Original Research Article

Prevalence and Antibiogram profile of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* among patients with Urinary Tract Infection in Abakaliki

ABSTRACT

Background and Objectives:

Carbapenem antibiotic are drug of last-resort from the treatment of bacterial infection, as a result of the prevalent and rapidly evolving enzymes from Carbapem resistant bacteria such *Escherichia coli* and *Klebsiella pneumoniae* make UTI difficult, and in some cases impossible to treat in health care settings. With limited progress of new antibacterial drugs, the best approach is monitoring the Prevalence and Antibiogram profile of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* among patients with Urinary Tract Infection in Abakaliki.

Methodology:

A non-repetitive, clean catch mid-stream urine was collected from five hundred (500) diagnosed UTI inpatient and outpatient. The samples were evaluated using routine microbiological protocol for isolation and identification of *Escherichia coli* and *Klebsiella pneumoniae*. Phenotypic screening of Carbapenem-resistant strains was performed using Modified Hodge testing. Antibiogram studies of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* was performed using the Kirby–Bauer disk diffusion method and the results were interpreted using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. Multiple antibiotic resistance index (MARI) was determined for MDR strain.

Result: The prevalence of *Escherichia coli* and *Klebsiella pneumoniae* isolate accounted for 148(29.6 %) consisting of 95(54.3 %) and 53(16.3 %) from In-patients and out-patients. *Escherichia coli* accounted overall isolation rate of 112(22.4 %) comprising of high proportion among in-patient 82(46.9 %) over out-patient 30(9.2 %). The proportion of *K. pneumoniae* accounted for 36(7.2 %) with 13(7.4 %) and 23(7.1 %) recorded among in-patients and out-patients. Association between presence of *Escherichia coli* and *Klebsiella pneumoniae* isolates in clinical samples was statistically significant with patient's population with p value <0.05. Carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* accounted for 37(7.4 %) comprising of 24(13.7) and 13(4.0 %) among In-patients and Out-patients respectively while carbapenem susceptible *Escherichia coli* and *Klebsiella pneumoniae* accounted for overall detection rate of 111(22.2 %) consisting of 71(40.6 %) and 40(12.3 %) among In-patients and Out-patients respectively. The isolate resistance rate to cephalosporins were relatively high i.e., Cefotaxime, Cefoxtin Ceftazidime, Ceftriaxone resistance was observed at 60-100% while amoxicillin/clavulanate, azetronam, tetracycline nitrofurantoin and Ticarcillin-clavulanic acid recorded 100 % with MDR index ranged from 0.5-0.8, but were 100 % and 85.0 % sensitive to ciprofloxacin and ofloxacin.

Conclusion:

These results strongly hypothesize that MDR bacteria, including Carbapenem-resistant isolate, have become common residents in various hospital environments, however with substantial evidence in this study, ciprofloxacin and ofloxacin as drugs of choice could be used for treatment of UTI. Therefore, its importance that good antibiogram evaluation of other drug classes beside fluoroquinoles reported in this study need to be establishes as baseline for empirical diagnosis, epidemiological surveillance, drug prescriptions and infection management.

Keyword: Carbapenem-resistant, *Escherichia coli*, *Klebsiella pneumoniae*

1. INTRODUCTION

Urinary Tract Infections (UTIs) are infectious disease that involves microbial invasion and colonization of any part of the urinary tract [1, 2]. UTI encompasses a wide variety of clinical entities involving microbial invasion of any tissue of the tract from the renal cortex to the urethral meatus [3]. Also, bacterium which may lead to the infection of the prostate, epididymis or the testes, bladder and kidney are also included in the definition of UTI [3]. The prevalence of UTI is much more common in women than in men, at a ratio of 8:1, due to their anatomical and physiological arrangement [4]. One in five adult women experiences UTI in her life time [5]. The symptoms of UTIs

such as fever, burning sensations while urinating, Lower Abdominal pain (LAP), itching, formation of blisters and ulcers in the genital area, genital and suprapubic pain, and pyuria generally depend on the age of the person infected and the location of the urinary tract infected [6]. The major causative agents of UTIs are Gram-negative pathogens, primarily from the Enterobacteriaceae family including *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter* species *Citrobacter* species and *Klebsiella oxytoca* [7, 8]. Suwaiba *et al.* [1] and Niranjan *et al.* [9] reported a prevalence of 41.0% and 44.0% among Gram negative Enterobacteriaceae, associated with UTI. UTIs are common bacterial infections worldwide and affects around 150 million people annually [8] and contribute a significant financial burden in community and health system. The prevalent and rapidly evolving enzymes from Carbapemase resistant bacteria such *Escherichia coli* and *Klebsiella pneumoniae* make UTI difficult, and in some cases impossible to treat and have been associated with mortality rates up to 50% [10]. An isolate is considered a Carbapenem *Escherichia coli* and *K. pneumoniae* if it is resistant to imipenem, meropenem, doripenem, or ertapenem by susceptibility testing or if it is identified to have a carbapenemase gene. Resistances to carbapenem group of antimicrobials among *Escherichia coli* and *K. pneumoniae* due to production of carbapenemases, pose serious challenges in the treatment of UTI in healthcare settings [11]. Carbapenem resistance is progressively spreading among clinical isolates of *E. coli and K. pneumoniae* [12, 13, 14] challenging the empiric treatment worldwide. Due to the movement of patients throughout the health care system, if Carbapem Resistant bacteria such *Escherichia coli* and *Klebsiella pneumoniae* is a problem in one facility, then typically they are problem in other facilities in the region as well. Carbapemase Resistant bacteria such *Escherichia coli* and *Klebsiella pneumoniae* are mostly endemic in specific geographical regions, but reports of their spread into other geographical locations are point of grave concern these days. In Nigeria there have been reports of carbapenemase producing clinical isolates of enteric bacteria particularly among *E. coli* and *Klebsiella* species [1, 15, 16]. These strains become a serious threat to public health, associated with high mortality rates and have the potential to spread widely. With limited progress of new antibacterial drugs, the best approach is monitoring of these highly resistant strains by focusing on samples collected from UTI adult patients in Alex Ekwueme Federal Teaching Hospital, Abakaliki. Such data will serve an important role in understanding the spread pattern of CR *Escherichia coli* and *Klebsiella pneumoniae* in adult with UTI.

2. MATERIALS AND METHODS

2.1 Inclusion and Exclusion Criteria

All adult inpatients and outpatients at Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi State during the period of this study was included. All adult inpatients and outpatients with frequent urination, burning sensations while urinating, lower abdominal pain (LAP), itching, formation of blisters and ulcers in the genital area, genital and suprapubic pain are included in the study. All adult inpatients and outpatients who has not been on an antibiotics for more than 2 weeks, and paediatrics and children were excluded. No arrangement was made for alternative ways of communicating with patients that has hearing/speech impairment, and patients who cannot understand English, Igbo, pidgin or the native Abakaliki dialect. Patients suspected to have other diseases was excluded from the study as well as patients who decline consent.

