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RESEARCH ARTICLE

Antibacterial Resistance and Phenotypic Detection of Extended Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae Isolated from Environmental Sources in Nasarawa State, Nigeria

Richard Rebecca[1,*], Tobias Innocent Ndubuisi Ezejiofor[2], Chijioke Adulberth Nsofor[2], and Istifanus Haruna Nkene[3]

ABSTRACT

The multidrug resistant member of Enterobacteriaceae is common causes of community related infections. A study on antibacterial resistance and phenotypic detection of Extended Spectrum Beta-Lactamase (ESBL) producing Enterobacteriaceae isolated from environmental sources in Nasarawa state, Nigeria was carried out. A total of 400 samples comprised of air, water, soil, vegetable, and sewage were collected and 89 samples were multidrug resistant. Enterobacteriaceae were isolated and identified using commercial biochemical kit Eneterosystem 18R. The antibacterial susceptibility testing of Enterobacteriaceae isolates was carried out using the clinical and laboratory standards institute (CLSI). The phenotypic confirmation test of ESBL was carried out by double disc synergy test (DDST) method. The occurrence of multidrug resistant isolates shows that *Escherichia coli* (24.71%) and *Klebsiella pneumoniae* (19.10%) find to be high while the occurrence of *Proteus mirabilis* and *Citrobacter freundii* (6.74%) were low. The Enterobacteriaceae isolates were more resistant to Cefuroxime, Cefexime, Amoxicillin Clavulanate and Imipenem/Cilastatin with percentage resistance ranges from 62.5%–100%. The occurrence of ESBL producers shows that *Klebsiella pneumoniae* (66.7%) and *Shigella flexneri* (57.14%) were found to be high while the occurrence of ESBL was low in *Proteus mirabilis* (20.0%). The Beta Lactam and gentamycin antibiotics were not effective against the MDR isolates and most of the isolates were ESBL producers.

Keywords: Antibacterial, Enterobacteriaceae, Phenotypic, Resistance.

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[1] *Department of Biological Science, Federal University Gashua, Nigeria.*
[2] *Department of Biotechnology, Federal University of Technology, Nigeria.*
[3] *Department of Microbiology, Nasarawa State University Keffi, Nigeria.*

* ***Corresponding Author:***
e-mail: beckycool4u@gmail.com

1. Introduction

Enterobacteriaceae are rod in shape, gram negative bacteria, oxidase negative, citrate negative, indole positive, methyl red positive, and voges-proskauer negative, Enterobacteriaceae are a family of bacteria commonly isolated from environmental and clinical cultures, which include *Escherichia coli*, *Salmonella* sp, *Klebsiella* sp, *Enterobacter* sp, *Proteus mirabilis, Citobacter freundii*, and *Shigella* sp. (David & Yohei, 2017). Enterobacteriaceae has been reported to be one of the most predominant organisms causing a variety of human illnesses ranging from urinary tract infections (UTIs), wound infections, pneumonia, gastroenteritis, meningitis, septicaemia, etc. They are very common reasons for consultation and antibiotic prescription in current practice (El-bouamri *et al*., 2015).

Antimicrobials such as β-lactams and quinolones are classes of molecules that have been used globally in the treatment of infectious diseases. Enterobacteriaceae which are Gram negative bacteria, have been treated with quinolones which are synthetic antibiotics. Fluoroquinolones have been reported to have broad-spectrum intrinsic activity greater than quinolones (Salah *et al*., 2019). Three major mechanisms of quinolone resistance

to bacterial are; there is an accumulation of mutations in the genes encoding quinolone target DNA gyrase and topoisomerase IV and there is a decrease of intracellular concentration of fluoroquinolones by porins down-regulation or modification of the efflux pumps activity (Salah *et al*., 2019).

