Original Research Article

PREVALENCE OF AMPC (AMPICILLIN RESISTANT GENE CLASS C) BETA-LACTAMASE-PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* ISOLATES FROM CLINICAL AND ENVIRONMENTAL SOURCES IN ENUGU METROPOLIS, NIGERIA

ABSTRACT

Background: Pathogenic bacteria with beta lactamase-resistant determinants have emerged, posing a worldwide health threat. They confer resistance to a large array of β-lactam agents and β-lactam inhibitors. Aim: This study evaluated the prevalence and resistance profiles of plasmid-mediated AmpC β-lactamase producing isolates among *Escherichia coli* and *Klebsiella pneumoniae* from clinical and environmental sources in the Enugu metropolis. Methodology: The work was done in the Microbiological Laboratory of the University of Nigeria Teaching Hospital Ituku-Ozalla. A total of 150 non-duplicate isolates processed in the microbiology laboratories of three referral hospitals and some private laboratories were used in this study including 85 and 65 isolates of *Escherichia coli* and *Klebsiella pneumoniae* respectively. Isolates were identified and characterized using standard microbiological protocols. Antimicrobial susceptibility was performed using the Kirby-Bauer disc diffusion procedure. Phenotypic detection of AmpC β-Lactamase production was determined using Cefoxitin/Cloxacillin double-disc synergy (CCDST). Results: Of the 67 isolates of *E. coli* from clinical samples, AmpC production was confirmed in 20(29.9%) and of the 18 isolates from environmental sources, only 5(27.8%) were confirmed. Of the 60 isolates of *Klebsiella pneumoniae* from clinical samples 16(26.6%) were AmpC producers while 1(20%) out of the 5 isolates from environmental samples was an AmpC producer. The prevalence of AmpC β-lactamase producers was 28%. There was no statistical difference in the proportion of AmpC producers in clinical and environmental isolates in *E.coli* and *Klesiella pneumoniae* $p = .86$ and $p = .74$ respectively. Antimicrobial resistance was high in both AmpC and non-AmpC β-lactamase producers. Impenem's susceptibility was found in 75.4 percent of the isolates.

Conclusion: These findings revealed a high prevalence of AmpC beta-lactamase in the Enugu metropolis. The identification of AmpC beta-lactamase enzymes regularly is crucial to preventing therapeutic failures. The proper usage of antimicrobial drugs is critical.

Keywords: Antimicrobial resistance, AmpC, Phenotypic detection, Escherichia coli, Klebsiella pneumoniae

1. INTRODUCTION

Antimicrobial resistance (AMR) is a global problem that puts the treatment of infectious diseases at risk [1]. It is hazardous to one's health and development [2]. AMR is a significant public health concern with broad health, economic, and societal consequences [3]. The advent of new antibiotics was preceded by an increase in antibiotic-resistant bacterial strains, as well as a variety of bacterial strategies for overcoming the agents' fatal effects [4]. AmpC β-lactamases are one of the most important mechanisms driving β-lactam drug resistance in Enterobacteriaceae. Except for fourth-generation cephalosporins and carbapenems, these enzymes confer resistance to most beta-lactam antibiotics [5]. Because of their low side effects and wide bacterial spectrum, β-lactam antibiotics are among the most widely administered antibiotics [6]. Many novel β-lactams have been produced, many of which are resistant to the hydrolytic effects of β-lactamases [7]. Antibiotic resistance raises medical costs, lengthens hospital stays, and increases mortality rates [6]. AmpC β-lactamases can be mediated by chromosomes or plasmids. In most Enterobacteriaceae species, they are chromosomally encoded. *E.coli, Proteus mirabilis, Salmonella*, and *Klebsiella* spp. on the other hand, can obtain plasmid-encoded AmpC β-lactamases (pAmpC), which are highly transferable between species. *Salomonella* and *Klebsiella* spp. lack chromosomally encoded AmpC enzymes, however, *E. coli* has a chromosomally encoded AmpC β-lactamase that is expressed at low baseline levels due to the existence of a weak promoter and attenuator, making *E. coli* sensitive to cephamycins (e.g. cefoxitin, cefotetan) [5]. Organisms carrying AmpC genes in plasmids, such as *E. coli* or *Klebsiella* spp., are multidrug-resistant and can complicate clinical management and infection-control strategies [8]. Except for cefepime and carbapenems, all beta-lactam antibiotics are hydrolyzed by plasmid-mediated AmpC β-lactamases. Inducible chromosomal genes that have been mobilized among many organisms are the source of the plasmid-mediated AmpC genes [9]. Infections produced by AmpC-positive bacteria are of significant clinical and epidemiological value because of their high rate of morbidity and mortality [10, 11]. After colonizing the gut, pAmpC β-lactamase producing bacteria may infect multiple anatomical areas [5]. Patients with AmpC-positive infections have been found to have mortality rates ranging from 14.3 percent to 46 percent [12]. In low-income nations, third-generation cephalosporins are used to treat infections empirically, failure to detect AmpC β-lactamase-related resistance may lead to poor therapeutic outcome. AmpC β-lactamases are often linked with multiple resistances and the susceptibility profiles are not routinely done in most clinical laboratories, leaving the clinician with few therapeutic options [10]. AmpC identification is thus critical for enhancing the therapeutic management of infected individuals and providing solid epidemiological data to the setting [13]. Despite the foregoing, and due to a lack of data on the prevalence of AmpC β-lactamase in Enugu Metropolis, Nigeria, this investigation was carried out to assess the prevalence of AmpC β-lactamase in *Escherichia coli* and *Klebsiella pneumoniae* isolates recovered from clinical and environmental samples. Antimicrobial treatments can be guided by the phenotypic screening of these AmpC beta-lactamases harboring resistant strains, which can help decrease the spread of these pathogens in hospitals and communities.

