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Low Prevalence of Carbapenem Resistance in Clinical Isolates of Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* in North Central, Nigeria

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Abstract

Extended Spectrum Beta Lactamase (ESBL) producing *Escherichia coli* is a global cause of life threatening infections. We determined the presence of ESBL and carbapenemase production in clinical isolates of *E. coli* and their antibiotic susceptibility. Clinical isolates of community and hospital acquired *E. coli* from 220 patients seen at a tertiary hospital were evaluated. Antibiotic susceptibility testing was by the modified Kirby-Bauer protocol while ESBL production was determined by the Double Disk Synergy Test (DDST). Carbapenem resistance was confirmed by the Modified Hodge Test. Of the 220 isolates, 122 (55.5%) were from females; 41 (18.6%) were ESBL positive. About 90% of the ESBL producing isolates were resistant to nine of the 15 antimicrobial agents tested. However, only one (2.4%) of the 41 ESBL producing isolates exhibited carbapenem resistance. The ESBL negative isolates were susceptible to Meropenem (100%), Cefepime (97.8%), Ceftriaxone (96.6%) and Cefotaxime (96.6%). All the ESBL producing isolates harbored detectable plasmids with sizes ranging from 2322 to 23,130 base pairs. Our findings show that although multidrug resistant ESBL producing *E. coli* are prevalent in both the hospital and the community in this environment, carbapenem resistance is still low. We recommend that institutions develop guidelines for the early phenotypic detection of ESBLs and carbapenem resistance.

Keywords

E. coli, ESBL, Plasmids, Carbapenem Resistance, Nigeria

1. Introduction

Paul Ehrlich described the concept of antimicrobial agents as "magic bullets" for killing microbes [1] [2]. However, shortly after the introduction of these magic bullets in clinical practice, it was discovered that bacteria were capable of developing resistance to the antimicrobials. Although resistance to antibiotics affects all countries, it has potentials for causing more harm in developing countries since alternative antimicrobials are often not available nor affordable to those who need them [3]. Infectious diseases that were originally curable at the advent of antibiotics chemotherapy are again becoming killers of patients of all ages especially in developing countries [3].

Extended spectrum beta lactamase (ESBL) enzymes which are mainly produced by *Escherichia coli* (*E. coli*) and *K. pneumoniae* render these antibiotics ineffective when used to treat infections caused by ESBL producing organisms, thus increasing morbidity and mortality as well as the cost of therapy [4]. In most cases, the drug of choice in the presence of an ESBL producing organism is a carbapenem (e.g. meropenem, imipenem, ertapenem, doripenem) which are mostly injectable drugs and quite expensive. Carbapenems are cell wall synthesis inhibiting antibiotics just like penicillins and cephalosporins with a different chemical structure. They have the characteristic penicillin-like five membered beta lactam ring, but differs at the sulfur at position C-1 which is replaced with a carbon atom and an introduction of a double bond between C-2 and C-3 of the ring [5]. Carbapenems currently have the broadest spectra of antimicrobial activity among all beta lactams with proven efficacy in severe infections due to ESBL producing bacteria [6]. Reports from several studies already show presence of carbapenemases conferring resistance to the carbapenems, a situation that is potentially devastating [5] [6]. In addition, studies have also demonstrated the great potential for the spread of carbapenemase and ESBL producing strains and their encoding plasmids [4] [5] [6] [7]. The situation is disturbing as this could be applicable and more devastating in developing countries like Nigeria, made even worse because there are no institutionalized surveillance for resistant organisms. It is known that inappropriate treatment with antimicrobials thought to be effective against resistant organisms leads to poor outcome [8]. The burden of ESBL and carbapenemase producing organisms in clinical practice is poorly characterized in Nigeria. Therefore, we determined the prevalence, distribution and plasmids in clinical isolates of ESBL and carbepenemase producing *E. coli* and assessed the patterns of susceptibilities to commonly used antimicrobial agents in a Northern Nigerian tertiary hospital.

2. Methods

The study was a prospective cross-sectional study carried out in a 600-bed tertiary health care institution from August 2013 to January 2014. Plasmid profile analysis was carried out at the molecular diagnosis unit of the Nigerian Institute of Medical Research (NIMeR).

