



Comparison of Antibiotic-Resistant Pattern of Extended Spectrum Beta-Lactamase and Carbapenem-Resistant *Escherichia Coli* Isolates from Clinical and Non-Clinical Sources

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Abstract



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The increasing rate of antibiotic resistance among *E. coli* especially those mediated by extended spectrum beta-lactamases and carbapenem-resistant (CR) presents a major threat to public health and healthcare delivery globally. The aim of this research was to compare the antibiotic resistant pattern of extended spectrum beta-lactamase and carbapenem-resistant *Escherichia coli* isolates from clinical and non-clinical sources. A total of two hundred and fifteen (215) clinical and non-clinical samples were collected for the study. The collected samples were analysis using Standard Microbiological protocol for isolation and identification. Phenotypic detection of ESBL Production and carbapenem resistant was performed using Double Disk Synergy Test (DDST) and Modified Hodge Test (MHT) respectively. Antibiogram studies of ESBL producing and Carbapenem-resistant *E. coli* was determined using the Kirby-Bauer disk diffusion method. The result of isolation and characterization revealed higher occurrence rate of clinical isolates of *E. coli* 66.1 % over non-clinical sample 54.0%. Phenotypic ESBL screening of isolated *E. coli* revealed overall detection rate of 35(30.4 %) and 13(13.0 %) in clinical and non-clinical source respectively while overall detection rate of carbapenem resistant ESBL producers accounted 9.6 % and 8.0 % in clinical and non-clinical isolates respectively. The isolates exhibited high percentage of resistance to nitrofurantoin 100 %, cefepime 100 % colistin 66.7 %, amikacin 50.0 % and also exhibit MDR with MARI value of ≥ 0.5 . Comparison of antibiotic resistant pattern of carbapenem-resistant extended spectrum beta-lactamase producing *E. coli* isolates from clinical and non-clinical sources showed no statistical significant difference P-value < 0.05 but were 100 % susceptible to ciprofloxacin and gentamicin. The pattern of similar MDR portrayed by clinical and non-clinical isolate should not be overlooked due to its hyper-motility and virulence domination leading to possible AMR transfer to other bacterial organisms and also, appropriate measures such as pretreatment of animal excrement before being used as fertilizers and the quality of irrigation water, hospital effluent discharge need to be taken into consideration to prevent the spread of resistant determinant.

Keywords: *Escherichia coli*, Extended Spectrum Beta-Lactamase, Carbapenem-Resistant, clinical, non-clinical

1. INTRODUCTION

Escherichia coli is a member of normal gut microbiota, some isolates are pathogenic and may cause diarrhea and extra-intestinal disorders in humans ¹. (El-Shaer *et al.*, 2021) *E. coli* can be classified into three subtypes from human health perspective ^{1, 2}. (El-Shaer *et al.*, 2021; Roussel *et al.*, 2017) First, commensal isolates colonizing the gut of healthy individuals. The second one is the diarrheagenic (DEC) isolates

that cause diarrhea and differs according to strain virulence ². (Roussel *et al.*, 2017). Finally, extraintestinal pathogenic *E. coli* (ExPEC) are similar to commensal ones in colonizing the human gut, but they can survive well in extra-intestinal environments causing serious human diseases ^{1, 2}. (Roussel *et al.*, 2017; El-Shaer *et al.*, 2021).

The pathogenic behavior of a bacterial strain can be determined by assessing the virulence factor (VFs) collection and/or the phylogenetic background. *E. coli* is classified into

eight phylogroups: A, B1, B2, C, D, E, F, and *Escherichia* cryptic clade I depending on the new quadruplex PCR-based method³. (Clermont *et al.*, 2013). Genetically, ExPEC carries many virulence factor which allow them to avoid or subvert host defenses, colonize within anatomical sites, and/or induce host inflammatory response, thereby causing a disease status¹. (El-Shaer *et al.*, 2021). These VFs form clusters named pathogenicity-associated island markers (PAIs) located in chromosome and/or plasmids⁴. (Sabate *et al.*, 2006). The PAIs contribute to the transmission of many genes that help bacteria survive and cause diseases.

The development of antimicrobial resistance in *E. coli* is one of the biggest challenges in public health⁵. (World Health Organization, 2014). *E. coli* exhibits resistance to variable antibiotics, mainly extended-spectrum β -lactams (ESBLs) and carbapenem. ESBLs producers are isolates that are resistant to penicillins and cephalosporins⁶. (Ingti *et al.*, 2020). They were observed either as variants of *TEM* and *SHV* or the CTX-M enzyme. It is more prevalent in environmental (non-clinical) isolates suggesting that the environment is a reservoir of ESBL-producers⁷. (Doi *et al.*, 2017). This is a major contributor to increased antibiotic load, increased therapeutic cost, poor health outcomes, and limited therapeutic choices⁸. (Saleem *et al.*, 2017).

Carbapenemase producers are isolates with resistance to cephamycins and carbapenems via the expression of a variety of genes including *KPC*, *OXA-48*, *IMP*, *VIM*, and *NDM*^{9, 10, 11}. (Nordmann *et al.*, 2011; Ogba *et al.*, 2022a; Nomeh *et al.*, 2023). The intestinal microbiota, feces, and rectal swabs are all typical reservoirs of carbapenemase production in hospital settings¹². (Vila *et al.*, 2016). In addition, several studies have reported that these species harbours clinically important carbapenemase encoding resistant genes such as blaKPC, blaVIM, blaIMP, blaNDM and blaOXA-48^{13, 14}. (Tshitshi *et al.*, 2020; Solgi *et al.*, 2020). Given the fact that carbapenem antibiotics are considered as the last resort for treating infections caused by ESBL-producing strains. Presence of carbapenemase and ESBL genes in *E. coli* strains coupled with lack of new therapeutic option is cause for concern^{13, 15}. (Teixeira *et al.*, 2020; Tshitshi *et al.*, 2020).