2. MATERIAL AND METHODS

2.1 Study design

The study was carried out between October 2020 and June 2021 in the Microbiology Laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla Enugu. The protocol was as per Maduakor et al., [14].

2.2 Bacterial isolates

The study was conducted in the Microbiology Laboratory of the University of Nigeria Teaching Hospital, Ituku-Ozalla. A total of 600 non-duplicate isolates were already processed in the laboratories of the University of Nigeria Teaching Hospital Ituku-Ozalla, National Orthopedic Hospital, Enugu State University Teaching Hospital, Emmanuel Research Laboratory, and Mac-Chuks Diagnostic Laboratory was examined. Eighty-five isolates of *Escherichia coli* and 65 *Klebsiella pneumoniae* were included in the study. Hospital samples were from wound swabs, urine, sputum, stool, and high vaginal swab while environmental isolates were from water, soya milk, and zobo drink. The isolates were identified by standard microbiology laboratory techniques. All isolates obtained were stored in nutrient agar slants and taken to the laboratory. The isolates were reactivated and cultured primarily on the MacConkey agar and incubated at 37^0C for 24 hrs on different agar plates to get pure culture.

2.3 Identification of bacteria isolates

Standard biochemical testing and the API 20E confirmatory method were used to identify the isolates [15, 16].

2.4 Antimicrobial susceptibility Test

Susceptibility tests were performed on all the isolates recovered using Kirby Bauer's disk diffusion method on Muller-Hinton agar medium as described in the guidelines of the Clinical Laboratory Standard Institute (CLSI) [17]. The antibiotics used include; Imipenem (10µg), cefoxitin (30µg), ofloxacin (5 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amoxicillin/clavulanate (20/10 µg), nitrofurantoin (50 µg), ceftazidime (30 µg), cefixime (30 µg), cefotaxime (30 µg), and cefuroxime (30 µg). A standardized suspension of each isolate equivalent to 0.5 McFarland turbidity standards was inoculated onto Mueller-Hinton agar plates and the antibiotics disc was aseptically placed on Muller-Hinton (MH) agar plates incubated at 37°C for 18-24 hours. After incubation, the Inhibition Zone Diameter (IZD) was measured and recorded after the incubation as per the guidelines of the CLSI.

2.5 The Phenotypic Detection of AmpC beta-lactamase production

Screening for AmpC β-lactamase-producers

Cefoxitin discs (30µg) were used to screen AmpC-producing isolates according to CLSI recommendations [CLSI, 2015]. The isolates were screened for the activity of the AmpC enzyme by determining their susceptibility to cefoxitin. Isolates having inhibition zones of ≤ 18mm in diameter were deemed probable positive AmpC producers [11].