2.2 Data Collection and Clinical Assessment

Information of the patients enrolled in the study was obtained from the hospital's electronic medical records in accordance with the objectives of this study while the clinical characteristics of each patient were composed of two parts: (1) basic information including Gender, (2) Admission status including Inpatient or outpatient. All the information obtained from the studied subjects was coded to maintain confidentiality.

2.3 Sample Collection

About 5 ml of a non-repetitive, clean catch mid-stream urine was collected from five hundred (500) diagnosed UTI inpatient and outpatient. All sample containers was labeled with the unique sample number; date and time of collection. The sample Containers was transported within 1-2hours of collection in an ice-pack to the Microbiology laboratory Unit of Ebonyi State University for routine microbiological protocol [17].

2.4 Isolation, Purification and Characterization of Test Organism

The collected urine samples were analyzed for the presence of *Escherichia coli* and *Klebsiella pneumoniae* by inoculating a loopful of each sample into a separate tube of sterile nutrient broth (Merck Co., Germany) and incubated at 37 °C for 24 h. After overnight incubation, a loopful of the turbid broth culture was aseptically seeded

by streaking on sterile solidified, Eosin Methylene blue agar and MacConkey agar (Merck Co., Germany) and was incubated at 37 °C for 24h. Suspected *Escherichia coli* and *Klebsiella pneumoniae* from positive cultures were identified by their characteristic appearance (color, consistency, shape) on the differential media. Each mucoid-pink and metallic sheen colonies were sub-cultured on sterilized solidified Nutrient agar (Merck Co., Germany) and incubated at 37 °C for 24 h for Gram staining reaction and biochemical testing profiles, using standard procedures [17]. *Escherichia coli* and *Klebsiella pneumoniae* were further confirmed using VITEK 2 System (bioMerieux, France) [18].

2.5 Modified Hodge testing

Bacterial isolates which were resistant to imipenem (IPM 10 µg), doripenem (10 µg), meropenem (MEM 10 µg) and ertapenem (ERT10 µg) based on CLSI breakpoints [19].

2.6 Antimicrobial Sensitivity Testing

Antimicrobial susceptibility was performed by employing Kirby Bauer disk diffusion method using sterilized Mueller Hinton agar in accordance with the guidelines of clinical and laboratory standards institute [19]. Bacteria suspension of the test isolate was prepared using 0.5 McFarland standards and seeded on solidified Mueller–Hinton agar. The plates were allowed to pre-diffuse for 5 minute. Thereafter, the following antibiotic: amoxicillin-clavulanic acid (20/10 µg), amoxicillin (30 µg), azetronam (30 µg), cefoxitin (30 µg) ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), colistin (10 µg), chloramphenicol (10 µg), ciprofloxacin (5 µg), gentamicin (15 µg), ofloxacin (5 µg), nitrofurantoin (100 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), ticarcillin-clavulanic acid (85 µg), imipenem (10 µg), ertapenem (10 µg), meropenem (10ug), doripenem (10µg) was impregnated on the inoculated Mueller-Hinton (MH) agar plates and incubated at 37 ℃ for 24 hours. After overnight incubation, the diameters of zones of inhibition were measured, and results interpreted in accordance with the criteria of Clinical and Laboratory Standards Institute [19].

2.7 Determination of Multiple antibiotics resistance index (MARI) Multiple antibiotic resistance index (MARI) was calculated to determine the multiple antibiotic resistance profile of the isolated *Escherichia coli* and *Klebsiella pneumoniae* isolates that were positive for Carbapenemase enzyme production. This was done according to Peter *et al*. [20]. MARI was calculated using the formular: MARI = a/b; where 'a' represents the number of antibiotics which the resistant bacteria was resistant to; and 'b' represents the total number of antibiotics to which the resistant bacteria has been evaluated for.

2.8 Data Analysis

The data collected were analyzed by SPSS software statistical application version 20 (SPSS INC, Chicago, IL, USA). Fisher's exact test (X^2) was used to determine the association between presence of *Escherichia coli* and *Klebsiella pneumoniae* isolates in clinical sample and patient's population. T-independent test was used to determine the difference in the prevalence of *Escherichia coli* and *Klebsiella pneumoniae* in male and female inpatient and out-patient with UTI. Statistical significant was set at p value <0.05 [21].

3. RESULT AND DISCUSSION

3.1 Distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates in urine samples of in and out UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA).

The overall frequency of *Escherichia coli* and *Klebsiella pneumoniae* isolate accounted for 148(29.6 %) consisting of 95(54.3 %) and 53(16.3 %) from In-patients and out-patients. *Escherichia coli* accounted overall isolation rate of 112(22.4 %) comprising of high proportion among in-patient 82(46.9 %) over out-patient 30(9.2 %). The proportion of *K. pneumoniae* accounted for 36(7.2 %) with 13(7.4 %) and 23(7.1 %) recorded among in-patients and out-patients. Association between presence of *Escherichia coli* and *Klebsiella pneumoniae* isolates in clinical samples was statistically significant with patient's population with p value <0.05 as shown in Table 1.

3.2 Distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates from urine samples of male and female UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA).

The distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates from urine samples of UTI patients revealed high proportion of *K. pneumoniae* among female in-patients 8(8.0 %) over male in-patients 5(6.7 %). *Escherichia coli* accounted for 60(60.0 %) and 22(29.3 %) in female in-patients over male in-patients respectively. Female Out-patients accounted for isolation rate of *Escherichia coli* 20(10.0 %) compare to male counterpart 10(8.0 %) while, *K. pneumoniae* among female out-patients accounted for 18(9.0 %) over male in-patients 5(4.0%) as shown Table 2. There was no statistically significant difference in the prevalence of *Escherichia coli* and *Klebsiella pneumoniae* in male and female inpatients and out-patients with UTI ($P < 0.05$).

3.3 Distribution of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from urine samples of in and out UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

Carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* accounted for 10(2.0 %) and 27(5.4 %) among *Klebsiella pneumoniae* and *Escherichia coli*. Among in-patients, carbapenem resistant *Klebsiella pneumoniae* was 4(2.3 %) while carbapenem resistant *Escherichia coli* accounted for 20(11.4 %). Out-patients harbor 6(1.8 %) and 7(2.2 %) carbapenem resistant *Klebsiella pneumoniae* and carbapenem resistant *Escherichia coli* respectively as shown Table 3.

3.4 Distribution of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in urine samples of male and female UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

The distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates from urine samples of UTI patients revealed high proportion of carbapenem resistant *K. pneumoniae* among female in-patients 3(3.0 %) over male in-patients 1(1.3 %). Carbapenem resistant *Escherichia coli* accounted for 13(13.0 %) and 7(9.3 %) in female in-patient over male in-patients respectively. Female Out-patients accounted isolation rate of Carbapenem resistant *Escherichia coli* 4(2.0 %) compare to male counterpart 3(2.4 %) while Carbapenem resistant *K. pneumoniae* among female out-patients accounted for 4(2.0 %) in male in-patients 2(1.6 %) as shown Table 4.