Only a few antibiotics (e.g. carbapenems, colistin, tigecycline) are available to treat infection caused by ESBL-producing bacteria, although the *in vivo* efficacy and/or toxicity of these drugs is not well known^{16, 17, 18}. (Giamarellou, 2006; Morrill *et al.*, 2015; Teklu *et al.*, 2019). Resistance accumulation and dissemination in clinical and environmental *E. coli* underlined the necessity of developing suitable strategies to tackle antimicrobial resistance, where it is difficult to anticipate the development of novel antimicrobial agents.

2. METHODS

2.1 Sample Collection, Culture and Identification

A total of two hundred and fifteen (215) clinical and non-clinical samples were collected for the study. A total of one hundred (100) samples including water sources [bore-hole water (10) and well water (10)] from College of agricultural studies (CAS) and Presco campus while selected edible vegetable (Tomatoes [20], Scent leaf [20], Garden egg leaf [10] and Carrot [15]) were collected from Hausa Quarters and International market vendors was sampled from Abakaliki metropolis for the study. A total of one hundred and fifteen (115) clinical samples were collected comprising of early morning mid-stream urine (70), wound swab (25) and pus (25) from patients attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FEUTHA) were collected in a sterile sample container.

Aseptically, a loopful of the collected clinical sample of urine, wound and pus swab samples were separately inoculated and suspended in a sterile nutrient broth (Hi media Co., India) and incubated aerobically at 37 °C for 24 hrs. After overnight incubation, turbid broth culture of clinical sample was streaked on solidified MacConkey agar (Hi media Co., India). The plates were incubated aerobically for 24 hrs at 37°C. Bacterial colonies with pinkish coloration on overnight MacConkey agar (Hi media Co., India) for clinical sample were infer as the presence *Escherichia coli*. Discrete colonies were purified by plating onto nutrient agar (Hi media Co., India). The sub-cultured plates were incubated at 37°C for 24 hrs. Exactly, 10 grams of the each selected edible vegetable from market vendors was rinsed in 100 ml milliliter volume of sterile water. Thereafter, 10⁻¹⁰ folds' serial dilution was carried out. Exactly 0.5ml of dilution factor three for each randomly rinsed and serially diluted selected edible vegetable samples were spread plated on plate count agar and incubate at 37 °C for 24 hrs. After 24 hrs of incubation, colonies were counted using Colony counter (Bioeuropeak Co., Ltd., China) and a loopful of each colony were aseptically streaked on solidified eosin methylene blue agar plate. The plates were incubated aerobically for 18-24 hrs at 37°C. Bacterial colonies with greenish-metallic sheen on eosin methylene blue agar plate for non-clinical samples were infer as the presence *Escherichia coli*¹⁹. (Cheesbrough, 2006). Discrete colonies were purified by plating onto nutrient agar (Hi media Co., India). Both clinical and non-clinical bacteria sub-cultured plates were incubated at 37°C for 24 hrs. Standard microbiological techniques for identification of pure culture strain was performed using Vitex 2 automated system (BIOMERIEUX, U.S.A).

2.2 Screening of the *Escherichia coli* from clinical and non-clinical source with 2nd and 3rd Generation Cephalosporin

This test was performed using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Hi media Co., India) as described by the Clinical and Laboratory Standards Institute²⁰. (CLSI, 2019) guidelines. The Modified Kirby-Bauer standardized disc diffusion testing was performed using the direct colony suspension method. A suspension was made from a 24-hour growth of the test organisms (from clinical and non-clinical source) in sterile water to match the 0.5 McFarland turbidity standard. This was seeded on the entire surface of solidified Mueller-Hinton agar plate. The following 2nd and 3rd generation Cephalosporin; cefuroxime (30 µg), cefotetan (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg) (Oxoid, UK) was impregnated aseptically and incubated at 37°C overnight. Any of the isolate that showed reduce susceptibility to any of the 2nd and 3rd generation cephalosporin was subjected to ESBL screening according to the guidelines of Clinical and Laboratory Standards Institute²⁰. (CLSI, 2019).

2.3 Phenotypic Detection of Extended-spectrum beta-lactamase *Escherichia coli*

2.3.1 Double-disk Synergy Method

An Aliquot of 0.5 MacFarland equivalent standard of the test bacteria was seeded on a sterile solidified Mueller-Hinton agar (Hi media Co., India) using a sterile swab stick. After 15 minutes of pre-diffusion, a disc containing 30 µg amoxicillin-clavulanic acid (Oxoid, UK) was placed at the centre of the agar plate, ceftazidime (30 µg) disc and cefotaxime (30 µg) disc (Oxoid, UK) was placed 15 mm apart from the centre disc. The plates were incubated at 37°C overnight. ESBL-positive strains were inferred by the expansion of the zone of inhibition of either cephalosporin in the presence of clavulanate than in its absence giving a dumbbell shape. This expansion occurred

because the clavulanic acid present in the Augmentin disc inactivated the ESBL produced by the test organism ^{13, 19}, (Tshitshi *et al.*, 2020; CLSI, 2019).

2.4 Phenotypic Detection of carbapenem resistant *Escherichia coli*

The isolates were screened for carbapenem resistance using Kirby-Bauer disc diffusion method. The turbidity of the cultures was adjusted to 1×10^6 CFU/mL (equivalent to 0.5 McFarland standard). An aliquot was seeded on a sterile solidified Mueller-Hinton agar (Hi media Co., India) using a sterile swab stick. After 15 minutes of pre-diffusion Four carbapenem antibiotic discs: imipenem (IPM, 10 µg), ertapenem (ETP, 10 µg), meropenem (MEM, 10 µg and doripenem (DOR, 10 µg g) (Oxoid, Uk) was placed on inoculated plates. The plates were incubated at 37°C for 24 h. The results were interpreted using Clinical Laboratory Standards Institute and CLSI guidelines ²⁰. (2019). Colonies of all the isolates showing intermediate or resistant phenotypes to one of the three tested antibiotics was further be subjected to Modified Hodge Test to confirm their ability to produce carbapenemase ^{21,22}. (Nomeh *et al.*, 2022; Ogba *et al.*, 2022b).