2.6 Confirmatory Tests: Phenotypic detection of AmpC β-lactamase production was performed Cefoxitin/cloxacillin double-disc synergy [18]. A 30µg cefoxitin disc was placed at a distance of 20mm away from a disc of cefoxitin supplemented with 200µg of cloxacillin on Muller-Hinton (MH) agar plated already inoculated with cefoxitin-resistant isolates (equivalent to 0.5 McFaland turbidity standards). The MH agar plates were incubated at 37°C for 18-24 hours, and the zones of Inhibition were recorded. The isolates that displayed a defined increase in zone diameter around the cefoxitin with cloxacillin in comparison to that cefoxitin alone was considered to be AmpC producer. A difference in zone diameter of ≥ 4mm was used as the cutoff value.

2.7 Statistical analysis

Data analyses were performed using SPSS for windows version 22(SPSS, Chicago, IL, USA). Categorical variables were described using descriptive statistics (frequencies and percentages). The resistance profile of AmpC and non-AmpC -lactamase producing isolates were compared using the Chi-square test. P-value ≤ 0.05 is considered statistically significant.

3. RESULTS

Table 1 shows the prevalence of AmpC β-lactamase and non-AmpC β-lactamase producers in *E.coli* according to sources of isolates. Urine isolates yielded the highest number of AmpC β-lactamase producers 13(52%) followed by the environmental isolates 5(20%) and the least

was from stool 3(12%). Statistically, there was no significant difference in the proportion of AmpC producers according to the distribution of the isolates (p> 0 .05, X2=2.403).

Table 1: Prevalence of AmpC β-lactamase and non-AmpC β-lactamase producers in *E. coli* bases on the source the of isolates

Source of isolate	No of Isolates	AmpC β-lactamase positive No (%)	AmpC β-lactamase negative No (%)
Urine	34	13(52)	21(35)
Stool	13	3(12)	10(16.7)
HVS	20	4(16)	16(26.7)
Environmental	18	5(20)	13(21.6)
Total	85	25	60
p<0.966	**X^2=2.403**		

Fig 1 shows the resistance and susceptibility profiles of AmpC β-lactamase producers in E. coli. AmpC β-lactamase producers were all (100%) resistant to cefixime, cefuroxime, augmentin, cefotaxime, and 80%, 92%, 96% resistance to ofloxacin, ciprofloxacin, and ceftazidime respectively. The average resistance percentage prevalence of the AmpC β-lactamase producers was 72%. AmpC β-lactamase producers were susceptible to nitrofurantoin and imipenem with a percentage prevalence of 72% and 88% respectively. Statistically, there was a higher proportion of resistant isolates than sensitive isolates among the AmpC β-lactamase producers (p< 0.0001, X2= 140.2).