3.5 Summary of distribution of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolate from urine samples of UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

Carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* accounted for 37(7.4 %) comprising of 24(13.7) and 13(4.0 %) among In-patients and Out-patients respectively while carbapenem susceptible *Escherichia coli* and *Klebsiella pneumoniae* accounted for overall detection rate of 111(22.2 %) consisting of 71(40.6 %) and 40(12.3 %) among In-patients and Out-patients respectively as presented in Table 5.

3.6 Antibiotic susceptibility profile of carbapenem resistant *K. pnuemoniae* and *Escherichia coli* from Urine samples of UTI in-patients and out-patient at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

Carbapenem resistant *K. pnuemoniae* from in-patient demonstrated high level of resistant to Amoxicillin-Clavulanic, Azetronam, Cefotaxime, Cefoxitin, Ceftazidime, Ticarcillin-clavulanic acid and Chloramphenicol recording 100% respectively while Carbapenem resistant *K. pnuemoniae* strain were 80.0%, 60.0% and 100% sensitive to Colistin, Ofloxacin and Ciprofloxacin respectively as shown in Table 6. Carbapenem resistant *K. pnuemoniae* strain from out-patient were susceptible to cefoxitin 40.0%, Colistin 80.0% Gentamicin 20.0%, Ofloxacin 60.0 % and ciprofloxacin 100% but were extremely resistant to Nitrofurantoin 100%, Ceftriaxone 100%, Cefotaxime 100%, Amoxicillin 100%, Chloramphenicol 100% and Trimethoprim-Sulfamethoxazole 100% as shown in Table 6. Amongst in-patient, majority of carbapenem resistant *Escherichia coli* strain were highly resistant to Amoxicillin 100%, Azetronam 100%, Nitrofurantoin 100%, Ceftazidime 100%, Ceftriaxone 100% and Gentamicin 42.9% but 71.4%, 71.4 % and 85.7% susceptible to Colistin, ofloxacin and Ciprofloxacin respectively as shown in Table 6. Majority of carbapenem resistant *Escherichia coli* strain from out-paitent were highly resistant to Amoxicillin 100%, Azetronam 100%, Nitrofurantoin 100%, Ceftazidime 100%, Ceftriaxone 100% and Gentamicin 42.9% but 71.4%, 71.4 % and 85.7% susceptible to Colistin, ofloxacin and Ciprofloxacin respectively as shown in Table 6. All the strain demonstrated multidrug resistant with MARI value within the range of 0.6 and 0.8 exhibited by *Klebsiella*

pneumonia and *Escherichia coli* isolate from In-patients while 0.5-0.6 was recorded against *Klebsiella pneumonia* and *Escherichia coli* isolate from Out-patients sample source as presented in Table 7.

Table 1: Distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates in urine samples of in and out UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA).

Patient's Population	No. sampled	*K. pneumoniae* (%)	*E. coli* (%)	Frequency (%)	p-*value**
In-patients	175	13(7.4)	82(46.9)	95(54.3)	.00001
Out-patients	325	23(7.1)	30(9.2)	53(16.3)	
Total	**500**	**36(7.2)**	**112(22.4)**	**148(29.6)**	

Table 2: Distribution of *Escherichia coli* and *Klebsiella pneumoniae* isolates from urine samples of male and female UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA).

Gender	Patient's Population	No. sampled	*K. pneumoniae* (%)	*Escherichia coli* (%)	Frequency (%)	p-*value**
	In-patients					
Male		75	5(6.7)	22(29.3)	27(36.0)	.1391
Female		100	8(8.0)	60(60.0)	68(68.0)	
		175	**13(7.4)**	**82(46.9)**	**95(54.3)**	
	Out-patients					
Male		125	5(4.0)	10(8.0)	15(12.0)	
Female		200	18(9.0)	20(10.0)	38(19.0)	
		325	**23(7.1)**	**30(9.2)**	**53(16.3)**	
Total		**500**	**36(7.3)**	**112(22.4)**	**148(29.6)**	

Table 3: Distribution of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from urine samples of in and out UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

		Klebsiella pneumoniae			***Escherichia coli***		
Patient's Population	**No. sampled**	**No. of isolate**	**CPR (%)**	**CPS (%)**	**No. of isolate**	**CPR (%)**	**CPS (%)**
In-patients	175	13(7.4)	4(2.3)	9(5.1)	82(46.9)	20(11.4)	62(35.4)
Out-patients	325	23(7.1)	6(1.8)	17(5.2)	30(9.2)	7(2.2)	23(7.1)
Total	**500**	**36(7.2)**	**10(2.0)**	**26(5.2)**	**112(22.4)**	**27(5.4)**	**85(17.0)**

Key: **CPR-** Carbapenem resistant, **CPS-** Carbapenem Susceptible

Table 4: Distribution of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates in urine samples of male and female UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

Gender	Patient's Population	No. sampled	*Klebsiella pneumoniae*			*Escherichia coli*		
			No. of isolate	CPR (%)	CPS (%)	No. of isolate	CPR (%)	CPS (%)
	In-patients							
Male		75	5(6.7)	1(1.3)	4(5.3)	22(29.3)	7(9.3)	15(20)
Female		100	8(8.0)	3(3.0)	5(5.0)	60(60.0)	13(13.0)	47(47.0)
		175	**13(7.4)**	**4(2.3)**	**9(5.1)**	**82(46.9)**	**20(11.4)**	**62(35.4)**
	Out-patients							
Male		125	5(4.0)	2(1.6)	3(2.4)	10(8.0)	3(2.4)	7(5.6)
Female		200	18(9.0)	4(2.0)	14(7.0)	20(10.0)	4(2.0)	16(8.0)
		325	**23(7.1)**	**6(1.8)**	**17(5.2)**	**30(9.2)**	**7(2.2)**	**23(7.1)**
	Total	**500**	**36(7.2)**	**10(2.0)**	**26(5.2)**	**112(22.4)**	**27(5.4)**	**85(17.0)**

Key: **CPR-** Carbapenem resistant, **CPS-** Carbapenem Susceptible

Table 5: Summary of distribution of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolate from urine samples of UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

Patient's Population		Enterobacteriaceae	
		CPR (%)	**CPS (%)**
In-patients (n=175)			
	Klebsiella pneumoniae	4(2.3)	9(5.1)
	Escherichia coli	20(11.4)	62(35.4)
		24(13.7)	**71(40.6)**
Out-patients (n=325)			
	Klebsiella pneumoniae	6(1.8)	17(5.2)
	Escherichia coli	7(2.2)	23(7.1)
		13(4.0)	**40(12.3)**
Total (n=500)		**37(7.4)**	**111(22.2)**

Key: **n=** number of isolate, **CPR-** Carbapenem resistant, **CPS-** Carbapenem Susceptible

Table 6: Antibiotic susceptibility profile of carbapenem resistant *K. pnuemoniae* and *Escherichia coli* from Urine samples of UTI in-patients and out-patient at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