2.4.1 Modified Hodge Test

A 10 µg meropenem or ertapenem susceptibility disk was placed in the center of the test area. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 37°C in ambient air for 16–24 hours. After 24 hrs, MHT Positive test showed a clover leaf-like indentation of the *Escherichia coli* streak within the disk diffusion zone. MHT Negative test showed no growth of a clover leaf-like indentation within the diffusion disk ^{21,22}. (Nomeh *et al.*, 2022; Ogba *et al.*, 2022b).

2.6 Antibiotic Susceptibility Testing

This test was performed using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Hi media Co., India) as described by the Clinical and Laboratory Standards Institute ²⁰. (CLSI, 2019) guidelines. The Modified Kirby-Bauer standardized disc diffusion testing was performed using the direct colony suspension method. A suspension was made from a 24-hour growth of the test organisms in sterile water to match the 0.5 McFarland turbidity standard. This was swabbed on the entire surface of solidified Mueller-Hinton agar plate. The following antibiotic discs with potencies were used: amikacin (15 µg), azetronam (30 µg), ceftriaxone (30 µg), cefepime (30 µg), colistin (10 µg), gentamicin (15 µg), ciprofloxacin (5 µg), nitrofurantoin (100 µg) (Oxoid, UK). The Mueller-Hinton agar plate was incubated at (35°C–37°C) for 18–24 hour, after which the diameter of the zones of growth inhibition around the discs was measured with a meter rule. These results were further interpreted using the Performance Standards for Antimicrobial Susceptibility Testing ^{20, 23}. (CLSI, 2019; Akpu *et al.*, 2023).

2.4.1 Determination of Multiple Antibiotic Resistance (MAR) index

Multiple antibiotic resistance (MAR) index was determined as the ratio of the number of antibiotics to which isolate showed resistance to the number of antibiotics to which the isolate was exposed, using the following formula:

MARI = X/Y; where 'X' is the number of antimicrobial agents which bacteria revealed resistance while 'Y' is the total number of antimicrobial agents tested ²⁴. (Peter *et al.*, 2022).

2.6 Statistical analysis

Graph Pad Prism software package (version 5.01) was used for statistical analysis of the data applying One-Way ANOVA test (Including Turkey HSD) for Comparison of antibiotic

resistant pattern of carbapenem resistant extended spectrum beta-lactamase *Escherichia coli* Isolates from clinical and non-clinical sources. The level of significance was set at a p-value < 0.05.

3. RESULTS

3.1 Results

The proportion of *Escherichia coli* in clinical sample accounted for 76 (66.1 %) comprising of high occurrence rate in urine 70.0 %, followed by Wound swab 64. 0% while low occurrence rate of 55.0 % in Pus sample was recorded as presented in Table 1.

Total bacteria count of non-clinical sample accounted for 14.9×10^3 CfU/ml as borehole water had high proportion of bacteria count of 2.7×10^3 CfU/ml followed by Scent leaf 2.6×10^3 CfU/ml while the least bacteria count of 1.8×10^3 CfU/ml was observed in Garden egg leaf as presented in Table 2.

Escherichia coli from non-clinical sample accounted for overall prevalence rate of 54.0% comprising of high proportion of *Escherichia coli* 80.0 % in Garden egg leaf followed by Scent leaf 70.0 %, Well water 60.0 %, Borehole water 50.0 % while least isolation rate of 33.3 % was in Uziza leaf as shown in Table 3.

In clinical samples, extended spectrum beta-lactamase producing *Escherichia coli* were highly predominant in Pus sample 50.0 % followed by Wound swab 28.0 % while the least proportion was found in Urine 25.7 %. ESBL producing *Escherichia coli* accounted overall detection rate of 35(30.4 %) while Non-ESBL producer recorded 35.7 % as shown in Table 4.

ESBL producer in non-clinical samples accounted for high prevalence rate of 13(13.0 %) consisting of 30.0 %, 20.0%, 20.0% and 10.0 % from scent leaf, tomatoes, borehole water and well water respectively while non-ESBL accounted for 41.0 % as presented in Table 5.

In clinical sample, carbapenem resistant ESBL producing *Escherichia coli* were highly predominant in Wound sample 16.0 % followed by Pus sample 10.0 % and Urine 7.1 % while overall detection rate of carbapenem resistant ESBL producers and carbapenem susceptible strain accounted for 9.6 % and 20.9 % as presented in Table 6.

Carbapenemase Susceptible. CR ESBL producer in non-clinical samples accounted for high prevalence rate of 8(8.0 %) consisting of 20.0%, 15.0 %, 10.0 % and 1.0 % from Borehole water, Tomatoes, well water and Scent leaf respectively while non-CPS ESBL strain accounted for 5.0 % as presented in Table 7.

Carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* from urine exhibit high level of resistance to azetronam 100 %, cefepime 100 %, nitrofurantoin 100 %, amikacin 80.0 %, ceftriaxone 80.0 % but were susceptible to colistin 60.0 %, ciprofloxacin 80.0 % and gentamicin 80.0 % as presented in Table 8. Also, CR ESBL producing *Escherichia coli* from wound were sensitive to colistin 50.0%, gentamicin 100 % and ciprofloxacin 100 % while majority of the isolate were highly resistant to cefepime 100%, ceftriaxone 100%, amikacin 100% and azetronam 100%. CR extended spectrum beta-lactamase producing *Escherichia coli* isolated from Pus sample were highly resistant to nitrofurantoin 100 %, azetronam 100 %, ceftriaxone 100 %, colistin 100 %, but were susceptible to amikacin 50.0 %, gentamicin 100 %, and ciprofloxacin 100 %.