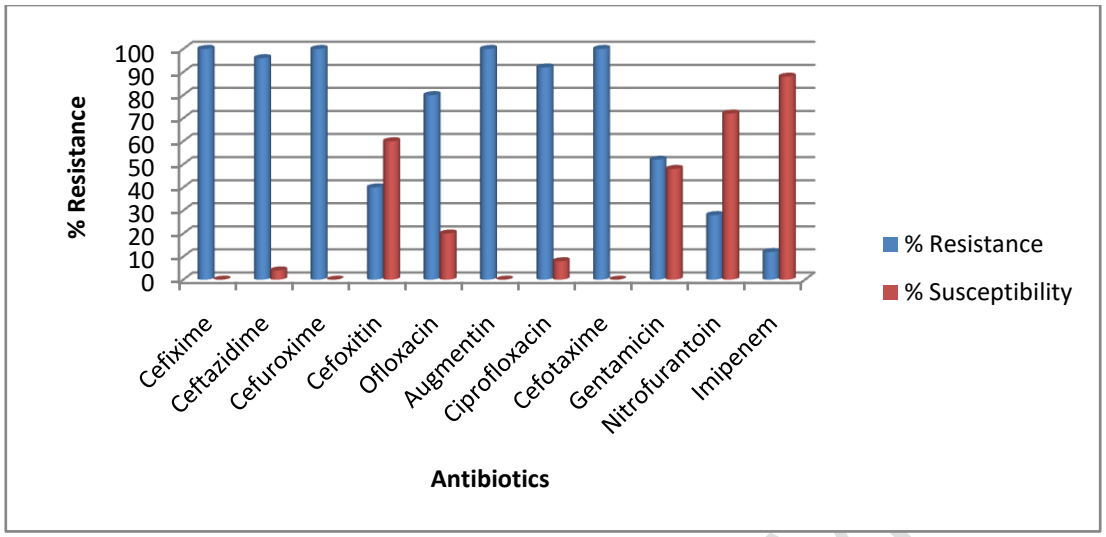

Fig 1: Resistance and Susceptibility Profiles of AmpC β-lactamase producers in *E. coli*

Fig 2 shows the resistance and susceptibility profiles of non-AmpC β-lactamase producers among *E. coli*. The non-AmpC β-lactamase producers were all (100%) resistant to cefixime, cefuroxime, augmentin, and 80%, 91.7%, 93.3% resistant to Gentamicin, cefotaxime, ciprofloxacin, and ceftazidime. The average resistance percentage prevalence of non-AmpC β-lactamase producers was 74.8%. Moderate susceptibility was seen in cefoxitin at 51%. Nitrofurantoin was the most potent with 81.7% and imipenem at 68%. There was a higher proportion of resistant isolates than the sensitive ones ($p< 0.0001$. $X2=275.2$).

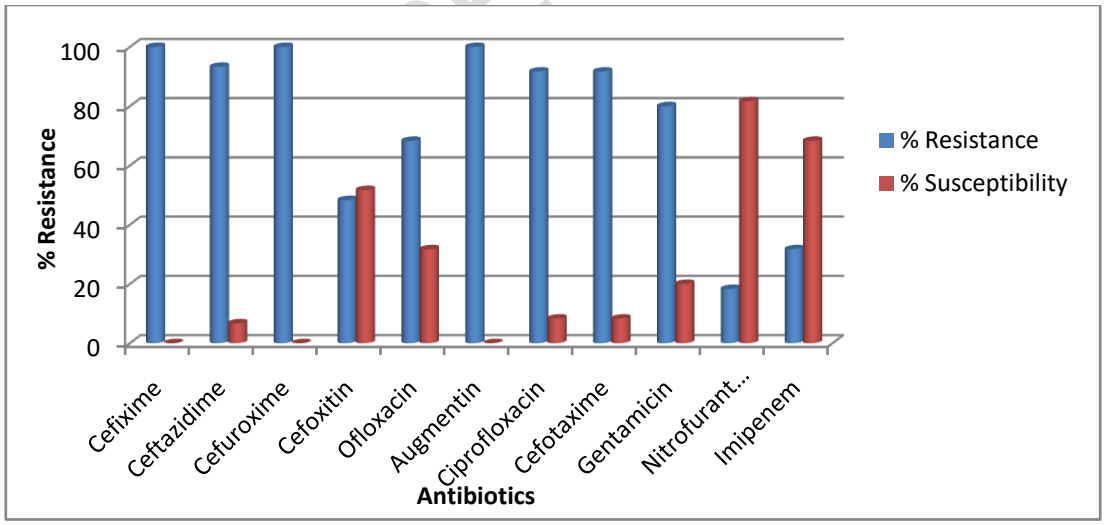

Fig 2: Resistance and Susceptibility Profiles of non-AmpC β-lactamase producers among *E. coli*: N= 60

Table 2 shows the comparison of AmpC producers and non-AmpC producers among Escherichia coli isolates from the hospital and environmental sources. Of the 67 isolates of *Escherichia coli* from hospital samples tested for phenotypic detection of AmpC, only 20

(29.9%) were confirmed to produce AmpC while 47 (70.1%) were confirmed to be AmpC non-producers. Out of the 18 isolates of *Escherichia coli* from environmental samples tested for phenotypic detection of AmpC, only 5 (27.8%) were confirmed to produce AmpC while 13 (72.2%) were confirmed to be AmpC non-producers. Statistically, there was no significant difference in the proportion of AmpC producers between hospital and environmental isolates of *Escherichia coli* (p>0.05, X2=0.02937).