	Carbapenem resistant *K. pnuemoniae*				**Carbapenem resistant *Escherichia coli***			
	In-patient (n=4)		**In-patient (n=6)**		**In-patient n=20)**		**(out-patient n=7)**	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
AMX CA (20/10)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
AMX (30)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
ATM (30)	4(100)	0(0.0)	6(100)	0(0.0)	19(95.0)	1(5.0)	7(100)	0(0.0)
CAZ (30)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
CRO(30)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
FOX (30)	4(100)	0(0.0)	3(60.0)	2(40.0)	20(100)	0(0.0)	7(100)	0(0.0)
CTX (30)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
CT (10)	2(50)	2(50.0)	1(20.0)	5(80.0)	4(20)	16(60)	2(28.6)	5(71.4)
C (10)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
CIP (5)	0(0.0)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	1(14.3)	6(85.7)
CN (15)	2(50.0)	2(50.0)	5(80.0)	1(20.0)	15(75.0)	5(25.0)	7(100)	0(0.0)
OFX (5)	1(25.0)	3(75.0)	2(40.0)	3(60.0)	3(15.0)	17(85.0)	7(100)	0(0.0)
F (100)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
TE (30)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
SXT (25)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)
TIC (85)	4(100)	0(0.0)	6(100)	0(0.0)	20(100)	0(0.0)	7(100)	0(0.0)

Key: CA- Amoxicillin CA **Clavulanic Acid, AMX-**Amoxicillin**, ATM-**Azetronam**, CAZ-**Ceftazidime**, CRO**-Ceftriaxone**, FOX-**Cefoxitin**, CTX-**Cefotaxime**, CT-**Colistin, **C-**Chloramphenicol**, CIP-** Ciprofloxacin, **CN-**Gentamicin, **OFX-**Ofloxacin, **F-** Nitrofurantoin, **TE-** Tetracycline**, SXT-** Trimethoprim-Sulfamethoxazole, **TIC**-Ticarcillin-clavulanic acid, **n=**Number of isolate, **R-**Resistance, **S-**Susceptible **%-** Percentage

Table 7: Multiple Antibiotic Resistant Index (MARI) of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from urine samples of UTI patients at Alex Ekwueme Federal University Hospital Teaching Hospital (AE-FEUTHA)

	MARI	
Patient's Population	***Klebsiella pneumoniae***	***Escherichia coli***
In-patients	0.6	0.8
Out-patients	0.5	0.6

4. DISCUSSION

The phenotypic detection of Carbapenem-resistant in this study was identified in 37(7.4 %) of the isolate using only MHT. Carbapenem-resistant among *E. coli* and *K. pneumoniae* has been reported by other author [22, 23, 24]. The prevalence of CRKP among *K. pneumoniae* was low recording 4(2.3 %) and 6(1.8 %) inpatient and outpatient. These findings also congruent with other results obtained by previous studies; were 4.05 % in Malaysia, 5.5 % in Israel, 5.0 % in Argentina, 5.6 % in Ethopia, 8.0 % in USA and 13.0 % in Greece was reported [25, 26, 27, 28, 29, 30] and other Asian studies [14, 31, 32]. The increasing prevalence of carbapenem-resistant *K. pneumoniae* is a public health concern of major importance in Europe, particularly in Greece reporting the highest percentages (60.5%) of carbapenem-resistant *K. pneumoniae* isolates [33]. In recent years, the rapid dissemination of carbapenem-resistant *K. pneumoniae* and *E. coli*, a critical priority pathogen listed by WHO, has become a global threat to human health due to high morbidity and mortality. Based on the data of the CHINET antimicrobial resistance surveillance program for 2005–2017, the prevalence of carbapenem-resistant *K. pneumoniae* in China has dramatically increased from 3% to 20.9% with children even higher than adults [34, 35]. The prevalence of CRKP worldwide varies, partially depending on the cultural or population exchange relationship between countries and possible reservoirs of the carbapenemase producer.

The proportion of carbapenem-resistant *E. coli* was 7(2.2 %) and 20(11.4 %) among inpatient and outpatient. This observation echoes with a Systematic Review and Meta-Analysis of Cross-Sectional Studies from Iran which revealed the pooled rates of resistance to carbapenem in *E. coli* 5.0% (95% CI 2.0–8.0), while In India, 29.03 % of Carbapenem-Resistant *Escherichia coli* isolates was reported [36]. Also, Carbapenem resistant has been widely reported mostly in *Escherichia coli* [37, 38, 39] and may result from the frequent occurrence and virulent nature of this bacteria in UTIs.

Carbapenem-resistant *Klebsiella pneumoniae* isolate accounted for 24(13.7 %) among inpatients. This partly due to increased use of carbapenems and broad spectrum cephalosporin and other antibiotic by physicians for treatment of serious and even non-serious cases in the Clinical settings.

CR strains 50.0 % and 100% resistant to colistin was commonly observed in this study and may depict the persistence of colistin resistant in this area. Colistin a polymyxin which has been used extensively in the past (1940s–1970s) against Gram-negative bacteria but was abandoned because of its nephrotoxic and neurotoxic side effects. However, this forgotten drug got back in use in the early 2000s due to the emergence of carbapenem-resistant Gram-negative bacteria which were found to be susceptible to polymyxins [40]. Unfortunately, as the use of colistin increased, the colistin resistance among carbapenem-resistant GNR increased as well [41]. Spread of colistin-resistant *K. pneumoniae* has also been described in Italy [42, 43]. In particular, an outbreak involving different wards of the ARNAS general hospital Civico, di Cristinae Benfratelli in Palermo, Italy was reported in the period from June to December 2011 [42]. Two recent multicenter clinical and laboratory studies on carbapenem-resistant *K. pneumoniae* isolates from medical centers in USA revealed colistin-resistance in 13% and 16% of the isolates [44, 45]. Additionally, Colistin-resistant *E. coli*, 3 (75.0%) and 2 (50%) of them harbored plasmid-mediated and chromosomal *mcr-1* gene respectively [46]. Percent of *E. coli* resistance to colistin was 33.3%, and for *Klebsiella*, it was 31.6% reported by Qadi *et al.* [47] while Alfoiuzan *et al.* [48] reported resistance of 4.3% for *E. coli* and 7.7% for *Klebsiella* (Alfoiuzan *et al*., 2018). According to Prim *et al.* [49] the overall prevalence of colistin resistance was 0.67%. The rates were higher in \ *Escherichia coli* (0.5%) over *Klebsiella pneumoniae* (0.4%). One third of the isolates were multi-drug resistant (MDR). The high prevalence of colistin resistance in this setting or studied area confirm the role of possible modifications in the *mgrB* gene based upon the findings of earlier studies identifying this gene as a critical target for the development of colistin resistance in enterobacteria [50, 51]. The isolates resistant to colistin was commonly observed in this study and may depict the persistence of colistin resistant in this study area. Such trend could be linked to exposure to sublethal doses of colistin by the female and male counterpart as last-line antibiotic in treatment of recurrent or complicated enterobacteria infections.