Majority of the strain from tomatoes sample were extremely resistant to nitrofurantoin 100 %, azetronam 100 %, ceftriaxone 100 %, but were susceptible to colistin 33.3 %, ceftriaxone 100 %, but were susceptible to colistin 33.3 %, ceftriaxone 100 %, but were susceptible to colistin 33.3 %,

amikacin 33.3 %, gentamicin 100 % and ciprofloxacin 100 %. Strain isolated from Scent leaf demonstrated resistant to cefepime 100 %, azetronam 100 %, colistin 100 %, ceftriaxone 100 % but were sensitive to amikacin 50.0 %, gentamicin 100 % and ciprofloxacin 100 % as shown in Table 9. Antibiotic Susceptibility profile of CR extended spectrum beta-lactamase producing *Escherichia coli* isolated from borehole water displayed 100% resistant to amikacin, azetronam, ceftriaxone, nitrofurantoin but were highly sensitive to ciprofloxacin, gentamicin and colistin recording 100 %, 100 % and 50.0 % respectively. Majority of CPR strain from well water were extremely resistant to colistin 100 %, cefepime 100 %, azetronam 100 %, nitrofurantoin 100 % but were susceptible to gentamicin, amikacin and ciprofloxacin recording 100 % respectively as presented in Table 9.

Comparison of antibiotic resistant pattern of carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* isolates from clinical and non-clinical sources showed no statistical significant difference p-value < 0.05 i.e., the both isolate had similar pattern of resistant to the test antibiotic as presented in Table 10. Multiple Antibiotic Resistant Index of Carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* from non-clinical sample revealed. MAR index value of 0.5, 0.7, 0.7 and 0.5 among strains isolated from tomatoes, scent leaf, borehole water and well water as presented in Table 10. Among clinical sample, carbapenem resistant in extended spectrum beta-lactamase producing *Escherichia coli* demonstrated multiple antibiotic resistant index of 0.6, 0.5 and 0.7 recorded in strains recovered from Urine, wound sample and Pus sample respectively as shown in Table 11.

Table 1: Distribution of *Escherichia coli* in clinical sample

Clinical Sample	No. of sample	<i>Escherichia coli</i> (%)
Urine	70	49(70.0)
Wound swab	25	16(64.0)
Pus	20	11(55.0)
Total	115	76(66.1)

Table 2: Total Bacteria count of non-clinical sample

Location	Samples	No. of colonies	Cfu/ml
International Market			
	Tomatoes	107	2.1 x10 ³
	Scent leaf	79	1.5 x10 ³
	Uziza leaf	134	2.6 x10 ³
	Garden egg leaf	91	1.8 x10 ³
Hausa Quarters			
	Carrot	104	2.0 x10 ³
CAS			
	Borehole water	139	2.7 x10 ³
Presco			
	Well water	110	2.2 x10 ³
Total Bacteria count		764	14.9 x10³

Key: Cfu-Colony Forming Unit, ml-Milligram, CAS-Campus for Administrative Studies

Table 3: Distribution of *Escherichia coli* from non-clinical sample

Location	Sample	No. of sample	<i>E. coli</i> (%)
International Market			
	Tomatoes	20	9(45.0)
	Scent leaf	20	14(70.0)
	Uziza leaf	15	5(33.3)
	Garden egg leaf	10	8(80.0)
Hausa Quarters			
	Carrot	15	7(46.7)
CAS			
	Borehole water	10	5(50.0)
Presco			
	Well water	10	6(60.0)
Total		100	54(54.0)

Key: CAS-Campus for Administrative Studies

Table 4: Distribution of extended spectrum beta-lactamase producing *Escherichia coli* in clinical sample

Clinical Sample	No. of sample	<i>E. coli</i>	ESBL (%)	Non-ESBL (%)
Urine	70	49(70.0)	18(25.7)	31(44.3)
Wound swab	25	16(64.0)	7(28.0)	9(36.0)
Pus	20	11(55.0)	10(50.0)	1(5.0)
Total	115	76(66.1)	35(30.4)	41(35.7)

Key: ESBL Extended Spectrum Beta-lactamase

Table 5: Distribution of extended spectrum beta-lactamase producing *Escherichia coli* from non-clinical sample

Location	Sample	No. of sample	<i>E. coli</i> (%)	ESBL (%)	Non-ESBL (%)
International Market					
	Tomatoes	20	9(45.0)	4(20.0)	5(25.0)
	Scent leaf	20	14(70.0)	6(30.0)	8(40)
	Uziza leaf	15	5(33.3)	0(0.0)	5(33.3)
	Garden egg leaf	10	8(80.0)	0(0.0)	8(80.0)
Hausa Quarters					
	Carrot	15	7(46.7)	0(0.0)	7(46.7)
CAS					
	Borehole water	10	5(50.0)	2(20.0)	3(30.0)
Presco	Well water	10	6(60.0)	1(10.0)	5(50.0)
Total		100	54(54.0)	13(13.0)	41(41.0)

Key: ESBL Extended Spectrum Beta-lactamase, CAS-College of agricultural science campus

Table 6: Distribution of carbapenem resistant in extended spectrum beta-lactamase producing *Escherichia coli* in clinical sample

