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Extended Spectrum Beta-Lactamases in Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* from University of Uyo Teaching Hospital, Uyo-Nigeria

Innocent Azekhueme[1], Anietie E. Moses[1*] and Samuel D. Abbey[2]

[1]Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, University of Uyo, Uyo, Nigeria.
[2]Department of Medical Laboratory Science, Rivers State University of Science and Technology, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author IA designed the study and wrote the protocol. Author AEM wrote part of the manuscript and performed the statistical analysis. Author SDA did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed to investigate the prevalence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in clinical samples from University of Uyo Teaching Hospital (UUTH), Uyo-Nigeria, and determine their antimicrobial susceptibility profile.
Study Design: This study involved sample collection and laboratory analysis of samples.
Place and Duration of Study: The study was conducted at the University of Uyo Teaching Hospital, Uyo between January and June 2013.
Methodology: Faecal, wound and urine samples were obtained from 280 in- and out-patients

**Corresponding author: E-mail: amoses264@gmail.com;*

attending University of Uyo Teaching Hospital, Uyo. *Escherichia coli* and *Klebsiella pneumoniae* isolates were identified using standard conventional microbiological methods. Antimicrobial susceptibility assay was performed using the Kirby-Bauer disc diffusion method and test for ESBL production was conducted using the Double Disk Synergy Test (DDST) following the reviewed Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: ESBL was detected in 40 (47.1%) of the 85 isolates and *E. coli* was the major ESBL producer, 21 (52.5%) followed by *K. pneumoniae,* 19 (47.5%). The detection rates of ESBL producing organisms was higher in female patients (57.5%) than their male counterparts (42.5%), and also high in infants under 1 year of age (20%). The ESBL-producers were most frequently detected in paediatric ward, 11 (27.5%); surgical ward, 10 (25.0%) and GOPD, 7 (17.5%). Majority of the isolates encountered were multidrug resistant strains. Tetracycline and trimethoprim/sulfamethoxazole were the least susceptible antimicrobials to *E. coli* and *K. pneumoniae* ranging from 14.6% - 24.3%, while less than 50% were ciprofloxacin sensitive. Carbapenems were the most effective antimicrobials. All isolates were susceptible to imipenem, while >90% of the isolates were susceptible to ertapenem.

Conclusion: This study reveals that there is a high prevalence of ESBL-producing organisms in clinical samples, especially isolates from infants, in UUTH, Uyo with attendant high multi-drug resistance to commonly used antimicrobials. There is need for increased ESBL surveillance as it poses serious threat to successful treatment of infections and exacerbates the problem of antimicrobial resistance especially with commonly used drugs in resource poor settings.

Keywords: ESBL; E. coli; K. pneumoniae; antimicrobial resistance.

1. INTRODUCTION

The progressive emergence and rapid dissemination of antimicrobial resistance is one of the biggest challenges facing global public health [1]. The accelerated bacterial resistance to antimicrobials has emerged as perhaps the greatest threat worldwide to the favourable outcome of infections both in the hospital settings and in the community. Beta-lactamase production by several gram negative and gram positive organisms is the most important single mechanism of resistance to penicillins, cephalosporins, monobactams and carbapenems [2], which are the most commonly used antimicrobials in treatment of bacterial infections in hospitals [3]. Extended-Spectrum Beta-lactamases (ESBLs) are a group of diverse, complex and plasmid-mediated rapidly evolving enzymes that pose a major therapeutic challenge in the treatment of patients. The ESBLs are able to hydrolyse a broader spectrum of beta-lactam antibiotics than the simple parent beta-lactamases from which they were derived, hence, the term extended spectrum. They are able to inactivate beta-lactam antibiotics containing an oxyimino-group such as oxyimino-cephalosporins (e.g. ceftriaxone, cefotaxime, ceftazidime), oxyimino-monobactam (e.g. aztreonam) [4], as well as the penicillins [5].