Table 2: Comparison of AmpC producers and non-AmpC producers among *Escherichia coli* isolates from hospital and environmental sources

Source of sample	No tested	AmpC producer No (%)	Non-AmpC producers No (%)	X^2	P-value
Hospital sample	67	20 (29.9%)	47 (70.1%)	0.02937	0.8639
Environmental sample	18	5 (27.8%)	13 (72.2%)		
Total	85	25	60		

Table 3 shows the Prevalence of AmpC β-lactamase and non-AmpC β-lactamase producers in *Klebsiella pneumoniae* based on the sources of isolates. The highest number of AmpC β-lactamase producers was recorded in Urine 10(58.8%) followed by sputum 3(17.6%), and the least was in high vaginal swab and environmental 1(5.9%) each. Statistically, there was no significant difference in the proportion of AmpC β-lactamase producers based on the source of the isolates p> .05, X2=0.513.

Table 3: Prevalence of AmpC β-lactamase and non-AmpC β-lactamase producers in *Klebsiella pneumoniae* based on the sources of isolates

Source of isolate	No of Isolates	AmpC β-lactamase positive No (%)	AmpC β-lactamase negative No (%)
Urine	35	10(58.8)	25(52.0)
Sputum	12	3(17.6)	9(18.8)
HVS	6	1(5.9)	5(10.4)
Wound	7	2(11.8)	5(10.4)
Environmental	5	1(5.9)	4(8.3)
Total	**65**	17(26.1)	48(73.8)

P= 0.999 **X2=0.513**

Fig 3 shows the resistance and susceptibility profiles of AmpC β-lactamase producers in *Klebsiella pneumoniae.* AmpC β-lactamase producers in *Klebsiella pneumoniae* were all 100% resistant to Augmentin and cefotaxime. High resistance was recorded in all the antibiotics tested except imipenem which recorded 29.4%. The average resistance percentage prevalence was 78.1%. There was a higher proportion of resistant isolates than the sensitive ones statistically (p< 0.0002, X2= 38.67.

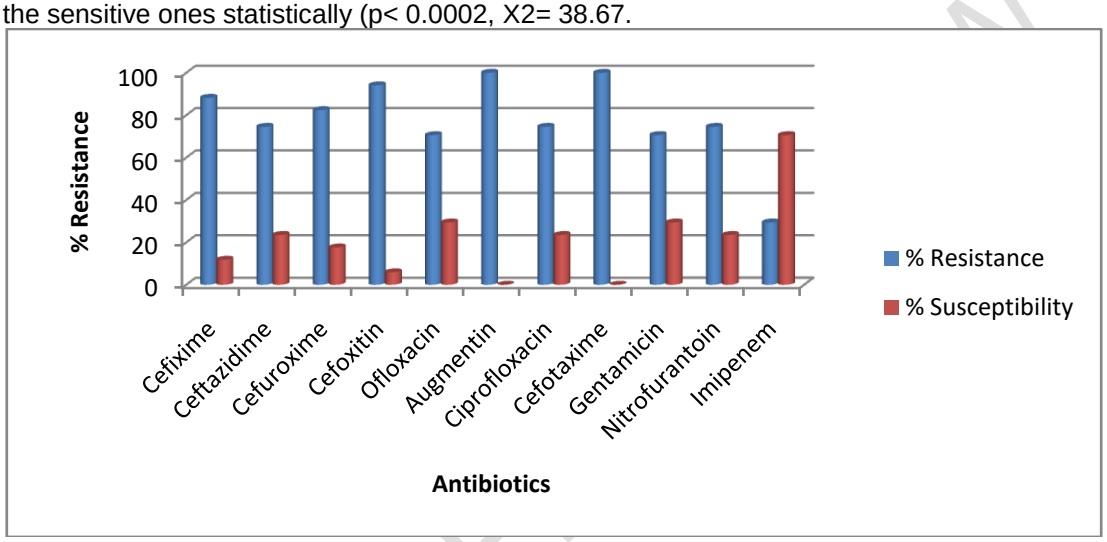

Fig 3: Resistance and Susceptibility Profiles of AmpC β-lactamase producers in *Klebsiella pneumoniae*: N=17

Fig 4 shows the resistance and Susceptibility Profiles of non-AmpC β-lactamase producers among *Klebsiella pneumoniae.* There was high resistance of the organisms to most of the antimicrobials ranging from 50%- to 100%. The average percentage resistance prevalence was 74.1%. Statistically, there was a higher proportion of resistant isolates than the sensitive isolates (p< 0.0001, X2=121.514).