Clinical Sample	No. of sample	<i>E. coli</i>	ESBL (%)	CPR (%)	CPS (%)
Urine	70	49(70.0)	18(25.7)	5(7.1)	13(18.6)
Wound sample	25	16(64.0)	7(28.0)	4(16.0)	3(12.0)
Pus	20	11(55.0)	10(50.0)	2(10.0)	8(40.0)
Total	115	76(66.1)	35(30.4)	11(9.6)	24(20.9)

Key: ESBL Extended Spectrum Beta-lactamase, CPR- Carbapenem Resistant, CPS-

Table 7: Distribution of carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* from non-clinical sample

Location	Sample	No. of sample	<i>E. coli</i> (%)	ESBL (%)	CPR (%)	CPS (%)
International Market						
	Tomatoes	20	9(45.0)	4(20.0)	3(15.0)	1(5.0)
	Scent leaf	20	14(70.0)	6(30.0)	2(1.0)	4(20.0)
	Uziza leaf	15	5(33.3)	0(0.0)	0(0.0)	0(0.0)
	Garden egg leaf	10	8(80.0)	0(0.0)	0(0.0)	0(0.0)
Hausa Quarters						
	Carrot	15	7(46.7)	0(0.0)	0(0.0)	0(0.0)
CAS						
	Borehole water	10	5(50.0)	2(20.0)	2(20.0)	0(0.0)
Presco	Well water	10	6(60.0)	1(10.0)	1(10.0)	0(0.0)
Total		100	54(54.0)	13(13.0)	8(8.0)	5(5.0)

Key: ESBL Extended Spectrum Beta-lactamase, CPR- Carbapenem Resistant, CPS-Carbapenem Susceptible, CAS-Campus for Administrative Studies.

Table 8: Antibiotic resistant pattern of Carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from clinical samples

Antibiotics (µg) (n=5)	Urine (n=5)		Wound (n=4)		Pus (n=2)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin (15)	4(80.0)	1(20.0)	4(100)	0(0.0)	1(50.0)	1(50.0)
Azetronam (30)	5(100)	0(0.0)	4(100)	0(0.0)	2(100)	0(0.0)
Ceftriaxone(30)	4(80.0)	1(20.0)	4(100)	0(0.0)	2(100)	0(0.0)
Cefepime (30)	5(100)	0(0.0)	4(100)	0(0.0)	2(100)	0(0.0)
Colistin (10)	2(40.0)	3(60.0)	2(50.0)	2(50.0)	2(100)	0(0.0)
Gentamicin (15)	1(20.0)	4(80.0)	0(0.0)	4(100)	0(0.0)	2(100)
Ciprofloxacin (5)	1(20.0)	4(80.0)	0(0.0)	4(100)	0(0.0)	2(100)
Nitrofurantoin (100)	5(100)	0(0.0)	4(100)	0(0.0)	2(100)	0(0.0)

R-Resistance, S-Susceptible, n=Number of isolate

Table 9: Antibiotic resistant pattern of Carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from non-clinical samples

Antibiotics (µg)	Tomatoes (n=3)		Scent leaf (n=2)		Borehole water (n=2)		Well water (n=1)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin (15)	2(66.7)	1(33.3)	1(50.0)	1(50.0)	2(100)	0(0.0)	0(0.0)	1(100)
Azetronam (30)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	1(100)	0(0.0)
Ceftriaxone(30)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	1(100)	0(0.0)
Cefepime (30)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	1(100)	0(0.0)
Colistin (10)	2(66.7)	1(33.3)	2(100)	0(0.0)	1(50)	1(50)	1(100)	0(0.0)
Gentamicin (15)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	1(100)
Ciprofloxacin (5)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	1(100)
Nitrofurantoin (100)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	1(100)	0(0.0)

R-Resistance, S-Susceptible, n=Number of isolate

Table 10: Comparison of antibiotic resistant pattern of carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* Isolates from clinical and non-clinical sources

Antibiotic (µg)	Urine	Wound	Tomatoes	Scent leaf	Borehole water	p-value
Amikacin (15)	4(80.0)	4(100)	2(66.7)	1(50.0)	2(100)	.9999
Azetronam (30)	5(100)	4(100)	3(100)	2(100)	2(100)	
Ceftriaxone(30)	4(80.0)	4(100)	3(100)	2(100)	2(100)	
Cefepime (30)	5(100)	4(100)	3(100)	2(100)	2(100)	
Colistin (10)	2(40.0)	2(50.0)	2(66.7)	2(100)	1(50)	
Gentamicin (15)	1(20.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Ciprofloxacin (5)	1(20.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
Nitrofurantoin (100)	5(100)	4(100)	3(100)	2(100)	2(100)	

Table 11: Multiple antibiotic resistant Index of carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* from clinical and non-clinical sample

	Location	Sample	Multiple Antibiotic Resistant Index
Clinical samples			
	International Market	Tomatoes	0.5
		Scent leaf	0.7
		Uziza leaf	-
		Garden egg leaf	-
	Hausa Quarters		
		Carrot	-
	CAS		
		Borehole water	0.7
	Presco		
		Well water	0.5
Non-clinical samples			
		Urine	0.6
		Wound sample	0.5
		Pus	0.7

Key: CAS-Campus for Administrative Studies

4. DISCUSSION

Among all positive 66.1 % clinical cultures, *E. coli* was the most common isolated bacteria in urine 70.0%. The major findings of this study reiterate with a retrospective review of urine culture results of human clinical sample which has reported 14.2%²⁵ (Kibret and Abera, 2011), 34.0 %²⁶ (Ghaima *et al.*, 2018) 36.6%²⁷ (Alsharapy *et al.*, 2018), 49.3%²⁸ (Kpalap *et al.*, 2019), 62.3%²⁹ (Dela *et al.*, 2022), 66.6%³⁰ (Gatya Al-Mayahie *et al.*, 2022) *E. coli* from clinical samples. The high predominance of *E. coli* in urine sample is expected and it is well known that these bacteria represents a normal component of the intestinal microbiota of humans and animals and has strains with the potential of causing UTI and other extra intestinal infections. These UPEC strains have virulence traits that allow their successful colonization. Also, the high prevalence of *E. coli* may indicate a general lack of adherence to hygienic procedures, which are simple but effective measures to reduce the transfer of fecal bacteria.