Globally, antimicrobial resistance in *Escherichia coli, Klebsiella pneumoniae, Salmonella sp., Acinetobacter* sp., *Staphylococcus aureus, and Streptococcus pneumoniae* has been reported to be high in their global antimicrobial resistance surveillance program (World Health Organization, 2019). Environment serves as the major reservoir of antibiotic resistant bacteria, the environment also plays an important role in the occurrence of multi drug resistant bacteria in human and animals through food chain. Anthropogenic activities often result into discharge of waste into the environment which has effects on food chain leading to increase in multi drug resistant. The increase in MDR all over the world is alarming which is considered a public health threat, several investigation recently have reported the emergence or MDR bacterial pathogen from difference origins including soil, flowing rivers, waste water, refuse dump sites and animals (Jesumirhewe *et al.*, 2022). The high prevalence of ESBL producing Enterobacteriaceae in South-eastern, Nigeria was recorded Ugah and Udeani (2020), where they isolated Enterobacteriaceae from clinical samples only. Also, in Western part of Nigeria high rate of Enterobacteriaceae was recorded by Abayomi *et al*. (2020) from clinical samples from LAUTECH teaching hospital Ogbomosho. This research study however aimed at antimicrobial resistance and phenotypic detection of ESBL Producing Enterobacteriaceae isolated from environmental sources in Nasarawa state, Nigeria.

2. Materials and Methods

2.1. Materials

Antibiotic Discs: The antibiotic discs and potency that were used in this study include Amoxicillin-Clavulanic acid (AMT: 30 µg), Cefotaxime (CTX: 30 µg), Ceftazidime (CAZ: 30 µg). All disc were products of Oxoid Ltd., (U.K.).

Enterosystem 18R: Enterosystem 18R kits were used for the identification of Gram negative, oxidase negative enterobacteria, which contains 18 well system containing desiccated biochemical substrata. Products of Liofilchem Diagnostic Italy.

2.2. Methods

2.2.1. Study Location

Samples were collected within selected local government areas in Nasarawa State which include: Keffi (Nasarawa West Senatorial District), Karu (Nasarawa West Senatorial District), Akwanga (Nasarawa North Senatorial District), and Lafia (Nasarawa South Senatorial District). Nasarawa State is located in the central part of Nigeria popularly known as the middle belt. The State lies between latitude 70 45*I* and 90 25*I*°N of the equator and between Longitude 70 and 90 37°E of the Greenwich meridian. Nasarawa State has a total landmass of about 27, 137.8 km^2, a population of 1,863.275 according to the 2006 census report, and a population density of 130 (Census, 2006). Nasarawa State is bounded in the north by Kaduna State, in the west by Abuja Federal Capital Territory, in the south by Kogi and Benue States, and, in the east by Taraba and Plateau States.

2.2.2. Sample Size

A total of 400 environmental samples were isolated from four different local government areas of Nasarawa State, the sample size used in this study was calculated adequately using the formula developed by Cochran (1963) with little modification by Ugah and Udeani (2020).

2.2.3. Sample Collection

A total of 400 samples were collected in Keffi, Karu, Akwanga, and Lafia local government areas of Nasarawa State.

2.3. Sampling Techniques

2.3.1. Air Sample

The air samples were collected using exposed plate techniques as described by Richard *et al*. (2018). In brief, the agar plates (MacConkey agar and or Eosin Methylene Blue agar) (Oxoid Ltd., Basingstoke, UK) were exposed to both indoor and outdoor air of the selected sites for about 5 min. After 5 min of exposure, the plates were covered and taken to the Microbiology Laboratory, Nasarawa State University, Keffi for incubation at 37 °C for 24–48 hours.