ESBLs are most commonly produced by *Escherichia coli and Klebsiella* species, with *Klebsiella pneumonia* seemingly the major ESBL producer [6]. Other members of the Enterobacteriaceae family producing ESBLs include *Salmonella* species, with a growing incidence [7], *Citrobacter freundii, Enterobacter aerogenes* and *Proteus mirabilis* [8]. Non-Enterobacteriaceae ESBL producers are relatively rare with *Pseudomonas aeruginosa* being the most important organism [9]. Others reported include *Acinetobacter* species, *Burkholderia cepacia* and *Alcaligenes faecalis* [4].

Organisms producing ESBL have been increasingly detected worldwide with prevalence rates varying from country to country and from institution to institution [6]. A survey carried out from 1997 to 2002 in some countries put the prevalence rate of ESBL producing *Klebsiella* species at 42.7% in Latin America, 21.7% in Europe and 5.8% in North America [7]. The Pan European Antimicrobial Resistance using Local Surveillance (PEARLS) study (2001-2002) showed that Egypt had the highest prevalence of 38.5%; Greece, 27.4%; while the lowest, 2% and 2.6% were reported in Netherlands and Germany respectively [10]. In the USA, the prevalence of ESBL producing *Klebsiella* species ranged from 0 – 25% with the national average being around 3% [11]. In Japan, the prevalence of ESBL producing *E. coli* and *K. pneumonia* organisms remain low at 0.1% and 0.3% respectively [12]. Some studies have been carried out in Nigeria

especially in the South East with reports of ESBL-producing bacteria ranging from as low as 2.5% to as high as 76.9% prevalence [13,14]. Nevertheless, the prevalence of ESBL-producing organisms in many hospitals in Nigeria as well as ESBL types remain unknown [15,16]. Therefore, the objective of this study was to investigate the prevalence of ESBL-producing *E. coli* and *K. pneumonia* from clinical samples in University of Uyo Teaching Hospital (UUTH), Uyo-Nigeria.

2. MATERIALS AND METHODS

2.1 Study Population

This study was carried out at the University of Uyo Teaching Hospital (UUTH), being a referral and tertiary health facility located in Uyo, the capital of AkwaIbom State, Niger Delta region of Nigeria. Patients attending the out-patient clinics and in-patients at UUTH, Uyo were recruited into the study following informed consent. They included patients from Surgical, Paediatric, General Outpatient Department (GOPD), Medical and Antenatal clinic attendees. Others were from Accident and Emergency, Gynaecology, Orthopaedic and Staff clinics of the Hospital. The ages of patients recruited ranged from 3 months to 82 years. Ethical Approval was sought and obtained from the institutional ethical review board of UUTH, Uyo.

2.2 Sample Collection, Culture and Identification of Isolates

A total of 280 samples were collected comprising stool, urine and superficial wound swabs. Samples were cultured on Blood and Mac Conkey agars (Biomark, India) overnight at 37^{o}C. *E. coli* and *K. pneumoniae* isolates were identified on the basis of conventional microbiological procedures [17].

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility assay was performed according to Clinical Laboratory Standards Institute (CLSI) guidelines (18) using the Kirby-Bauer disc diffusion method to evaluate the sensitivity of the test organisms. Antimicrobial agents used include Ciprofloxacin (5 µg), Tazobactam-Piperacillin (10/100 µg), Cefotaxime (30 µg), Gentamicin (10 µg), Aztreonam (30 µg), Cefpodoxime (10 µg), Ceftazidime (30 µg), Tetracycline (10 µg), Ertapenem (10 µg), Imipenem (10 µg) and Trimethoprim-Sulfamethoxazole (1.25/23.75 µg) (Oxoid, UK). The results were interpreted according to the CLSI guidelines [18]. *Escherichia coli* American Type Culture Collection (ATCC) 25922 reference strain was used as control to test the performance of the method.

2.4 Double Disk Synergy Test for ESBL

Isolates with zone diameters suspicious of ESBL production as pre-determined by the susceptibility test results (Cefpodoxime: ≤17 mm; Ceftazidime: ≤22 mm; Aztreonam: ≤27 mm; Cefotaxime: ≤27 mm) (18) were subjected to the Double Disk Synergy Test [19] to test for the presence of ESBL producing enzymes.