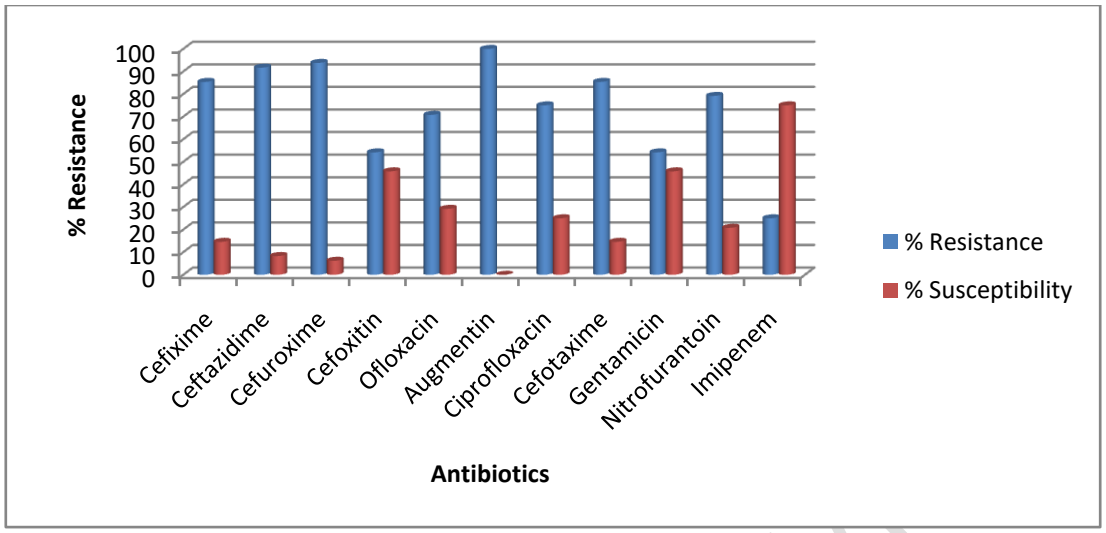

Fig 4: Resistance and Susceptibility Profiles of non-AmpC β-lactamase producers among *Klebsiella pneumoniae:* N=48

Table 4 shows the comparison of AmpC producers and non-AmpC producers among *Klebsiella pneumoniae* isolates from the hospital and environmental sources. Of the 60 isolates of *Klebsiella pneumoniae* from hospital samples tested for phenotypic detection of AmpC, only 16 (26.7%) were confirmed to produce AmpC while 44 (73.3%) were confirmed to be AmpC non-producers. Out of the 5 isolates of *Klebsiella pneumoniae* from environmental samples tested for phenotypic detection of AmpC, only 1 (20%) was confirmed to produce AmpC while 4 (80%) were confirmed to be AmpC non-producers. Statistically, there was no significant difference in the proportion of AmpC producers between hospital and environmental isolates of *Klebsiella pneumoniae* ($p>0.05$, $X2=0.1062$).

Table 4: Comparison of AmpC producers and non-AmpC producers among *Klebsiella pneumoniae* isolates from hospital and environmental sources

Source of Sample	No. tested	AmpC producer No (%)	Non-AmpC producers No (%)	X^2	P-value
Hospital sample	60	16 (26.7%)	44 (73.3%)	0.1062	0.7445
Environmental sample	5	1 (20%)	4 (80%)		
Total	65	17(26.2)	48(73.8)		