2.3.2. Soil Sample

Soil samples were collected from dump site, agricultural site and residential site at a depth of 0–15 cm, using soil auger, a method described by Sylvester and Ayobami (2017). The enterobacteriaceae were isolated from soil using soil dilution method with little modification of the method earlier described by Murugalatha *et al*. (2018). Ten-fold serial dilutions of soil samples were prepared using Ringer's solution and 100 μl of the distilled samples were spread on MAC plates and incubated at 37 °C for 24 hours Pinkish and colourless colonies on MAC were selected as presumptive Enterobacteriaceae. Spread plate techniques were used to isolate the enterobacteriaceae from the diluted samples. From each of the dilutions, 1 mls from the aliquot in tube (4) 10^{-4}, tube (5) 10^{-5}, tube (6) 10^{-6}, and tube (7) 10^{-7} was pipetted into sterile petri dishes with MacConkey agar and or EMB agar and spread with a glass L shape rod. The agar plates were incubated at 37 °C for 24–48 hours. Pinkish and non-pinkish colonies on MAC were selected as lactose and non-lactose fermenting colonies.

2.3.3. Water Sample

Water samples were collected from boreholes, pipe borne, streams and well. Sampling was done using standard procedure by American Public Health Association (APHA) (2005). The water samples were taken into pre-sterilized bottles and kept in ice-cooler for onward transportation to the Microbiology Laboratory, Nasarawa State University, Keffi within 6 hours of collection for bacteriological analyses (Raji *et al*., 2010). Ten-fold serial dilutions of the water samples were prepared using sterile distilled water. From all the dilutions, 0.1 ml of the

sample was aseptically transferred to the center of the prepared MacConkey agar (Oxoid Ltd., Basingstoke, UK), the plates were swirled for an even distribution of the inoculum. The plates were in duplicates and incubated at 37 °C for 24–48 hours (Raji *et al*., 2010).

After incubation, typical presumptive Enterobacteriaceae colonies were purified and sub-cultured on nutrient agar and incubated at 37 °C for 24–48 hours. The positive growth was Gram stained and viewed under the microscope using oil immersion ×100 objectives lens (Holt *et al*., 1994).

2.3.4. *Vegetable Sample*

In total, three types of commonly consumed fresh vegetables namely; Spinach (*Spinacia oleracea*), Pumpkin leaves (*Telfairia occidentalis*) and Tomatoes (*Solanum lycopersicum*) were purchased from different markets in the selected study sites for bacterial analysis. The samples were collected in sterile polythene bags with a zip to avoid contamination from handling and kept in ice-cooler for onward transportation to the Microbiology Laboratory, Nasarawa State University, Keffi for microbial analysis within one hour (Kaur and Rai, 2015).

The vegetable samples were taken into the laboratory rinsed for each with 100 ml of distilled water, and diluted 10 fold serially. After washing the vegetables surface 10 ml of aqueous of washed aqueous suspension of each sample was mixed with 90 ml MacConkey Broth (Oxoid Ltd., Basingstoke, UK), Selenite F Broth (Oxoid Ltd., Basingstoke, UK) and Nutrient Broth (Oxoid Ltd., Basingstoke, UK) incubated at 37 °C for 24 hours. The over-night culture was used for the isolation and identification of selective media using streak plate method.

2.3.5. *Sewage Sample*

Sewage Samples were collected from domestic and industrial waste. The sewage samples were taken into pre-sterilized bottles and kept in ice-cooler for onward transportation to the Microbiology Laboratory, Nasarawa State University, Keffi for bacteriological analyses.

Ten-fold serial dilutions of the sewage samples were prepared using sterile distilled water. From all the dilutions, 0.1 ml of the sample was aseptically transferred to the center of the prepared MacConkey agar (Oxoid Ltd., Basingstoke, UK) and Eosin Methylene Blue (E.M.B) agar (Oxoid Ltd., Basingstoke, UK), the plates were swirled for an even distribution of the inoculum. The plates were in duplicates and incubated at 37 °C for 24–48 hours.