Isolates from out-patient and in-patient exhibited high level resistant to nitrofurantoin 100 % respectively. When these are considered in relation to other findings they seem to be in contrary. Odongo *et al.* [52] from their study showed that 70% of the isolates were susceptible to nitrofurantoin while Onanuga *et al.* [53] documented 100% susceptibility to nitrofurantoin. An earlier study involving non-pregnant women had shown high sensitivity of the isolates to nitrofurantoin at 100% [54]. Likewise, another study reported a similar susceptibility of 95.9 % and 78 %

to nitrofurantoin [23, 55]. Result from this study shows a decline in the sensitivity rates of these isolates to this antibiotic. The increasing resistance could be due to increased overuse and misuse of nitrofurantoin in the study area due to the cheap costs and ready availability of this drug as it's mostly recommended in the empirical treatment of urinary tract infections. The persistor cells (defined as metabolically inactive cells that neither grow or die when exposed to bactericidal concentrations of the antibiotics) presents another important challenge as these cells tend to be associated with treatment failure, recurrence, and chronic infections as they continue to replicate after the antibiotic therapy is discontinued.

In addition, the resistance rate to cephalosporins were relatively high i.e., Cefotaxime, Cefoxtin Ceftazidime, Ceftriaxone was observed at 60-100% and is in support with the findings of Yan *et al.* [56] were Carbapenem-resistant *Klebsiella pneumoniae* isolates demonstrated 78.2% and 75.6% resistant to ceftriaxone and ceftazidime with similar findings reporting cefoxitin 83.3%, ceftriaxone 100%, ceftazidime 95.8% [40]; ceftazidime 66.7% and ceftriaxone 92.3% [57] and more recent study documented carbapenem-resistant *Klebsiella pneumoniae* isolates 100% resistant to ceftriaxone and ceftazidime [58]. Also, this finding correlate with an earlier study in the same setting (Abakaliki) were resistant to cefotaxime (83.6%), ceftazidime (79.5%) and ceftriaxone (57.5%) were reported [59] while Adabara *et al.* [60] in Minna reported resistant to cefotaxime (84.6%).. This study conducted also established that most Carbapenem-resistant isolates are multidrug resistant (MDR), especially to 3rd and 4th generation cephalosporins. The high rate of resistance in this study was ascertained to be due to indiscriminate use and abuse of beta-lactam antibiotics by individuals have caused problems in the treatment of microbial infections and diseases caused by these antibiotic-resistant organisms as a result of carbapemase production.

The MDR resistant trend of carbapenem-resistant isolates to trimethoprim/sulfamethoxazole, amoxicillin/clavulanate, azetronam and tetracycline in this study has not changed from the reported pattern in earlier studies [40, 59, 61, 62, 63, 64]. Additionally, the high percentages of Carbapenem-resistant isolates cross-resistance observed to various antimicrobials are of concern in clinical medicine, especially in intensive care units and other relevant unit. It is possible that the genes encoding carbapenemases are located on genetic elements, such as integrons and transposons, in association with conjugative plasmids typically carrying genes for resistance to other antimicrobials [65] as seen in this study. Additionally, beta-lactamase inhibitor (amoxicillin/clavulanate) resistance reiterate or suggested that the *in vitro* resistance to amoxicillin/clavulanate acid in Gram-negative bacteria could be used as a pointer or rationale to the actual level of *in vitro* resistance to Ticarcillin-clavulanic acid and other beta-lactamase inhibitor i.e., the resistance to any antimicrobial agent in the beta-lactamase inhibitor class has an impact on the resistance of other agents within this drug class.

Carbapenem-resistant *E.coli* and *Klebsiella pneumoniae* strain evaluated in this study exhibited inconsistent frequencies of susceptibility to Gentamicin. This observation raises concern on the stability of this aminoglycoside antibiotics during treatment of enterobacteria infection in the study setting. Resistance to Gentamicin could be particularly common among women with a history of prior UTI. Interestingly, aminoglycoside are widely used for the treatment of UTIs in patient. It is possible that infections may be more difficult to eradicate because of the higher rates of antibiotic resistance observed in strains isolated from males and female, which may lead to recurrent infections. Susceptibility analysis of isolates to antibiotics prior to treatment choice is recommended. The high resistance to aminoglycoside in this study could be that, this antibiotic may have been misused or abused in the study location.

Additionally, the MDR profile of Carbapenem-resistant strain from in-patient corresponded with data from out-patient in this study with MARI value of 0.5-0.8 and thus corroborate with existing literature [12, 38, 64]. Such pattern of MDR between the two entities (in-patients and out-patients) may likely depict similar genetic clone of isolates been responsible for the distribution of Urinary tract infection in the studied hospitals. Although, clonal spread was not assessed. This finding strongly suggests the role of cross-transmission within and between hospital community in our epidemiological setting.

In accordance to the susceptibility profile of ofloxacin, ciprofloxacin in this study. Hassuna *et al.* [66] showed susceptibility to ciprofloxacin at 86.25% while Isegohi *et al.* [63] affirmed in their studies that isolates were highly susceptible to Ofloxacin (65%) also majority of the Carbapenem-resistant *Klebsiella pneumoniae* strain were sensitive to ciprofloxacin 100% this observation congruent with some other studies which reported different trend of susceptibility 57.6%, 70.9%, 75.0%, 65%, [32, 67, 68, 69] while recent report in Pretoria South African documented PMQR gene resistant determinant (*aac-lb-6-cr, Qnr*- potent Plasmid Mediated Quinolone Resistant gene capable of hydrolying fluoroquinolone) in Carbapenem-resistant *Klebsiella pneumoniae* strain [70], this study thus advocate for

judicious use of ciprofloxacin against this strain. In contrast to the susceptibility profile of ciprofloxacin and ofloxacin few studies has documented resistant among CR strain [31, 57] these indicate that the force driving antibiotic resistant differ between two setting and may change overtime.

5. CONCLUSION

This study reports the prevalence of carbapenem-resistant *Klebsiella pneumoniae* and *E.coli* among UTI patient. These isolates exhibited a high level of resistance to carbapenems, another antibiotic studied, and their infections are typically associated with a high mortality and morbidity rate. Although the current study's findings do not clearly distinguish whether CR isolates clones are of hospital or community origin using molecular methods, these findings strongly suggest that MDR bacteria, including CR isolates, have become common residents in various hospital environments, particularly wards. However, ciprofloxacin and ofloxacin, as drugs of choice in this study, could be used for the treatment of UTI. As a result, it is critical to establish good antibiogram evaluation as a baseline for empirical diagnosis, epidemiological surveillance, drug prescriptions, and infection management.

CONSENT

All authors declare that written informed consent was obtained from the patient or care-giver of the patient before collection of sample.

ETHICAL CONSIDERATION

The approval for this study was gotten from the research and ethics committee of Ministry of Health Ebonyi State, with Ethical clearance number SMOH/ERC/042/21

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Suwaiba M, Dadah AJ, Sanusi SB. Prevalence of Carbapenem resistant *Escherichia coli* and *klebsiella pneumoniae*in urine samples of Patients Attending Selected General Hospitals within Kaduna Metropolis. *Sci. World J.* 2020; 15(4):23-67.