2.1. Recruitment of Participants

Participants were outpatients and inpatients attending the Jos University Teaching Hospital for various infections during the study period. Point of entry into the study was from a laboratory isolate of *E. coli* from a sample (urine, blood, swabs or aspirates) obtained from a clinically defined focus of infection in a patient in the hospital. An isolate of *E. coli* from specimen(s) from other site(s) or the same site in a patient from whom *E. coli* was previously isolated during same hospital visit or admission was excluded from the study in order not to have duplicate isolates in the study.

2.2. Data Collection

Clinical and demographic data were collected from all patients by means of a proforma. Data collected included age, gender, occupation, place of residence (*urban, semi-urban, urban slum* or *rural*) and patient status (*in-patient* or *out-patient*). All data collected were checked thoroughly by two independent reviewers for accuracy of the data.

2.3. Ethical Considerations

Ethical clearance was obtained from the institutional research and ethics review board. An informed consent form was signed by all participants. Parents or guardians signed on behalf of patients who were unable to do so themselves. Immediate feedback was provided to the primary physicians of patients from whom ESBL or carbapenemase producing *E. coli* was isolated for appropriate therapy to be commenced based on antibiotic susceptibility and resistance testing results.

2.4. Laboratory Methods

Standard microbiological procedures were followed in handling all samples and isolates used in the study in accordance with the Clinical and Laboratory Standard Institute (CLSI) stipulations [9] [10]. Media and reagents were prepared according to the manufacturers' specifications. Each batch of media and reagent was quality controlled before use. These included sterility testing and performance testing with the use of control strains from the American Type Culture Collection (ATCC). *E. coli* ATCC 35218 and *E. coli* ATCC 25922 were used as control strains. Motility was determined by the hanging drop procedure. Motile, lactose fermenting, indole positive, citrate negative; gram negative bacilli isolates processed by standard techniques were identified as *E. coli*. These isolates were

processed further for antibiotic susceptibility and production of ESBL and carbapenemases [8] [11].

2.4.1. Antibiotic Susceptibility and Carbapenem Resistance Testing

Antibiotic susceptibility test was performed using Mueller-Hinton agar (Oxoid, Basingstoke, UK) by standard disk diffusion procedure [10]. The modified hodge test as described in the CLSI protocols was used to confirm carbapenem resistance [10]. The control strains were run simultaneously with the test organisms. Results were interpreted with the CLSI criteria for disk diffusion [10].

2.4.2. Extended Spectrum Beta Lactamase Detection

This was carried out by the Double disk synergy test (DDST). All isolates with reduced susceptibilities or resistant to an extended-spectrum cephalosporin namely ceftriaxone were subjected to DDST as described by Jarlier [7] with modifications suggested by Thomson and Sanders [11] to detect the presence of ESBL enzyme. Mueller Hinton agar plates were inoculated with a 0.5 McFarland standard inoculum of *E. coli*. Control strains: *E. coli* ATCC 35218 served as positive control while *E. coli* ATCC 25922 served as negative control.

2.4.3. Plasmid Analysis

This was carried out at the molecular diagnosis laboratory of NIMeR. The plasmid extraction was by the protocol contained in the TENS Mini Prep method [12]. The TENS reagent composition is Tris 25 mM, ethylene diamine tetra acetic acid (EDTA) 10 mM and sodium hydroxide (NaOH) 0.1 N. This was used to determine the quantity and sizes of the plasmids in the ESBL producing *E. coli* isolates. The results were read by observing the gel on an ultraviolet (UV) transilluminator. Non random selection of consecutive odd numbered ESBL producing isolates was used to select 21 isolates for plasmid profile analysis. An ESBL negative isolate labeled four (4) was included in the plasmid profile analysis as a blinded control to double check the reliability of the result obtained from the electrophoresis procedure. The identity of the isolate was only revealed after the run.

2.5. Data Processing and Statistical Analysis

All data generated were collated and analyzed using EPI info version 3.5.3 statistical software. Continuous variables were expressed as means ± standard deviation (SD), while categorical variables were expressed as proportions. P value of <0.05 was considered significant.

3. Results

Two hundred and twenty (220) isolates of *E. coli* obtained from biological samples collected from 122 (55.5%) female and 98 (44.5%) male patients were evaluated. A total of 41 (18.6%) of the 220 *E. coli* isolates studied were ESBL positive. One (2.4%) of the 41 ESBL producing isolates was carbapenemase positive and meropenem resistant. The age range of participants was between one and 93

years as shown in Table 1. Table 1 also shows the distribution of ESBL positive and ESBL negative isolates across the different age groups; gender, occupation, location of patient residence and inpatient or outpatient status.