2.4. *Identification and Biochemical Characterization of Enterobacteriaceae Isolates*

The presumptive Enterobacteriaceae was identified by microscopy (gram staining) and some relevant biochemical characterization tests were carried out which includes citrate utilization test, indole test and Methyl red-Voges Proskauer was chosen according to Bergy, manual of determinative bacteriology by Holt *et al*. (1994), Cheesebrough (2006) and further identified using Commercial Biochemical Kit Enterosystem 18R. In brief, presumptive Enterobacteriaceae to be identified was recently isolated (18–24 hours); one or more morphologically similar well isolated colonies from the agar culture medium were suspended into physiological solution and the suspension was thoroughly homogenized.

A system was taken away from its wrapper and brought to room temperature, the system was properly labelled with the date and origin of the of the isolates, about 0.2 ml of bacterial suspension was transferred into each well of the system and overlay with 1 drop Vaseline oil the wells 2-LDC, 3-ODC, 4-ADC, 7-UR and 8-H_2S. The system was covered with lid provided and incubated at 36 °C ± 1 °C for 12–18–24 hours.

At the end of the incubation period, about 2 drops of alpha-napthol and 1 drop of NaOH 40% was added to the well 10-VP development of pink-red colour in about 20 minutes indicates positive reaction, about 2–3 drops of KOVAC'S Reagents into the well 11-IND, the development of a red colour within 2–3 minutes indicates positive reaction, colour change was watched at each well and the results were read and interpreted using standard given in the identification index.

2.5. *Antibacterial Susceptibility Testing of Enterobacteriaceae Isolates*

The antibacterial susceptibility testing of the bacterial isolates was carried out as earlier described by Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2017). Briefly, three (3) pure colonies of the isolates were inoculated in to 5 ml sterile 0.85% (w/v) NaCl (normal saline), and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ (BDH Chemicals Ltd., England) was added into 99.5 ml of 1% (w/v) H_2SO_4 (BDH Chemicals Ltd., England).

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK) plates and the antibiotic discs was aseptically placed at the center of the plates and allowed to stand for 1 hour for pre-diffusion. The plates were incubated at 37 °C for 24 hours. The diameter zone of inhibition in millimeter will be measured and the result will be interpreted in accordnce with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2017).

2.6. *Determination of Multiple Antibiotic Resistance (MAR) Index*

The MAR index of the isolates was determined using the method of Krumperman (1983) and Tsaku *et al*. (2019) with little adjustments:

$$\text{MAR Index} = \text{No. antibiotics isolate is resistant to} / \text{No. of antibiotics tested.}$$

2.6.1. *Classification of Antibiotic Resistance*

Antibiotic resistance in the isolates were classified into: multidrug resistant (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistant (XDR: non-susceptible to ≥1 agent in all but ≤2

antimicrobial categories); pan drug resistant (PDR: non-susceptible to all antimicrobial listed) (Magiorakos *et al*., 2012).

2.7. *Phenotypic Confirmatory Test for ESBL Production*

The phenotypic confirmatory test of ESBL production by isolates resistant to both third and generation Cephalosporins (Ceftazidime and Cefotaxime) was carried out using Double Disc Synergy Test (DDST) method as described earlier by Jarlier *et al*. (1988). 10^5 cfu/ml bacterial suspension was streaked on sterile Mueller-Hinton agar plates and Amoxicillin-Clavulanic acid (AMT: 30 µg) disc was placed at the center of the plate. Cefotaxime (CTX: 30 µg) and Ceftazidime (CAZ: 30 µg) discs were placed at the 15 mm (edge to edge) from the center disc. Enhancement of zone of inhibition in the area between the Amoxicillin-Clavulanic acid disc and any one of the β-lactam discs compared with the zone of inhibition on the far side of the drugs disc was interpreted as indicative of the presence of an ESBL in the test strain.