Non-clinical sample had bacteria count of 2.7×10^3 CfU/ml and 2.2×10^3 CfU/ml from borehole and well water respectively. Both Nigerian national standards for drinking water and world Health Organization³¹. (WHO, 2004) earlier stated that the level of *E. coli* or thermo-tolerant bacteria should be zero in a 100 mL sample of water directly intended for drinking, as such all the borehole and well water sample analyzed in this study were not suitable for human consumption. Also the source of contamination might have come from the point of distribution, or leaking water pipes since the bore-hole water was allowed to flow for 5 min before the sample was taken. Additionally, that borehole water, which ought to be treated, must have been contaminate as a result of non-treatment, poor water treatment or as a result of leaky water pipes, which are buried in agricultural runoff, municipal drainage systems thus getting contaminated³². (Ateba *et al.*, 2020). Well water found with positive bacteria count might have been contaminated by water hoses use in fetching of water, which was normally left on the ground after used and reused without cleaning. Generally, the noted bacteria count from the two source may also be influence by the deep (shallow nature) of

the underground water and seasonal factor as this study was performed during rainy season and they may be a positive correlation between the prevalence of bacteria and season.

Non-clinical sample such as Scent leaf had 2.6×10^3 CfU/ml while the different in other fruit and vegetable were almost similar. The higher bacterial load in this sample may be partly due to the use of contaminated irrigation water and organic fertilizers in the farms, coupled with the poor hygienic environment in the area. Together these factors contribute to the microbial contamination during pre-harvest, harvesting, and poor handling practices at the post-harvesting, open display for marketing. These poor sanitary conditions were clearly observed when the vegetables and fruit were being sourced from the various markets. Additionally, the types of nutrients that are frequently present on the leaf surface of leafy green vegetables can be categorized into two categories: inorganic (ions) and organic molecules (organic acids and carbohydrates). Carbohydrates (dominant phyllosphere sugars are glucose, fructose and sucrose) are of special interest due to their capacity to readily support growth of enteric bacteria such as *E. coli*^{33, 34}. (Mogren *et al.*, 2018; Chelaghma *et al.*, 2022). The present of such nutrient may facilitate their presence in Scent leaf.

In addition, season, months may also show a significant effect on the isolation rate, bacterial contamination was higher for the fruit and vegetable samples since this study was performed during rainy seasons. Earlier study provided an insight into the influx of fecal pollution deterioration in the wet season, which was the opposite of organic pollution. This may have been due to rainfall. In the wet season, the feces of domestic animals and contaminated soils are easily washed into rivers by rain, and lead to the rise of bacteriological values. On the contrary, the absence of rain in the dry season reduces the dilution effect of water for pollution, which could cause a higher level of organic pollution³⁵. (Chen *et al.*, 2017).

Occurrence of *E. coli* in most water, vegetable and fruit is destructive to food safety. Safe food is essential and fundamental for a healthy, productive and reproductive life. Lack of access to safe food causes a destructive cycle of

disease, specifically affecting people with ill-health, children, and the elderly ³⁶. (WHO, 2020). According to the World Health Organization (WHO) report, 550 million people become ill, and 230,000 die yearly due to diarrheal diseases associated with the ingestion of foods already contaminated by microbial pathogens such as *E. coli* ³⁶. (WHO, 2020). In another report by World Bank (2018), the overall productivity loss linked to FBD in developing countries is estimated to cost \$95.2 billion annually, and the amount spent in treating FBD per year is estimated at \$15 billion. According to the report, the highest occurrence of food-borne diseases is in Asia and sub-Saharan Africa compared to other continents. Since man's existence, FBD has been a critical challenge for all nations and people of the world ^{37,38}. (WHO, 2008; Beshiru *et al.*, 2022).

The high prevalence of *E. coli* in non-clinical samples; water vegetable and fruit in this study ranged from 33.3-80.0 % and in the context of fruit and vegetable could further be ascribed to its processing (minimally processed) to retain the quality of nutrients and enhance palatability to the consumers. Thus, vegetables harboring *E. coli* could be traced to the farming activities via human and animal waste manure and wastewater/untreated water used for irrigation or various processing stages such as slicing and peeling, favoring its interaction with *E. coli* ³⁸. (Beshiru *et al.*, 2022).

In 2003, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) launched an initiative to support the consumption of fruit and vegetables to promote health around the world, with a recommended minimum intake of 400 g per day ^{34,39}. (Chelaghma *et al.*, 2022; Holzel *et al.*, 2018). Because vegetables are often eaten raw, their consumption may result in the ingestion of bacteria able to pose a serious threat to consumer health ^{34, 40} (Chelaghma *et al.*, 2022; Van Hoek *et al.*, 2015).