Age had statistical significance in relation to the ESBL result ($p < 0.001$).

Table 1. Socio-demographic characteristics of patients with *Escherichia coli* isolates in the University Teaching Hospital.

socio-demographic characteristics	Total, n = 220	ESBL positive, n = 41	ESBL negative, n = 179		
	Frequency (%)	Frequency (%)	Frequency (%)	Chi-square	P value
Age (Years)					
0 - 10	26 (11.8)	7 (26.9)	19 (73.1)	46.11	< 0.001
11 - 20	35 (15.9)	3 (8.6)	32 (91.4)		
21 - 30	37 (16.8)	7 (18.9)	30 (81.1)		
31 - 40	38 (17.3)	1 (2.6)	37 (97.4)		0.019*
41 - 50	28 (12.7)	17 (60.7)	11 (39.3)		
51 - 60	15 (6.8)	3 (20.0)	12 (80.0)		
>60	41 (18.6)	3 (7.3)	38 (92.7)		
Mean age	36.7 ± 21.6	36.4 ± 21.6	36.0 ± 21.6		
Median age	34 years	42 years	33 years		
Gender					
Female	122 (55.5)	27 (22.1)	95 (77.9)	2.21	0.137
Male	98 (44.5)	14 (14.3)	84 (85.7)		
Type of location					
Urban	110 (50.0)	22 (20.0)	88 (80.0)	5.66	0.129
Semi-urban	24 (10.9)	8 (33.3)	16 (66.67)		
Rural	29 (13.2)	3 (10.3)	26 (89.7)		
Urban slum	57 (25.9)	8 (14.0)	49 (86.0)		
Occupation					
Artisan/Applicant	10 (4.5)	1 (10.0)	9 (90.0)	18.35	0.005
Business/Trader	35 (15.9)	15 (42.9)	20 (57.1)		
Civil servant/Teacher	46 (20.9)	6 (13.0)	40 (87.0)		
Farmer	24 (10.9)	5 (20.8)	19 (79.2)		
House wife	21 (9.5)	1 (4.8)	20 (95.2)		
Student/Pupil	79 (35.9)	12 (15.2)	67 (84.8)		
**Others	5 (2.3)	1 (20.0)	4 (80.0)		
Patient status					
In-patient	97 (39.0)	25 (25.8)	72 (74.2)	5.83	0.01
Out-patient	123 (61.0)	16 (13.0)	107 (87.0)		

**Others: Religious leaders, traditional ruler, military personnel, *Bonferronis correction.

However, with Bonferroni's correction, age group 30 - 41 years was found to be responsible for this variation (P = 0.019). Furthermore, a statistically significant relationship was observed between occupation and the ESBL result (p = 0.005). However, Bonferroni's correction did not identify any of the occupations as being responsible for this statistical variation. Patient status as inpatient or outpatient was found to have a significant relationship with ESBL positivity (p = 0.010) as more inpatients had a positive ESBL isolate than outpatients. See Table 1.

The antibiotic susceptibility patterns of the *E. coli* isolates tested against 15 antibiotics were as shown in Table 2. The ESBL producing isolates were completely non susceptible (resistant) to the penicillins as well as the second and third generation cephalosporins. The ESBL producing isolates also showed very poor *in vitro* susceptibility (greater than 80% resistance) to cefepime, amoxicillin-clavulanic acid, cotrimoxazole, tetracycline, ciprofloxacin and gentamicin and moderate susceptibility to amikacin (54%) and chloramphenicol (59%). However, both the ESBL producing and the non-producing *E. coli* isolates were highly susceptible to Meropenem. The ESBL non producers were also highly susceptible (greater than 95%) to Cefepime, Ceftriaxone and Cefotaxime (Table 2).

The numbers of plasmids ranged from four in isolate two to nine in isolate six. Figure 1 shows the gel electrophoresis picture of the plasmids seen in isolates 1

Table 2. Antibiotic susceptibility patterns of the *Escherichia coli* isolates studied in the University Teaching Hospital.