2. Al Yousef SA, Younis S, Farrag E, Moussa HS, Bayoumi FS, Ali AM. Clinical and Laboratory Profile of Urinary Tract Infections Associated with Extended Spectrum β-Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae. Annals of Clin and Laboratory Sci.* 2016; 46(4):393-400.
3. Pokharel B, Koirala J, Dahal R, Mishra S, Khadga P, Tuladhar N. Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella enterica* (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. *Int J Infect Dis.* 2006; 6: 434–438.
4. Shirishkumar P, Taviad PP, Mala S, Javadekar TB, Chaudhari VP. Urinary Tract Infections among Patients (UTI) among patients at GG Hospital and Medical College, Jamnagar. *National J Community Med.* 2012; 3(1):138–41
5. Ngong IN, Fru-Cho J, Yung MA, Akoachere JKT. Prevalence, Antimicrobial Susceptibility Pattern and Associated Risk Factors for Urinary Tract Infections in Pregnant Women Attending ANC in some Integrated Health Centers in the Buea Health District. *BMC Pregnancy Childbirth.* 2021; 21:673-674.
6. Amali O, Indinyero MD, Umeh EU, Awodi NO. Urinary tract infections among female students of the university of agriculture, Makurdi, Benue State, Nigeria, *Internet J Microbiol.* 2009; 7(1):1–5.
7. Tenney J, Hudson N, Alnifaidy H, Li JTC, Fung KH. Risk factors for aquiring multidrug-resistant organisms in urinary tract infections: A systematic literature review, *Saudi Pharm J.* 2018; 26:678–684.
8. Nasir F, Khan MI, Kashif S, Uddin F, Naseer A, Masood S. Prevalence of ESBLs secreting and carbapenem-resistant *E. coli* from urinary tract infection. *Rawal Med J.* 2021; 46:3-23.

9. Niranjan BP, Shetye S, Manjusha A, Mihir V, Zaheer V, Parijat G, Bharat S. Prevalence and Susceptibility Analysis of Gram Negative Pathogens in Tertiary Care Transplant Hospital, Mumbai. *Asian J Res Med Pharm Sci.* 2018; 4(20):1-8

10. Eshetie S, Unakal C, Gelaw A, Ayelign B, Endris M, Moges F. Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. *Antimicrob Resist Infect Cont.* 2015; 4:12-23.
11. Grover SS, Doda A, Gupta N, Gandhoke I, Batra J, Hans C, Khare, S. New Delhi metallo-β-lactamase - type carbapenemases producing *Escherichia coli* Isolates from Hospitalized Patients: A pilot study. *Indian J Med Res.* 2017; 146:105-110
12. Thapa A, Upreti MK, Bimali NK, Shrestha B, Sah AK, Nepal K, Dhungel B, Adhikari S, Adhikari N, Lekhak B, Rijal KR. Detection of NDM Variants (*bla*NDM-1, *bla*NDM-2, *bla*NDM-3) from Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae*: First Report from Nepal. *Infect and Drug Resist.* 2022; 15 4419–4434.
13. Tian D, Pan F, Wang C, Sun Y, Zhang H. Resistance Phenotype and Clinical Molecular Epidemiology of Carbapenem-resistant *Klebsiella pneumoniae* among Pediatric Patients in Shanghai. *Infect and Drug Resist.* 2018; 11:1935–43.
14. Tian X, Zheng X, Sun Y, Fang R, Zhang S, Zhang X, Lin J, Cao J, Zhou T. Molecular Mechanisms and Epidemiology of Carbapenem-Resistant *Escherichia coli* Isolated from Chinese Patients During 2002–2017. *Infect and Drug Resist.* 2020; 13 501–512
15. Akinduti PA, Ejilude O, Motayo BO, Adeyokinu AF. Emerging multidrug resistant AmpC beta-lactamase and carbapenemase enteric isolates in Abeokuta, Nigeria. *Nature and Sci.* 2012; 10(7):70-74.
16. Abdullahi SA, Arzai AH, Yusuf I, Adamu SM, Adamu S, Koki YA, Rabi'u MA, Abbas AM. Molecular Detection of New Delhi Metallo BetaLactamase 1 (NDM-1) Producing Bacterial Isolates inKano-Northwestern Nigeria. *Annual Res Review in Biol.* 2017; 14(4):1-6.

17. Iroha IR, Orji JO, Onwa NC, Nwuzo AC, Okonkwo EC, Ibiam EO, Nwachi AC, Afuikwa FN, Agah VM, Ejikeugwu EPC, Agumah NB, Moses IB, Ugbo E, Ukpai EG, Nwakaeze E A, Oke B, Ogbu L and Nwunna E. Microbiology Practical Handbook. (Editor; Ogbu. O), 1st Edition. Charlieteximage Africa (CiAfrica Press), 2019; Pp:344.

18. Edemekong, CI, Iroha IR, Thompson, MD, Okolo, IO., Uzoeto HO, Ngwu JN, Mohammed ID, Chukwu EB, Nwuzo AC, Okike BM, Okolie SO, Peter IU. Phenotypic Characterization and Antibiogram of Non-Oral Bacteria Isolates from Patients Attending Dental Clinic at Federal College of Dental Technology and Therapy Medical Center Enugu. *Int J Pathog Res.*2022; 11(2): 7-19.

19. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-eighth edition (M100). Wayne, PA: Clinical and Laboratory Standards Institute; 2019.

20. Peter IU, Ngwu JN, Edemekong CI, Ugwueke IV., Uzoeto HO., Joseph OV., Mohammed ID., Mbong EO, Nomeh OL., Ikusika BA., Ubom IJ., Inyogu JC., Ntekpe ME., Obodoechi IF., NseAbasi, PL., Ogbonna IP., Didiugwu CM, Akpu PO., Alagba EE., Ogba RC, Iroha, IR. First Report Prevalence of Livestock Acquired Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) Strain in South Eastern, Nigeria . *IOSR J Nurs Health Sci*. 2022a; 11(1):50-56.

21. Peter IU, Okolo IO, Uzoeto HO, Edemekong CI, Thompson MD, Chukwu EB, Mohammed, ID, Ubom, IJ, Joseph, OV, Nwuzo, AC, Akpu PO, Iroha, IR (2022a). Identification and Antibiotic Resistance Profile of Biofilm-forming Methicillin Resistant *Staphylococcus aureus* (MRSA) Causing Infection among Orthopedic Wound Patients. *Asian J Res Med Pharm Sci.* 2022b; 11(4)*:* 45-55.

22. Mabrouka B, Rahima T, Yousra O. Frequency and Antibiotic Resistance of *Enterobacteriaceae* Isolated in Community Urinary Tract Infections at Tebessa Region. *Biomed J Sci Technol Res,* 2018; 12(2).