Briefly, test organisms (suspected of ESBL production) were cultured overnight on nutrient agar, and a suspension prepared to match a 0.5 McFarland turbidity standard was inoculated on the surface of each of the molten Mueller Hinton agar plates using a sterile swab. Amoxicillin (20 µg)/ clavulanic acid (10 µg) combination disc was placed at the center of each inoculated Mueller–Hinton agar plate. Cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30µg) and cefpodoxime (10 µg) single discs were then placed 20 mm (center to center) from the amoxycillin/clavulanic acid disc and incubated at 37ºC overnight (18-24 hr).

Enhancement of the zones of inhibition of any of the cephalosporin beta-lactam antibiotic discs (i.e. cefotaxime, aztreonam, cefpodoxime or ceftazidime) towards the amoxycillin/clavulanic acid disc caused by the synergy with clavulanatewas taken as an evidence of ESBL production. *E. coli* ATCC 25922 (β-lactamase negative), *K. pneumoniae* ATCC 700603(ESBL-producer) were used as reference strains for quality control.

2.5 Data Analysis

Data from this study were presented in frequency tables and in percentages. Data were analysed using SPSS version 17 statistical package and the association between ESBL producing organisms and patient demographics was determined using Chi square test at significance level of $p = 0.05$.

3. RESULTS

The antimicrobial susceptibility pattern of *E. coli* and *K. pneumoniae* isolates from clinical samples in UUTH, Uyo are shown in Table 1.

Imipenem was the most effective antimicrobial agent with 100% activity against all the isolates. Ertapenem was sensitive to 93.7% of *E. coli* and 91.9% of *K. pneumonia* isolates. Tetracycline and trimethoprim/sulfamethoxazole were the least effective antimicrobials to *E. coli* with susceptibility rates of 16.7% and 14.6% respectively and 24.3% each for *K. pneumoniae.*

Table 2 shows the prevalence of extended-spectrum Beta-lactamase organisms isolated from clinical samples in UUTH, Uyo. Of the 85 bacterial isolates obtained, 40 were ESBL-producing organisms giving a prevalence rate of 47.1%. Out of a total of 40 ESBL producing organisms, *E. coli* was the major ESBL producer 21 (52.5%) followed by *K. pneumoniae,* 19 (47.5%).

The age and gender distribution of ESBL-producing organisms in UUTH, Uyo are shown in Table 3. Generally, infants under 1 year had relatively high prevalence (20%) of ESBL-producing organisms than older children, while females were detected with the highest rate, 23 (57.5%) of ESBL-producing bacteria compared with their male counterparts, 17 (42.5%). The gender difference in the isolation rates was not statistically significant (P>.05). Specifically, ESBL-producing *K. pneumoniae* were more frequently isolated in females than in males, so also were ESBL-producing *E. coli* isolates.

The distribution of ESBL producers according to ward/clinic in UUTH, Uyo is shown in Table 4. ESBL-producing organisms were most frequently encountered in the Paediatric ward, 11 (27.5%) closely followed by the Surgical ward, 10 (25.0%), then GOPD, 7 (17.5%). No ESBL producer was encountered among isolates from the orthopaedic and surgical wards.

4. DISCUSSION

The prevalence of ESBL-producing organisms has been increasing rapidly worldwide. This situation is alarming because ESBL producers have been reported to exhibit co-resistance to many other classes of antibiotics resulting in limited therapeutic options [5]. In addition, the prevalence of ESBL, type of enzyme, gender and age group of persons affected varies in different geographical areas.

Table 1. Antimicrobial sensitivity pattern of *E. coli* and *K. pneumonia* isolates from clinical samples

Antimicrobial (µg)	*E. coli* (n=48)			*K. pneumoniae* n=37)		
	S %	I %	R %	S %	I %	R %
ATM(30)	58.3	0	41.7	32.4	0	25
CTX (30)	58.3	0	41.7	32.4	0	67.6
CPD (10)	58.3	2.1	39.6	45.9	2.7	51.4
CAZ (30)	54.2	4.2	41.7	40.5	2.7	56.8
CIP (5)	45.8	4.2	50.0	48.6	13.6	37.8
ETP(10)	93.7	6.3	0	91.9	0	8.1
CN (10)	66.8	8.3	22.9	51.4	10.8	37.8
IPM(10)	100.0	0	0	100.0	0	0
TZP (10/100)	62.5	29.2	8.3	67.6	21.6	10.8
TE (30)	16.7	0	83.3	24.3	2.7	73.0
SXT (1.25/23.75)	14.6	0	85.4	24.3	0	75.7