On the phenotypic level, the prevalence of CR producing ESBL strain accounted for 11(9.6 %) among clinical isolate. Co-expression of carbapenem resistant and ESBL production in clinical isolate is not surprising but harmonizes the findings of other researcher ^{28, 30, 41, 42, 43, 44, 45}. (Kpalap *et al.*, 2019; Gatya Al-Mayahie *et al.*, 2022; Nasiri *et al.*, 2020; Thapa *et al.*, 2022 Aminu, *et al.*, 2022; Alegría *et al.*, 2020; Adabara *et al.*, 2020). This partly due to increased use of carbapenems and broad spectrum cephalosporin and other antibiotics by physicians for treatment of serious and even non-serious cases in the Clinical settings. In Spain, it was revealed that the Carbapenem resistant *E. coli* occurred after a continuous increase in resistance to third-generation cephalosporins and fluoroquinolones ⁴⁶. (Ortega *et al.*, 2016). The production of all kinds of carbapenemases by *E. coli* isolates represent a major issue with further problem in enteric bacterial infections treatment ^{30, 47}. (Gatya Al-Mayahie *et al.*, 2022; Shaik *et al.*, 2017). From a therapeutic perspective, CR producing ESBL strain represent a threat as only a few antibiotics may retain activity against them. This is due to the ability of carbapenemases to hydrolyze most other β -lactam antibiotics, and to frequent coexistence in CRE isolates of additional mechanisms of resistance against other antibiotics such as fluoroquinolones and aminoglycosides ⁴⁸. (Moxon and Paulus, 2016).

CR producing ESBL strain was identified in 8.0 % of non-clinical sample. The presence of CR producing ESBL strain in Tomatoes and scent leaf is unknown and contamination may occur through animal manure fertilization. Although bla_{IPM}, bla_{NDM-1}, bla_{VIM-4} and bla_{OXA-48} genes has been found in vegetables in Algeria and Ethiopia ^{34, 49}. (Chelaghma *et al.*, 2022, Yigrem *et al.*, 2021) but the presence of carbapenem-resistant bacteria on fresh vegetables not only raises questions about the role of agriculture but could be linked to human contamination during post-harvest stages ^{50, 51}. (Liu *et*

al., 2018; Amato *et al.*, 2021). Various studies have investigated the potential sources of produce contamination in the supply chain at the post-harvest periods linked to humans, where they have reported that poor or inappropriate personal hygienic practices during transport, methods of storage, processing and preparation by handlers including sellers and consumers and the market environment also contribute to vegetable contamination ⁵². (Berger *et al.*, 2010). The potential implications of wastewater used for irrigation in the dissemination of antibiotic-resistant human pathogens should also not be neglected as suggested by recent studies ^{53, 54}. (Antwi-Agyei *et al.*, 2015; Makkaew *et al.*, 2016).

CR producing ESBL strain was noted in water source (well and borehole water). Therefore, these water sources may contain contaminants resulting from agricultural and anthropogenic activities which is probably due to incoming runoffs during rainfalls. This finding is in agreement with a study conducted by ⁵⁵. Mahmoud *et al.* (2020) in Sudan where OXA-48 gene was the most predominant gene, detected in 15.5% of the isolates from drinking water. The bla_{KPC} and bla_{SPM} genes were also detected in 4.4% and 8.8% of the isolates, respectively. In another study by ⁵⁶. Amaya *et al.* (2012) in Nicaragua; they reported that gene encoding for bla_{OXA} was detected in 1% of well-water samples. There was another study done by ⁵⁷. Tanner *et al.* (2019) they identified TEM, SHV and bla_{OXA-48}-type genes (3.9%) in US drinking water the result obtained by ⁵⁸. Haberecht *et al.* (2019) in Northern Colorado in which *E. coli* isolated from environmental water represented 4.2% of total suspected bla_{KPC}. There is evidence of the presence of Carbapenemase and ESBL-producing bacteria in drinking water in both low-and high-income countries worldwide ^{59, 60, 61, 62}. (De Boeck *et al.*, 20112; Abera *et al.*, 2016; Madec *et al.*, 2016; Larson *et al.*, 2019). The presence of a strain of CR-ESBL-producing bacteria in the present study suggests that drinking water may serve as a route of transmission and potential source of community-acquired infections with CR ESBL-producing *E. coli* with limited therapeutic option. In fact, one of the critical priorities of the World Health Organization is carbapenem-resistant ESBL-producing bacterial pathogens for which new antibiotics should be designed ^{30, 63}. (Gatya Al-Mayahie *et al.*, 2022; Martinez-Medina, 2021).

Both clinical and non-clinical CR producing ESBL strain portrayed high resistance to antibiotics such as Azetronam, Ceftriaxone, Cefepime, Nitrofurantoin, Amikacin and this observation concurred with other previous studies that had been conducted in Ghana ^{64, 29}. (Dela *et al.*, 2022; Akuffo *et al.*, 2017), in Iraq ³⁰. (Gatya Al-Mayahie *et al.*, 2022), in Algeria ³⁴. (Chelaghma *et al.*, 2022) and in Nigeria ³⁸. (Beshiru *et al.*, 2022).

Clearly, antibiotics resistance arises quickly and spreads rapidly, especially when resistance genes are horizontally transferred via plasmids and integrons among individuals, among species, and even among bacterial kingdom ³⁰. (Gatya Al-Mayahie *et al.*, 2022). Much of the problem of antimicrobial resistance has been shown to be due to the presence of transferable plasmids encoding MDR and their dissemination among different enterobacterial species and it is common for a single plasmid to simultaneously mediate resistance to multiple antimicrobials and to be shared among different bacterial genera ³⁰. (Gatya Al-Mayahie *et al.*, 2022).

On the context of clinical isolate, this is indicative of a gradual narrowing of the most routinely prescribed and readily available treatment options for diarrhoeagenic *E. coli* infection in the country due to the selective pressure that may have been placed on these antibiotics over the years, as well as the over-the-counter availability of some of these antibiotics as well.

In this context, resistant noted among isolate from vegetable and fruit opines that vegetables and fruit could be a possible route for the dynamic diffusion of antibiotic-resistance genes in the community. The ingestion of antibiotic-resistant bacteria is a potential public health threat since they are able to colonize the gut or pass through the intestines and exchange resistance genes with intestinal bacteria, facilitating their widespread dissemination in the environment ⁶⁵. (Blaak *et al.*, 2014).