23. Gurung S, Kafle S, Dhungel B, Adhikari N, Shrestha UT, Adhikari B, Banjara MR, Rijal KR, Ghimire P. Detection of OXA-48 Gene in Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae* from Urine Samples. *Infect Drug Resist.* 2020; 13:2311–2321
24. Paveenkittiporn W, Lyman M, Biedron C, Chea N, Bunthi C, Kolwaite A, Janejai N. Molecular Epidemiology of Carbapenem-resistant *Enterobacterales* in Thailand, 2016–2018. *Antimicrobial Resist Infect Cont.* 2021; 10:88-89.
25. Beyene D, Bitew A, Fantew S, Mihret, A, Evans M. Multidrug-resistant Profile and Prevalence of Extended Spectrum β-lactamase and Carbapenemase Production in Fermentative Gram-negative Bacilli Recovered from Patients and Specimens Referred to National Reference Laboratory, Addis Ababa, Ethiopia. *PLOS.* 2019*;* 14(9):222-911.
26. Hamzan NI, Yean CY, Rahman RA, Hasan H, Rahman ZA. Detection of blaIMP4 and blaNDM1 harboring *Klebsiella pneumoniae* isolates in a university hospital in Malaysia. *Emerg Health Threats J*. 2015; 8: 260-11
27. Centers for Disease Control (CDC). Carbapenem-resistant Enterobacteriaceae (CRE). 2013. Available from: http://www.cdc.gov/HAI/organisms/cre/
28. Kotlovsky T, Shalginov R, Austin L, Sprecher H. Rapid Detection of Positive *Klebsiellapneumoniae* in a Clinical Setting. *European J Clin Microbiol Infect Dis*, 2009; **28**(3):309-311.
29. Pasteran F, Mendez T, Guerriero L, Rapoport M, Corso A. Sensitive Screening Tests for Suspected Class A carbapenemase Production in species of Enterobacteriaceae. *J Clinical Microbiol*. 2009; **47**(6):1631-1639.
30. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of Carbapenem-resistant *Klebsiella pneumoniae* Acquisition among Hospitalized Adults and Effect of Acquisition on Mortality. *Antimicrob Agents Chemother.* 2008; 52 (3):1028–1033.
31. Chen Y, Liao K, Huang Y, Guo P, Huang H, Wu Z, Liu M. Determining the susceptibility of carbapenem resistant *Klebsiella pneumoniae* and *Escherichia coli* strains against common disinfectants at a tertiary hospital in China. *BioMedl Complement Infect Dis.* 2020; 20:88-123.
32. Wang B, Pan F, Wang C, Zhao W, Sun Y, Zhang T, Shi Y, Zhang H. Molecular Epidemiology of Carbapenem-resistant *Klebsiella pneumoniae* in a Paediatric Hospital in China. *Int J Infect Dis.* 2020; 93:311–319.
33. European Centre for Disease Prevention and Control. Annual Epidemiological Report 2014. Antimicrobial Resistance and Healthcare-associated Infections (2015). Available online:https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/antimicrobial resistanceannual- epidemiological-report.pdf
34. Guo Y, Hu FP, Zhu DM, Wang CQ, Wang AM, Zhang H. Antimicrobial Resistance changes of Carbapenem-resistant Strains Isolated from Children. *Zhonghua Er Ke Za Zhi*, 2018; 56(12):907–14.
35. Hu F, Zhu, D, Wang F, Wang M. Current Status and Trends of Antibacterial Resistance in China. *J Clinical Microbiol Infect Dis;* 2018; 67(2):128–34.
36. Murugan MS, Sinha DK, Vinodh Kumar OR, Yadav AK, Pruthvishree BS, Vadhana P, Nirupama KR, Bhardwaj M, Singh BR. Epidemiology of Carbapenem Resistant *Escherichia coli* and First Report of blaVIM Carbapenemases Gene in Calves from India. *Epidemiol Infect.* 2019; 147:159, 1–5.
37. Li X, Fu Y, Shen M, Huang D, Du X, Hu Q. Dissemination of blaNDM-5 Gene via an IncX3-type plasmid among Non-clonal *Escherichia coli* in China. *Antimicrobial Agent Resist Infect Cont.* 2018; **7**:59-87.
38. Olalekana A, Onwugambab F, Iwalokunc B, Mellmann A, Beckerb K, Schaumburg F. High Proportion of Carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* among Extended-spectrum β-lactamase-producers in Nigerian Hospitals. *J Glob Antimicrob Resist.* 2020; 21:8–12.
39. Yuan Y, Li Y, Wang G, Li C, Chang YF, Chen W. bla NDM-5 carried by a hypervirulent *Klebsiella pneumoniae* with Sequence type 29. *Antimicrobial Resist Infect Cont.* 2019; 8:140-145.
40. Sakkas H, Bozidis P, Ilia A, Mpekoulis G, Papadopoulou C. Antimicrobial Resistance in Bacterial Pathogens and Detection of Carbapenemases in *Klebsiella pneumoniae* Isolates from Hospital Wastewater. *J Microb Drug Resist.* 2019; 8:85-90
41. Satlin MJ. The Search for a Practical Method for Colistin Susceptibility Testing: Have We Found It by Going Back to the Future? *J Clin Microbiol*, 2019; 57:1608-18.

42. Mammina C, Bonura C, Di Bernardo F, Aleo A, Fasciana T, Sodano C. Ongoing Spread of Colistin Resistant *Klebsiellapneumoniae* in Different Wards of an Acute General Hospital, Italy, June to December 2011. *EuroSurveilliance*. 2012; 17:202-48
43. Mezzatesta ML, Gona F, Caio C, Petrolito V, Sciortino D, Sciacca A. Outbreak of KPC-3-producing, and Colistin-resistant, *Klebsiella pneumoniae* Infections in Two Sicilian Hospitals. *J Clin Microbiol Infect Dis*. 2011; 17:1444–7.
44. Rojas LJ, Salim M, Cober E, Richter SS, Perez F, Salata RA, Kalayjian RC, Watkins RR, Marshall S, Rudin SD. Antibacterial Resistance Leadership Group. Colistin resistance in carbapenem-resistant *Klebsiella pneumoniae*: Laboratory detection and impact on mortality. *Clin Infect Dis.* 2017; 64:711–718.
45. Satlin MJ, Chen L, Patel G, Gomez-Simmonds A, Weston G, Kim AC, Seo SK, Rosenthal ME, Sperber SJ, Jenkins SG. Multicenter Clinical and Molecular Epidemiological Analysis of bacteremia due to Carbapenem-resistant Enterobacteriaceae (CRE) in the CRE Epicenter of the United States. *Antimicrob Agents Chemother*. 2017; 61:2349-16.
46. Karki D, Dhungel B, Bhandari S, Kunwar A, Joshi PR, Shrestha B, Rijal KR, Ghimire P, Banjara MR. Antibiotic resistance and detection of plasmid mediated colistin resistance *mcr-1* gene among *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples. *Gut pathogen.* 2021; 45;23-56.
47. Qadi M, Alhato S, Khayyat R, Elmanama AA. Colistin Resistance among *Enterobacteriaceae* Isolated from Clinical Samples in Gaza Strip. *Canadian J Infect Dis Med Microbiol.* 2021; 23:34-67.
48. Alfouzan W, Dhar R, Nicolau D. *In vitro* Activity of Newer and Conventional Antimicrobial Agents, Including fosfomycin and colistin, against Selected Gram-negative Bacilli in Kuwait. *Pathogens.* 2018; 7:75-89.
49. Prim N, Turbau M, Rivera A, Rodríguez-Navarro J, Coll P, Mirelis B. Prevalence of colistin resistance in clinical isolates of Enterobacteriaceae: A four-year cross-sectional study. *J Infect.*,2017; 75(6):493-498.
50. Bonura C, Giuffrè M, Aleo A, Fasciana T, Di Bernardo F, Stampone T. An Update of the Evolving Epidemic of blaKPC Carrying *Klebsiella pneumoniae* in Sicily, Italy, 2014: Emergence of Multiple Non-ST258 Clones. *PLOS.* 2015; 10:23-25.
51. Poirel L, Jayol A, Bontron S, Villegas MV, Ozdamar M, Türkoglu S. The *mgrB* gene as a Key Target for Acquired Resistance to Colistin in *Klebsiella pneumoniae*. *Journal of Antimicrob Agent Chemother.*2015; 70:75–80.
52. Onanuga A, Mahindroo J, Singh S, Taneja N. Phenotypic and molecular characterization of antimicrobial resistant *Escherichia coli* from urinary tract infections in Port-Harcourt, Nigeria. *PanAfrican Med J.* 2019; 23:34-56
53. Odongo I, Ssemambo R, Kungu JM. Prevalence of *Escherichia coli* and Its Antimicrobial Susceptibility Profiles among Patients with UTI at Mulago Hospital, Kampala, Uganda. *Hindawi Interdiscipl Perspect Infect Dis.* 2020; 12:23-56.
54. Mwaka AD, Mayanja-Kizza H, Kigonya E, Kaddu-Mulindwa D. Bacteriuria among Adult Non-pregnant Women Attending Mulago Hospital Assessment Centre in Uganda. *Afri Health Sci.* 2011; 11(2):182–189.
55. Kabugo D, Kizito S, Ashok DD. Factors Associated with Community-acquired Urinary Tract Infections among Adults Attending Assessment Centre, Mulago Hospital Uganda. *Afri Health Sci.* 2016; 16(4):1131–1142.
56. Yan J, Pu S, Jia X, Xu X, Yang S, Shi J, Sun S, Zhang L. Multidrug Resistance Mechanisms of Carbapenem Resistant *Klebsiella pneumoniae* Strains Isolated in Chongqing, China. *Annal Laboratory Med.* 2017; 37:5-398