Antibiotic	ESBL Positive (n = 41)		ESBL Negative(n = 179)	
	Susceptible	Resistant	Susceptible	Resistant
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
Amikacin	22 (53.7)	19 (46.3)	163 (90.5)	16 (9.5)
Amoxiclav	4 (9.8)	37 (90.2)	97 (54.2)	82 (45.8)
Ampicillin	0 (0.0)	41 (100.0)	26 (14.0)	153 (86.0)
Cefepime	4 (9.8)	37 (90.2)	175 (97.8)	4 (2.2)
Cefotaxime	0 (0.0)	41 (100)	173 (96.6)	6 (3.4)
Cefoxitin	13 (31.7)	28 (68.3)	109 (60.9)	70 (39.1)
Ceftazidime	0 (0.0)	41 (100)	167 (93.3)	12 (6.7)
Ceftriaxone	0 (0.0)	41 (100)	173 (96.6)	6 (3.4)
Cefuroxime	0 (0.0)	41 (100)	140 (78.2)	39 (21.8)
Chloramphenicol	24 (58.5)	17 (41.5)	133 (74.3)	46 (25.7)
Ciprofloxacin	6 (14.6)	35 (85.4)	111 (62.0)	68 (38.0)
Cotrimoxazole	4 (9.8)	37 (90.2)	60 (33.5)	119 (66.5)
Gentamicin	8 (19.5)	33 (80.5)	123 (68.7)	56 (31.3)
Meropenem	40 (97.6)	1 (2.4)	179 (100.0)	0 (0.0)
Tetracycline	4 (9.8)	37 (90.2)	63 (35.2)	116 (64.8)

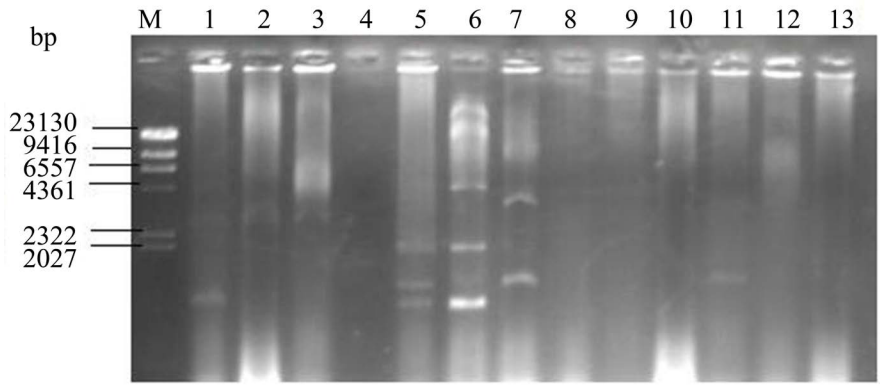

Figure 1. Plasmids for first 13 ESBL producing *E. coli* isolates (Lanes 1 to 13). M = ladder with known molecular weights, bp = base pairs.

to 13. The plasmid results showed that the ESBL producing isolates harbored detectable plasmids with sizes ranging between 2027 - 23,130 base pairs. Lane 4 shows the profile of an ESBL negative isolate which was included in the plasmid profile analysis as a negative control. The carbapenem resistant isolate is shown in Lane 12 with two bands of high molecular weight plasmids (23,130 base pairs).

4. Discussion

The prevalence of ESBL producing *E. coli* in the present study was 18.6%. This ESBL prevalence is much lower than the prevalence obtained from several other studies where investigators had a prevalence of 36.8% in Kano, Nigeria [13], 59.4% in Enugu [14], 66% in Lagos [15], 41% in Pakistan [16] and 53% in Sudan [17]. Lower prevalence rates were found in a study in the Palestinian Gaza strip [18] (3.3%) and in a Macedonian study [19] (11.8%). However, a study in India [20] had a comparable prevalence of 18.5%. These differences may be due to variations in data and sample collection protocols or subtle differences in the presumptive identification of ESBL producing isolates. Furthermore, the populations investigated may differ in various socio-demographic, immune-epidemiologic and clinical parameters. For instance, the Lagos study [15] was amongst patients who had a malignant disease while the Palestinian study [18] was on isolates obtained in an outpatient setting only.

Our findings also show that infection with ESBL producing isolates which used to be confined to hospitalized patients is now being acquired in the community as 13% of the outpatients had ESBL positive isolates. Indeed, there have been several reports of community acquired ESBL *E. coli* from Europe [19] [21], Canada [22], and Asia [20] [23]. A major factor that may be responsible for the emergence of these multidrug resistant phenotypes from the community is the indiscriminate use of antimicrobial agents in the community. This is even more common in our environment where antibiotics are bought over the counter without doctors prescriptions.