3. Results

3.1. *Occurrence of Enterobacteriaceae*

The occurrence of Enterobacteria isolated from different environmental source in Nasarawa state, Nigeria is shown in Table I. Out of 89 MDR environmental samples namely air, water, soil, vegetable, and sewage, the occurrence of *Escherichia coli* (24.71%) and *Klebsiella pneumoniae* (19.10%) were highest while the occurrence of *Proteus mirabilis* and *Citrobacter freundii* (6.74%) were low. The occurrence of *Escherichia coli* (80.0%) was also highest in Air sample but *Proteus mirabilis, Salmonella enterica*, *Klebsiella pneumoniae* and *Shigella flexneri* was low with their percentage occurrence (6.6%), respectively.

3.2. *Antimicrobial Resistance*

The antimicrobial resistance of Enterobacteria isolated from different environmental sources in Nasarawa state Nigeria is shown Table II. The *Escherichia coli, Proteus mirabilis, Salmonella enterica*, *Enterobacter cloacae, Klebsiella pneumoniae*, *Shigella flexneri* and *Citrobacter freundii* were more resistance Amoxicillin Clavulanic acid, Cefexime, Cefuroxime, Cefotaxime and Ceftriaxone Sulbactarm with resistance ranges from 62.5%–100% but less resistance to levofloxacin and ofloxacin with percentage resistance ranges from 14.28%–28.57%, respectively as shown in Table II.

3.3. *Categories of Antimicrobial Resistance*

The antimicrobial resistant Enterobacteria isolated from different environmental sources in Nasarawa state, Nigeria was classified into different categories of antimicrobial resistance namely, multi-drug resistant (MDR), extensive

TABLE I: Occurrence of MDR Enterobacteriaceae Isolated from Environmental Sources in Selected Local Government Areas of Nasarawa State, Nigeria

Samples	No of MDR	No. (%) enterobacteriaceae						
		EC	PM	SE	ET	KP	SF	CF
AIR	5	4(80.0)	1(20.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
WATER	22	1(4.54)	1(4.54)	2(9.09)	3(13.63)	9(40.90)	2(9.09)	4(18.18)
SOIL	22	1(4.55)	0(0.00)	9(40.90)	5(22.73)	4(18.18)	3(13.64)	0(0.00)
VEGETABLE	25	10(40.0)	3(12.0)	4(16.0)	3(12.0)	3(12.0)	2(8.0)	0(0.00)
SEWAGE	15	6(40.0)	1(6.6)	1(6.6)	3(20.0)	1(6.6)	1(6.6)	2(13.33)
Total	89	22(24.71)	6(6.74)	16(17.97)	14(15.73)	17(19.10)	8(8.98)	6(6.74)

Keys: MDR = Multidrug resistant, EC = *Escherichia coli*, PM = *Proteus mirabilis*, SE = *Salmonella enterica*, ET = *Enterobacter cloacae*, KP = *Klebsiella pneumoniae*, SF = *Shigella flexneri*, CF = *Citrobacter freundii*.

TABLE II: The Antimicrobial Resistance of Enterobacteriaceae Isolated from Environmental Sources in Nasarawa State, Nigeria

Antimicrobials	Disc contents (μg)	No. (%) resistance						
		EC (N = 22)	PM (N = 5)	SE (N = 16)	ET (N = 14)	KP (N = 18)	SF (N = 7)	CF (N = 7)
CXM	30	21(95.45)	4(80.0)	12(75.0)	13(92.85)	18(100.0)	7(100.0)	7(100.0)
CTX	25	15(68.18)	5(100.0)	13(81.25)	12(85.71)	17(94.4)	7(100.0)	7(100.0)
IMP	10/10	22(100.0)	5(100.0)	14(87.5)	14(100.0)	17(94.4)	7(100.0)	7(100.0)
OFX	5	6(27.27)	1(20.0)	4(25.0)	3(21.42)	6(33.3)	2(28.57)	1(14.28)
GN	10	7(31.81)	3(60.0)	8(50.0)	7(50.0)	9(50.0)	4(57.14)	4(57.14)
NA	30	20(90.90)	3(60.0)	10(62.5)	11(78.57)	18(100.0)	7(100.0)	5(71.42)
LBC	5	4(18.18)	1(20.0)	4(25.0)	3(21.42)	4(22.2)	4(57.14)	2(28.57)
CRO	45	21(95.45)	5(100.0)	13(81.25)	12(85.71)	18(100.0)	6(85.71)	7(100.0)
AMC	30	22(100.0)	5(100.0)	10(62.5)	14(100.0)	17(94.4)	7(100.0)	5(71.42)
ZEM	5	22(100.0)	5(100.0)	14(87.5)	14(100.0)	18(100.0)	7(100.0)	7(100.0)

