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Prevalence and Antibiogram Profile of Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* among Patients with Urinary Tract Infection in Abakaliki, Nigeria

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Authors' contributions

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

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ABSTRACT

Background and Objectives: Carbapenem antibiotic are drug of last-resort from the treatment of bacterial infection, as a result of the prevalence and rapidly evolving enzymes from Carbapenem resistant bacteria such *Escherichia coli* and *Klebsiella pneumoniae* make urinary tract infection 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 UTI in Abakaliki, Nigeria.

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 isolates 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.

Keywords: Urinary tract infection; 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]. Earlier study has reported a prevalence of 41.0% and

44.0% among Gram negative Enterobacteriaceae, associated with UTI [1,9]. UTIs are common bacterial infections worldwide and affect 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. Carbapem-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 University Teaching Hospital, Abakaliki, Ebonyi State Nigeria. Such data will serve an important role in understanding the spread pattern of Carbapenem-resistant *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 University Teaching hospital Abakaliki, Ebonyi State Nigeria 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 have not been on antibiotics for more than 2 weeks, and pediatrics 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 in-patient or out-patient. 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 were labeled with the unique sample number; date and time of collection. The sample containers were 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]. All phenotypic carbapenem-resistant bacteria suspension of the test isolates 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 °C 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 phenotypic 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 in-patient and out-patient with UTI. Statistical significant was set at p value <0.05 [21].

3. RESULTS

3.1 Distribution of *Escherichia coli* and *Klebsiella pneumoniae* Isolates in Urine Samples of UTI in-patients and out-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-patients 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.

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 %) in-patient and out-patient. 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.

Carbapenem-resistant strains 50.0 % and 100% resistant to colistin were 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]. According to earlier report, the percent of resistance to colistin was 33.3%, and 31.6% for *E. coli* and *Klebsiella* respectively [47] while in another study reported resistance of 4.3% for *E. coli* and 7.7% for *Klebsiella* was found [48]. Also another researcher reported that the overall prevalence of colistin resistance was 0.67% [49]. 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 observed in this study 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.

The 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. Existing studies showed that 70% and 100% of the isolates were susceptible to nitrofurantoin [52,53]. Also, 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. Additionally, the occurrence of persistor cells (defined as metabolically inactive cells that neither grow nor 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.

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

Patient's Population	No. sampled	*Klebsiella pneumoniae*			*Escherichia coli*		
		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

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 were not assessed. This finding strongly suggests the role of cross-transmission within and between hospital community in our epidemiological setting. Additionally, the observed pattern of MDR could be linked to indiscriminate use of broad and narrow spectrum antibiotic in the treatment of complicated and recurrent UTI.

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

Antibiotics (µg)	Carbapenem-resistant *K. pnuemoniae*				Carbapenem-resistant *Escherichia coli*			
	In-patient (n=4)		In-patient (n=6)		In-patient n=20)		(out-patient n=7)	
	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)

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

In accordance with the isolates susceptibility profile of ofloxacin and ciprofloxacin in this study. Earlier study showed susceptibility to ciprofloxacin at 86.25% [66] while another researcher affirmed in their studies that isolates were highly susceptible to Ofloxacin (65%) [63], 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.

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.

CONSENT

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

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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