The antibiotic susceptibility pattern of the *E. coli* isolates showed multidrug

resistance to several antimicrobial agents usually used in their treatment. This is even more so for the ESBL positive isolates where all the isolates were resistant to at least five of the antibiotics tested while greater than 90% of the ESBL producing isolates were resistant to nine of the 15 antimicrobial agents tested. This mirrors the findings by several investigators in Nigeria [24] other parts of Africa [25], Asia [26], Europe [27] and Canada [22]. The antibiotic susceptibility patterns also suggest concomitant presence of Amp C genes in some of the isolates. The therapeutic options in these cases are therefore highly limited and pose a huge challenge for outpatients in the community whose only therapeutic option in such cases is a carbapenem; often injectable, expensive and hard to come by. For those who can afford it, administration of 8 or 12 hourly injectable drugs in the outpatient setting also attracts challenges of compliance which might further endanger the effectiveness of carbapenems in the future. With an isolate already exhibiting resistance to meropenem, there is an urgent need to preserve the carbapenems. Although, these challenges are ominous, our study reveals that there is a low prevalence of carbapenem resistance to *E. coli* in this setting.

Unlike the pattern seen in the ESBL positive isolates, the ESBL negative *E. coli* isolates showed excellent susceptibility to meropenem and the cephalosporins. Less than five percent of ESBL negative isolates were resistant to cefepime, ceftriaxone and cefotaxime thereby making these antibiotics appropriate choices for treatment of infections caused by these organisms. However, amoxicillin-clavulanic acid which hitherto was thought to be a good choice for infections with these organisms and being mostly used as mono-therapy in our facility was associated with a high rate of resistance. This finding was in contrast with the Macedonian study where resistance to amoxicillin-clavulanic acid was only 20% [19]. Lack of antibiotic prescription policies in our environment and the frequent dispensing of antibiotics such as cefuroxime, ciprofloxacin, cotrimoxazole and amoxicillin-clavulanic acid as over the counter drugs may have contributed to the high level of resistance in the current study. Consequently, making decisions on the appropriate empirical oral antibiotic becomes difficult in the clinics. Cefuroxime had the highest susceptibility profile amongst the oral drugs for non ESBL producing *E. coli* and may be used as first line empirical therapy in such cases.

Although the ESBL genes are known to be either plasmid or chromosomally mediated, they seem to be mostly plasmid mediated in this study. Plasmid profile analysis of the ESBL producing isolates revealed a variety of different sizes ranging from 2 kbp to 23 kbp; (small, medium and high molecular weights). The small molecular weight plasmids also carry resistance genes as typically seen in TEM-1 type beta lactamase implicated in nosocomial isolates of *E. coli* [14]. Several of the tested isolates showed a unique plasmid pattern, with the number of plasmids ranging from four in strain two to nine in strain six. The negative control carried only two plasmids indicating that the higher the number of plasmids carried by the strain, the more the resistance genes carried by the or-

ganism. However, this is in contrast to the findings in a related study by Nariman and colleagues in Egypt where some strains with large numbers of plasmids were susceptible to many common antibiotics [28].

This study appears to be the first study that has reported carbapenem resistance in *E. coli* isolates from Jos in North Central Nigeria. There is a need for an expanded study to determine the extent of these resistance phenotypes in a wider population. It is noteworthy to state that we had several limitations including an inability to determine the actual ESBL genotypes, the carbapenem resistance gene and a complete plasmid profile analysis due to funding restrictions.

5. Conclusion

Our findings show that multidrug resistant ESBL producing *E. coli* are prevalent in both the hospital and the community but are still mostly susceptible to meropenem in this environment. We recommend that institutions develop guidelines for the early phenotypic detection of ESBLs and carbapenem resistance in their microbiology laboratories and incorporate the tools and emanating records into a national or regional antimicrobial resistance surveillance protocol in developing countries. In addition, governments should put in place enabling laws to check the over-the-counter availability and purchase of antibiotics. Carbapenems should only be prescribed when there is laboratory evidence and in consultation with the Microbiologists to preserve their usefulness in the future and in life threatening *E. coli* infections. Finally, the search for newer antimicrobial agents should be intensified as alternatives for treating multidrug resistant organisms such as ESBL producing *E. coli* are limited.

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Data Availability

All the raw data for this study are available on request from the corresponding author.

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There were no external funding sources for this study.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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