Additionally, it has been speculated that the extensive application of antibiotics on crops might augment the incidence of antibiotic resistance in bacteria existing on plant surfaces ⁶⁶. (Stockwell & Duffy, 2012). In addition to their use for the control of bacterial diseases of plants, antibiotics can be introduced into agriculture via soil fertilization through the use of manure from animal farming. Furthermore, the soil may well represent an important source of antibiotic-resistant bacteria and genes to the plant ⁶⁷. (Iseppi *et al.*, 2018). In spite of this, soil fertilization with animal manure is a common agricultural practice worldwide. Antibiotic-resistant bacteria associated with manure and soil may enter the plant microbiome through colonizing the roots, which are in direct contact with soil, or the above ground parts of plants, possibly via the motility of root endophytes or air particulates. In this context, manure from dairy cows, usually used as a soil fertilizer, may harbor different resistant bacteria and resistance encoding genes from the gut microbiota of cattle ⁶⁸. (Zalewska *et al.*, 2021). This phenomenon has become a major problem for public health, not only in underdeveloped countries but also in high-performing, socio-economically developed countries ⁶⁷. (Iseppi *et al.*, 2018).

E. coli clinical isolates susceptible to Colistin was also noted in 50-60% of the bacteria, and this implies future resistance, which makes such isolates awkward to handle and must not be eliminated by the antibiotic. Additionally, colistin resistant proportion 66.7-100% was noted among non-clinical isolate. However, MCR-1 producers have been reported from vegetable in Algeria ³⁴. (Chelaghma *et al.*, 2022). The colistin resistant reported in study might have originated from wastewater. In addition, several studies have reported foodborne human outbreaks linked to the consumption of fresh vegetables irrigated with wastewater, and have highlighted that the type of irrigation practice to play a vital role in the contamination of farm produce ⁶⁹. (Adegoke *et al.*, 2018). Several studies have demonstrated a correlation between the presence of resistance genes in vegetables, fruits, soil and irrigation water ^{70, 71, 72, 73, 74}. (Cerqueira *et al.*, 2019a; 2019b; H'olzel *et al.*, 2018; Araújo *et al.*, 2017; Jongman and Korsten, 2016). This is a main issue in the development of resistant bacterial strains, which can infect humans, increasing the difficulty to treat severe infections ⁷⁵. (Conde-Cid *et al.*, 2018).

Comparison of antibiotic resistant pattern of carbapenem resistant extended spectrum beta-lactamase producing *Escherichia coli* isolates from clinical and non-clinical sources showed no statistical significant difference P -value < 0.05 i.e., the both isolate had similar pattern of resistant to the test antibiotic. This indicate that force driving antibiotic resistance do not differ between these two settings. The high resistance in these isolates can be linked with the widespread application of these antibiotics in agriculture and human medicine.

The increased prevalence of MDR *E. coli* pathotypes with MAR index value 0.5-0.7 in the studied non-clinical isolate proposes the necessity for expanding our acquaintance of the reservoirs, transmission pathways and sources of such MDR.

Notably, MAR index value 0.5, 0.6 and 0.7 of the clinical *E. coli* isolates in this study shows MDR, which is bothersome.

Although MDR patterns from one country to another differed but MAR index from ⁷⁶. Fallah *et al.* (2021) was 0.75, compared to ³⁸. Beshiru *et al.* (2022) (0.13–0.94). This high prevalence of MDR among the isolates of the present study is alarming and necessitate the need for the clinicians to ensure the use of appropriate antibiotics for recommended periods in adequate doses in order to prevent emergence of multidrug resistant organisms. The existence of specific MDR phenotypes in diverse samples designates cross-contamination amid procurement from similar farms or markets, irrigation and hospital effluent return flows can be a point source of ARGs that ultimately discharge into environment, and may later percolation to underground water, animals waste can also contaminate water or may be used as manure, bringing antibiotics residues and resistant bacteria into crops ⁷³. (Araújo *et al.*, 2017). In this way, antibiotics can enter into human food chain through consumption of raw fruit, vegetables, which can also carry antibiotic-resistant food-borne pathogenic bacteria.

Both isolate of ESBL producing *E. coli* resistance to carbapenem showed high sensitivity to ciprofloxacin and gentamicin in this study. For bacterial infections, these antibiotics are the most reliable last-resort treatment ^{77, 30, 34}. (Gatya Al-Mayahie *et al.*, 2022; Chelaghma *et al.*, 2022; Mhawesh *et al.*, 2021). Therefore, the limited use of these antibiotics might be helpful to inhibit/avoid the emerging or spreading of multidrug-resistant bacteria.

5. CONCLUSION

This study revealed that clinical and non-clinical isolates of ESBL producing *E. coli* are possible reservoirs for co-existence carbapenem resistant ESBL strain and highlighted that the vegetables/fruits (Scent leaf and Tomatoes), well water and borehole water samples were the most contaminated sample types. As both clinical and non-clinical isolate had similar pattern of resistant to the test antibiotic, this may be due to the excessive use of broad spectrum antibiotic agriculture and clinical practice. MDR portrayed by majority of the tested isolates shows that they originated from areas of high antibiotic use. However, these bacteria strains identified are of clinical importance and may pose treatment failures in the host during antibiotic therapy but retaining activity of ciprofloxacin and gentamicin may be sufficient for infection treatment. Therefore, further investigations are needed for PCR-based detection of antimicrobial resistance genes and ongoing surveillance of the spread of antibiotic resistance in epidemiological and environmental studies. This calls for studies to determine the extent to which transmission of antibiotics-resistant *E. coli* occurs and how such transfer impacts the efficacy of anti-*E. coli* strains used in environmental and human medicine becomes imperative. In addition, appropriate measures such as pretreatment of animal excrement before being used as fertilizers and the quality of irrigation water, hospital effluent discharge need to be taken into consideration

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