Key: S=Susceptible, I=Intermediate, R=Resistant, ATM=Aztreonam, CTX=Cefotaxime, CPD=Cefpodoxime, CAZ=Ceftazidime, CIP=Ciprofloxacin, ETP=Ertapenem, CN=Gentamicin, IPM=Imipenem, TZP=Tazobactam/Piperacillin, TE=Tetracycline, SXT=Sulfamethoxazole/Trimethoprim

Table 2. Distribution of ESBL producing bacteria among clinical samples in UUTH, Uyo

Sample	No. tested	No. of Isolates (ESBL producers)		
		E. coli	*K. pneumoniae*	Total
Stool	47	21 (5)	15 (6)	36 (11)
Urine	173	21 (10)	11 (5)	32 (15)
Wounds	60	6 (6)	11 (8)	17 (14)
Total	280	48 (21)	37 (19)	85 (40)
Percentage ESBL producer	-	43.8	52.5	47.1

Table 3. Age and gender distribution of ESBL-producing bacteria from clinical samples in UUTH, Uyo

Patient group	No. isolates tested	ESBL-*Escherichia coli*	ESBL-*Klebsiella pneumoniae*	Total (%)
Age group (yr)				
<1	23	3	5	8 (20.0)
1-10	15	2	1	3 (7.5)
11-20	2	0	1	1 (2.5)
21-30	5	1	1	2(5.0)
31-40	18	3	5	8 (20.0)
41-50	9	7	4	11 (27.5)
51-60	8	4	1	5 (12.5)
61-70	3	0	0	0
71-80	2	1	1	2 (5.0)
Total	85	21	19	40 (100.0)
Gender				
Male	41	9	8	17 (42.5)
Female	44	12	11	23 (57.5)
Total	85	21	19	40 (100.0)

Table 4. Distribution of ESBL producing organisms according to Ward/Clinic in UUTH, Uyo

Location	No. of isolates	No. ESBL organisms		
		E. coli	*K. pneumoniae*	Total No. (%)
GOPD (General outpatient department)	24	5	2	7 (17.5)
Staff Clinic	2	1	0	1 (2.5)
A&E (Accident and emergency)	6	1	1	2 (5.0)
Medical wards	7	2	0	2 (5.0)
GYNAE (Gynaecology ward)	2	0	1	1 (2.5)
ANW (Antenatal clinic)	4	1	1	2 (5.0)
Paediatric ward	22	5	6	11 (27.5)
Orthopaedic ward	12	2	2	4 (10.0)
Surgical ward	6	4	6	10 (25.0)
Total (%)	85	21	19	40(100.0)

In this study, the overall prevalence of ESBL producing isolates from clinical samples was 47.1%. This is comparable to 39.8% reported in Enugu [13] and 33.6% in Abuja [20]. Higher prevalence rates of 58.6% (Enugu) and 76.9% (Ibadan) have been reported in other Nigerian studies [15,16]. Global reports show that Pakistan [21], Sudan [22] and India [23] recorded 29.45%, 59.6% and 57.5% prevalence respectively. The variation in ESBLs prevalence rates reported between geographical areas, institutions and countries may be attributed to the complex epidemiology of ESBLs, specific type of bacteria involved and methods used for ESBL detection among other factors [6,24].

In this study, *E. coli* was identified as the major ESBL producer (52.5%) followed by *K. pneumoniae* (47.5%). *E. coli* has recently been reported as the major ESBL producer in similar studies conducted in Enugu [16], Osun [25] and Ogun States [14] as well as in Bangladesh [26] and Pakistan [21]. However, other Nigerian reports, Ogun [27], Benin [28] and Abuja [20] as well as Ghana [29] and Sudan [22] indicated *K. pneumonia* as the major ESBL producer. Even though both organisms have been identified in various studies within and outside Nigeria as the major ESBL producing microorganisms, there has been no consensus on which has a greater capacity to produce ESBL.