Key: CXM = Cefuroxime, CTX = Cefotaxime, IMP = Imipenem/Cilastatin, OFX = Ofloxacin GN = Gentamycin, NA = Nalidixic Acid, LBC = Levofloxacin, CRO = Ceftriaxone Sulbactarm, AMC = Amoxicillin Clavulanate, ZEM = Cefexime, EC = *Escherichia coli*, PM = *Proteus mirabilis*, SE = *Salmonella enterica*, ET = *Enterobacter cloacae*, KP = *Klebsiella pneumoniae*, SF = *Shigella flexneri*, CF = *Citrobacter freundii*.

TABLE III: The Classes of Antimicrobial Resistance in Antimicrobial Resistant of Enterobacteriaceae Isolated from Environmental Sources in Nasarawa State, Nigeria

Classes of antimicrobial resistant	No. (%) isolates						
	EC (N = 22)	PM (N = 5)	SE (N = 16)	ET (N = 14)	KP (N = 18)	SF (N = 7)	CF (N = 7)
MDR	100.0	100.0	100.0	100.0	100.0	100.0	100.0
XDR	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PDR	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Keys: MDR = Multi-Drug Resistant, XDR = Extensive Drug Resistant, PAN = Pan Drug Resistant, EC = *Escherichia coli*, PM = *Proteus mirabilis*, SE = *Salmonella enterica*, ET = *Enterobacter cloacae*, KP = *Klebsiella pneumoniae*, SF = *Shigella flexneri*, CF = *Citrobacter freundii*.

TABLE IV: The Phenotypic Detection of Extended Spectrum Beta Lactamase Production from Enterobacteriaceae Isolated from Environmental Sources Nasarawa State, Nigeria

ESBL production	No. (%) enterobacteriaceae						
	EC (N = 22)	PM (N = 5)	SE (N = 16)	ET (N = 14)	KP (N = 18)	SF (N = 7)	CF (N = 7)
POSITIVE	8(36.36)	1(20.0)	7(43.75)	7(50.0)	12(66.7)	4(57.14)	3(42.86)
NEGATIVE	14(63.64)	4(80.0)	9(56.25)	7(50.0)	6(33.3)	3(42.86)	4(57.14)

Keys: MDR = Multi-Drug Resistant, XDR = Extensive Drug Resistant, PAN = Pan Drug Resistant, EC = *Escherichia coli*, PM = *Proteus mirabilis*, SE = *Salmonella enterica*, ET = *Enterobacter cloacae*, KP = *Klebsiella pneumoniae*, SF = *Shigella flexneri*, CF = *Citrobacter freundii*.

drug resistant (XDR) and pan drug resistant (PAN) with occurrence of MDR as the highest with percentage occurrence of 100% but none of the antimicrobial resistant bacteria were either extensive or pan drug resistance as shown in Table III.

3.4. *Extended-Spectrum Beta Lactamase Production*

The production of ESBL by different species of MDR enterobacteria isolated from different environmental sources is as shown in Table IV. The occurrence of ESBL producers was high in *Klebsiella pneumoniae* (66.7%) and *Shigella flexneri* (57.14%) but low in *Escherichia coli* (36.36%) and *Proteus mirabilis* (20.0%) respectively as shown in Table IV.

