* isolates, with blaNDM being the most prevalent. The presence of blaTEM, blaCTX-M, blaOXA, blaIMP, and blaSHV genes highlights the diversity of ESBL genes in our studied group.

6.0 Recommendations

Efforts should be concentrated on improving water quality in Oyo State, addressing concerns such as low pH and dissolved oxygen levels. 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. Advocacy programs aimed at educating the public on the debilitating effects of contaminated water and the importance of using safe and clean water, should be initiated and encouraged.

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