Regarding gender classification, the fact that more ESBL producing organisms were recovered from females than their male counterparts, the difference was however not statistically significant and similar results have been documented elsewhere, Abuja, Nigeria [20] and Pakistan [21]. Children under one year of age were among the age brackets with high

prevalence of ESBL producing organisms in this study (20%). Similar high prevalence have been reported elsewhere [14,30]. This observation has serious therapeutic implications that may impact on clinical outcomes in infants. More so, ESBL producing organisms have been associated with increased mortality and morbidity [31].

In this study, it was observed that ESBL producers were most frequently encountered in the Paediatric ward (27.5%) closely followed by surgical wards (25.0%) and General Outpatient Department (GOPD) (17.5%). A study by Alipourfard and Nili [26] reported most of the ESBL-producing isolates (29.6%) were from the medical wards, followed by outpatient's clinic (24.3%).

Majority of the isolates encountered in this study were multidrug resistant strains showing resistance to 4 to 5 antimicrobials or more. The gross misuse or over-use of antimicrobials in the hospital, non-compliance or adherence of antimicrobial use by patients, could lead to development of multidrug resistant bacteria [2,4]. The existence of ESBL-producers in an individual could lead to increased antibiotic resistance because the plasmid which carries the ESBL enzymes also harbour resistance genes to other classes of antimicrobials [32].

Findings from this study shows that Imipenem is the drug of choice against ESBL-producers followed by ertapenem as equally reported in other studies [33,34]. Commonly used antimicrobials in our locality such as trimethoprim/sulfamethoxazole, Tetracycline and ciprofloxacin were among the least effective against the ESBLs as observed in this study. The reason could be attributed to the gross misuse and abuse of these inexpensive antimicrobials which are readily available as over-the-counter (OTC) drugs and can even be purchased without doctor's prescription [35]. Consequently, this poses problems for treatment of infections caused by ESBL producers because these commonly used antimicrobials are usually ruled out in therapeutic options. However, Imipenem and which is the most effective drug against ESBL-producing organism is very expensive and is a broad spectrum antimicrobial. Also, its indiscriminate use could result in the development of imipenem-resistant strains leaving no therapeutic alternative in the future.

The reviewed CLSI guidelines [36] advise that when using the new interpretive criteria, routine ESBL testing is no longer necessary before reporting results (i.e. it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins from susceptible to resistant) except for epidemiologic purposes. However, some researchers recently argued against the new breakpoint categorization and posited thatit is prudent to continue to seek ESBLs and carbapenemases directly to avoid using substrate drugs as therapy [37]. Nevertheless, it is imperative to adhere to the CLSI guidelines [36] when seeking ESBL-producing Enterobacteriaceae and promptly report cases as appropriate. The need for rapid screening of patients for ESBL production for cost-effective isolation, effective medical treatments and to facilitate improved prevention of nosocomial infections, had earlier been advocated [34,38]. It is also important to note that, failure to detect ESBL-mediated resistance can lead to therapeutic failure and facilitate the spread of ESBL enzyme among non-ESBL producing organisms.

5. CONCLUSION

The data from this study showed that there is a high prevalence of ESBL-producing organisms in clinical samples, especially isolates from infants in University of Uyo Teaching Hospital, Uyo-Nigeria. Majority of the ESBL enzyme producers in this study were *E. coli* strains. Most of the organisms isolated were multidrug resistant strains while the ESBL producers further compounded the problem by exhibiting co-resistance to other classes of antimicrobials including the fluoroquinolones and aminoglycosides. However, the carbapenems remain the most effective therapeutic option for both ESBL and non-ESBL producing organisms, imipenem being the most effective followed by ertapenem. There is need for increased surveillance of ESBL producing organisms as they pose serious threat to successful treatment of infections and exacerbates the problem of antimicrobial resistance in the hospitals, especially in resource poor settings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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