4. Discussion

The prevalence of MDR ESBL producing Enterobacteria isolated from environmental sources such as air, water, soil, vegetable, and sewage in Nasarawa state, Nigeria in our study was 22.25% which is consistent with the study earlier reported 20.04% by Dela *et al*. (2022) but higher 61.5% when compared to the study reported by Ugah and Udeani (2020). The isolation of Enterobacter such as *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* from environmental sources in our study may have public health implication, this is because the Enterobacteria mention are known to cause wide range of community related infection such as UTI, respiratory tract infection and diarrhea (Dela *et al*., 2022; Raya *et al*., 2020; Oghenevo *et al*., 2016). The isolation of *Salmonella enterica* from the environmental sources such as water, soil and sewage also have health implication since *Salmonella enterica* are widely known as true pathogens responsible for either diarrhea or typhoid fever (Kuijpers *et al*., 2017; Carl *et al*., 2020). Most community related infection caused by Enterobacteria may be as a result of individual coming in contact with those environmental sources directly or indirectly. Hence, the environmental sources in which the enterobacteria were isolated may serve as possible sources of community related infection. The percentage occurrence of *Escherichia coli* from air, water, soil, vegetable, and sewage was 24.71% in this study which is in line with 26.8% isolated among humans, chicken, and poultry environment as reported by Aworh *et al*. (2020) and higher than 61.8% (Ugah & Udeani, 2020). Public health thread due to the fact that both intestinal and extraintestinal pathogenic *Escherichia coli* have been isolated from soil, water, and air in the study earlier described by Shah *et al*. (2018). Multi-Drug Resistant *Escherichia coli* is an example of antibiotic resistance which is responsible for life threatening infections. Antibiotics resistance can be developed when there is a prolonged exposure of *Escherichia coli* to antibiotics (Pormohammed *et al*., 2019; Reinthaler *et al*., 2013; Sahoo *et al*., 2012; Carattoli, 2008). While, occurrence of *Klebsiella pneumoniae* in this study is 19.10% which is consistent with 19.3% (Ugah & Udeani, 2020). The occurrence of MDR *Citrobacter freundii* and *Proteus mirabilis* isolated from environmental sources in this study is 6.74% each respectively which was higher than 10.3% and slightly lower than 4.3% as reported by Ugah and Udeani (2020).

The occurrence of ESBL producing isolated from environmental sources may also pose public health implication because resistance to cephalosporin antibiotics in being considered to be critical by World Health Organization (Egbule *et al*., 2021). The occurrence of ESBL producing Enterobacteriaceae isolates in our study suggest that the enzymes may be responsible for β-lactam resistant isolates were ESBL producers and this suggest that the resistant to β-lactam may be due to other mechanism like modification of target site of β-lactam or decrease in cellular uptake of the antibiotics. The percentage occurrence of ESBL producing *Escherichia coli* (36.36%) was lower than 68% Igbinosa *et al*. (2023) and higher than 29.6% Kimera *et al*. (2021), *Salmonella enterica* (43.75%) which was lower than 58.1% as reported by Parvin *et al*. (2020) and *Klebsiella*

pneumoniae (66.7%) was higher than 45.5% in the study conducted by Kimera *et al*. (2021).

5. Conclusion

The high resistance of the Enterobacteriaceae isolates to β-lactam antibiotics such as cefuroxime, cefotaxime, cefexime, ceftriaxone sulbactarm, and imipenem/cilastatin as well as gentamycin may be due to their indiscriminate use as therapeutics options for infection caused by gram negative Enterobacteriaceae. The high resistance of the isolates to antibiotics mentioned suggest that the agents may not be effective for the treatment of infection caused by Enterobacteria. The low resistance of the isolates to levofloxacin and ofloxacin suggest that the agents may not have been abused or misused in the study area. The β-lactam antibiotics and aminoglycosides were not effective against the isolates and most of the isolates were multidrug resistant and ESBL producer levofloxacin and ofloxacin were effective against the isolates. The molecular characterization of the ESBL producing Enterobacteria is ongoing.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

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