57. Makanjuola OB, Fayemiwo SA, Okesola AO, Gbaja A, Ogunleye VA, Kehinde AO, Bakare RA. Pattern of Multidrug Resistant Bacteria Associated with Intensive Care Unit Infections in Ibadan, Nigeria. *Annals of Ibadan Postgrad Med.* 2018; 16(2):162-169
58. Azim NS, Nofal MY, AlHarbi MA, Al-Zaban MI, Somily AM. Molecular-diversity, Prevalence and Antibiotic Susceptibility of Pathogenic *Klebsiella Pneumoniae* under Saudi Condition. *Pakistan J Biol Sci.* 2020; 22:174-179.
59. Ugbo EN, Anyamene CO, Moses IB, Ariom TO, Agumah NB, Chukwunwejim CR, Egbule CU, Emioye AA, Okata-Nwali OD, Aneke CJ, Ugadu IO, Osu BO. Isolation and molecular characteristics of extended spectrum beta-lactamase-producing uropathogenic *Escherichia coli* isolated from hospital attendees in Ebonyi State, Abakaliki. *Afri J Biotechnology*. 2020; 19(11):829-835
60. Adabara NU, Bakinde ND, Enejiyon SO, Salami T, Iorzua D.Detection of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* from Urinary Tract Infection in General Hospital, Minna. *Tanzania J Sci.* 2020; 46(3): 613-619.
61. Nkene IH, Ngwai YB, Bassey EB, Pennap GRI, Makut MD, Abimiku RH, Ibrahim T Tsaku PA. Antibiotic Resistance Profile of *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infections in Federal Medical Centre, Keffi, Nigeria. *J Adv Microbiol*. 2019; 18(1): 1-8.
62. Lohani B, Thapa M, Sharma LH, Adhikari AK, Sah-Khanal AB, Basnet RB. Aryal M. Predominance of CTX-M Type Extended Spectrum β-lactamase (ESBL) Producers Among Clinical Isolates of Enterobacteriaceae in a Tertiary Care Hospital, Kathmandu, Nepal. *The Open Microb J*. 2020; 14:13-22.
63. Iseghohi F, Igwe JC, Galadima M, Kuta AF, Abdullahi AM, Chukwunwejim. CR. Prevalence Of Extended Spectrum Beta-Lactamases (ESBLs)- Producing *Escherichia Coli* Isolated From UTI Patients Attending some Selected Hospitals In Minna, Nigeria. *Niger J Biotechnol*. 2020; 37(2): 56-73.
64. Alizadeh N, Rezaee MA, Kafil HS, Hasani A, Barhaghi MHS, Milani M, Sefidan FY, Memar MY, Lalehzadeh A, Ghotaslou R. Evaluation of Resistance Mechanisms in Carbapenem-Resistant Enterobacteriaceae. *Infect Drug Resist*. 2020; 13 1377–1385
65. Ribeiro PCS, Monteiro AS, Marques SG, Monteiro SG, Monteiro-Neto V, Coqueiro M MM, Marques AG, Turri RG, Santos SG, Bomfim MRQ. Phenotypic and Molecular Detection of the blaKPC Gene in Clinical Isolates from Inpatients at Hospitals in São Luis, M.A, Brazil. *Biomed Complement Infect Dis.* 2016; 16:737-768
66. Hassuna NA, Khairalla AS, Farahat EM, Hammad AM, Abdel-Fattah M. Molecular Characterization of Extended Spectrum β lactamase-producing *E. coli* Recovered from Community-acquired Urinary Tract Infections in Upper Egypt. *Scientific Report*, 2020; 10(1): 1-8.
67. Mohsen SMY, Hamzah HA, Al-Deen MMI, Baharudin R. Antimicrobial Susceptibility of *Klebsiella pneumoniae* and *Escherichia coli* with Extended-Spectrum β-lactamase associated Genes in Hospital Tengku Ampuan Afzan, Kuantan, Pahang. *Malaysian J Med Sci*. 2016; 23(2): 14-20
68. Moghadampour M, Rezaei A, Faghri J. The Emergence of blaoxa-48 and blaNDM Among ESBL-Producing *Klebsiella pneumoniae* in clinical Isolates of a Tertiary Hospital in Iran. *Acta Microbiologica et Immunologica Hungaric.* 2018;65 (3):335–344.
69. Hashemizadeh Z, Hosseinzadeh Z, Azimzadeh N, Motamedifar M. Dissemination Pattern of Multidrug Resistant Carbapenemase Producing *Klebsiellapneumoniae* Isolates Using Pulsed-Field Gel Electrophoresis in Southwestern Iran. *Infect Drug Resist.* 2020; 13:921–929.
70. Mbelle NM, Feldman C, Sekyere JO, Maningi NE, Modipane L, Essack SY. Pathogenomics and Evolutionary Epidemiology of Multi-Drug Resistant Clinical *Klebsiella pneumoniae* Isolated from Pretoria, South Africa. *Scientific Report*, 2020; 10:1232-1236.