3. DISCUSSION

To minimize nosocomial outbreaks and treatment failures, plasmid-mediated AmpC β-lactamases are becoming relevant clinically, and their detection will be advantageous for surveillance, epidemiological measures, and infection control [19, 20, 21]. Several detection methods have been proposed for AmpC β-lactamases. Modified cefoxitin Hodge test [22], Tris-EDTA disc test [12], inhibitor-based assays (e.g., utilizing boronic acid compounds [18] or cloxacillin [23]), and rapid chromogenic assays [24] are only a few examples. Cefoxitin-cloxacillin double-disc synergy was used for the detection of AmpC enzymes in this study because cloxacillin is a superior AmpC enzyme inhibitor than boronic acid because it has a higher sensitivity and specificity [11, 18]. Of the 39 cefoxitin-resistant *E. coli*, and 42 *Klebsiella pneumoniae*, 25 and 17 strains were found to be AmpC producers respectively. This agrees with the work of many researchers that reported that cefoxitin-resistant isolates were not all that AmpC beta-lactamase producers [11, 25, 26l]. This according to them may have been due to other enzymatic mechanisms such as extended beta-lactamases (ESBLs) or metallo beta-lactamases (MBL) or non-enzymatic mechanisms like porin channel mutation [27] or it could due to over-expression of the chromosomal AmpC gene as a result of mutations in the promoter and/or attenuator regions in E. coli [28]. Cefoxitin has also been shown in clinical isolates to be a substrate for active efflux pumps [29].

Our study showed that the overall prevalence of AmpC beta-lactamase producers was 42/150 (28%). A total of 25 E. coli isolates (29.4%) and 17 *Klebsiella pneumoniae* isolates (26.29%) harbored AmpC enzymes. Comparing this prevalence rate with similar studies in the same country Nigeria but different states, showed some variations. Ogefere et al reported 15.3% in Benin City South-south Nigeria [10], Akujiobi et al, 39.6% in Owerri South-east Nigeria [30]. Differences in antimicrobial type selection, antibiotic selection pressure and local antibiotic prescribing practices, which differ by state, country, and institution, could explain the differences [31]. Selective pressure is usually produced by the large-scale use of oxyimino-cephalosporins contributing to increased prevalence in AmpC-production. However, this prevalence of 28% agrees with the work of Fam et al that reported 28.8% in Egypt [25], Bahramian et al 25% in Iran [32] but higher than what was documented in Uganda (13.1%) [5]. The prevalence of AmpC beta-lactamase producers is known to be influenced by geographic regions, strain and species of organisms under study, period of study, and methods used [31]. Very low prevalence has been reported in Europe (4.7%) [33] and Brazil (1.8%) [34]. The reason for the lower prevalence may have been due to good antibiotic stewardship.

The study also revealed a higher number of isolates harboring AmpC enzymes in both *E. coli* and *Klebsiella pneumoniae* from urine isolates but the difference was not statistically significant ($p > 0.05$). This agrees with the work of Ibadin et al [35] that there was no significant statistical difference in the source of the isolates but is at variance with the findings of Ogefere et al [10] that reported a significantly higher proportion in the sputum and Yusuf et al in urine [31].

There was high antibiotic resistance in both AmpC and non-AMPC producers. This could be due to unregulated antibiotic usage, over-the-counter drug sales without a prescription, and treatment of patients without laboratory tests or results, which are all too prevalent in underdeveloped nations [10]. Overexpression of AmpC has been shown to result in clinical resistance to nearly all beta-lactam antibiotics, except for imipenem, therefore it remains the best therapeutic option for serious infections caused by pAmpC-producing isolates [11]. In this investigation, the average percentage susceptibility of isolates to imipenem was 75.4 percent. Detection of pAmpC isolates may be clinically relevant not only because of their broad cephalosporin resistance, but because carbapenem resistance may emerge as a result of subsequent mutations, leading to lower porin expression [11, 36, 37]. Plasmid-

mediated AmpC genes have the power to transfer and multiply in other organisms within the hospital, leading to nosocomial infections and therapeutic failures [8]. AmpC beta-lactamase phenotyping should be done routinely before antibiotic therapy, especially in cefoxitin-resistant isolates. There are several phenotypic tests available that are inexpensive and simple to perform along with routine susceptibility tests in the clinical laboratories. The primary drawback of phenotypic approaches is that they don't distinguish between chromosomal and plasmid-mediated AmpC producers, thus molecular testing is still the gold standard.

4. CONCLUSION

These findings showed a high prevalence of 28% of AmpC beta-lactamase-producing isolates of *Escherichia coli* and *Klebsiella pneumoniae* from clinical and environmental sources in Enugu Metropolis Southeast Nigeria. There was high antibiotic resistance in both AmpC and non-AMPC producers. The identification of AmpC beta-lactamase enzymes regularly is crucial to preventing therapeutic failures. The proper usage of antimicrobial drugs is critical**.**

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