

Detection of Extended Spectrum β -Lactamase Producing *Klebsiella pneumoniae* and *Escherichia coli* in Two Hospitals in the Federal Capital Territory, Abuja, Nigeria

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ABSTRACT

In this study, the prevalence of Extended Spectrum Beta-lactamase (ESBL) producing *Klebsiella pneumoniae* and *Escherichia coli* isolates from the University of Abuja Teaching Hospital and the National Hospital was determined. A total of two hundred and fifteen (215) clinical isolates were examined, of which 60% were *E. coli* and 40% *K. pneumoniae* respectively. The isolates were collected from various samples namely: Stool, Urine, Pus, High Vagina Swab, Sputum and Wound swab. Out of these isolates, 54 of *K. pneumoniae* were screened to be ESBL negative and 32 as ESBL positive isolates, while 88 and 40 *E. coli* were also screened as ESBL negative and ESBL positive isolates respectively. These represent 37.9% of all *K. pneumoniae* isolates and 31.25% of *E. coli* isolates respectively. The prevalence of ESBL among the species was not however statistically different ($p > 0.05$). Multiple resistance in these isolates was common and there is the need for routine screening of ESBL in our hospitals to guide rational and effective use of antibiotics.

Keywords: Extended Spectrum Beta-Lactamase; *Klebsiella pneumoniae*; *Escherichia coli*; Nigeria; Multidrug Resistance

1. Introduction

Antimicrobial resistance has arisen across the globe in both nosocomial and community settings as a consequence of widespread antibiotics consumption [1]. The Beta-lactam antibiotics are among the most widely used antimicrobial agents worldwide. Destruction of these antibiotics by the bacterial enzyme, beta-lactamase is the most frequently encountered mechanism of resistance among Gram-negative microorganisms [2]. Multidrug resistant gram negative bacilli belonging to the family Enterobacteriaceae have been increasingly responsible for infections in many countries [3]. The emergence of multiresistance in the Enterobacteriaceae family needs attention, because these are important causative agents of hospital infections, typically associated with pneumonias, blood stream infections, urinary tract infections, bacteremia and other intra-abdominal infections [4,5]. By definition, ESBLs are β -lactamases capable of conferring

bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, which are inhibited by β -lactamase inhibitors such as clavulanic acid [6].

The presence of ESBLs has tremendous clinical significance due to the fact that ESBLs are frequently plasmid encoded and also in most cases the plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes therefore limiting antibiotic options in the treatment of ESBL-producing organisms [6]. The selection pressure that drives the emergence of ESBLs has usually been attributed to the intense use of oxyimino-beta lactams, mainly the third generation cephalosporins in addition to extensive use of broad spectrum antibiotics, prolonged hospitalization, indwelling devices and severe underlying diseases [7-9].

Several studies have demonstrated that ESBL-pro-

ducing bacteria are isolated with increasing frequency in many parts of the world [3,10-12] and a number of reports are also available in other parts of this country [13,14]. The aim of this study was to detect ESBL-producing *K. pneumoniae* and *E. coli* from clinical isolates from both inpatients and outpatients to evaluate the risk factors that may be inherent in this location. This is necessary because the prevalence of resistant strains of these organisms varies from one geographical location to another. Two hospitals receiving the highest number of patients from the federal capital territory and its environs namely, the University of Abuja Teaching Hospital and National Hospital were chosen for this study. To our knowledge, no published study exists on ESBL in *Klebsiella pneumoniae* and *E. coli* in this locality, probably reflecting the lack of appreciation of the problem.

2. Materials and Methods

2.1. Isolation and Identification of *Klebsiella pneumoniae* and *Escherichia coli*

The isolates were collected from the University of Abuja Teaching Hospital Gwagwalada and National Hospital, Abuja. The organisms were isolated from stool, pus, urine, sputum as well as wound and high vaginal swabs (HVS) samples of both out-patients and In-patients. Isolates presumed to be the etiologic agent responsible for the disease condition were used for the study. Isolates considered to be contaminants were not included in the screening for ESBL. Isolates from pus and wound swabs were however included in the screening even though the organisms are not usually associated with infection at these sites.

Briefly, swabs and clinical specimens were inoculated on eosin methylene blue agar (EMB, Oxoid) and MacConkey agar (Oxoid). After inoculation, the plates were incubated at 37°C for 24 hr. The mucoids and smooth colonies suggesting *K. pneumoniae* strains were Gram stained. Routinely, Indian ink was used to detect the presence of capsules and isolates were also inoculated onto the screening media for biochemical identification: TSI (triple sugar iron), SIM (sulphate/indole/motility) and citrate agar (Oxoid), and incubated at 37°C for 24 h. Colonies showing green metallic sheen on EMB and non mucoid round pinkish colonies on MacConkey were tested for production of indole, methyl red, Voges Proskauer and citrate utilization (IMVIC).

2.2. Antimicrobial Susceptibility Tests

The *K. pneumoniae* and *E. coli* strains isolated were submitted to antimicrobial susceptibility testing according to the recommendations of the Clinical and Laboratory Standards Institute [15]. The turbidity of the suspensions used for sensitivity testing was adjusted to 0.5

McFarland standard and inoculated onto Mueller-Hinton agar medium followed by incubation at 35°C ± 1°C for 18 - 24 hrs. The following antimicrobial discs were used: ceftriaxone (CRO) (30 µg), ceftazidime (CAZ) (30 µg), cefepime (FEP) (30 µg), gentamicin (GEN) (10 µg), amikacin (AK) (30 µg), ciprofloxacin (CIP) (5 µg), chloramphenicol (C) (30 µg) and trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 µg).

Isolates that exhibited a zone of inhibition of growth for ceftazidime and ceftriaxone ≤22 mm and ≤25 mm, respectively, were submitted to the combined disc test in order to check for ESBL-producing strains. The combined disc methodology used to detect ESBL-producing *K. pneumoniae* and *E. coli* was performed as recommended by CLSI [15]. The antimicrobials used were: cefotaxime (30 µg) and cefotaxime (30 µg) plus clavulanic acid (10 µg), and ceftazidime (30 µg) and ceftazidime (30 µg) plus clavulanic acid (10 µg). Results were interpreted according to the criteria established by the CLSI [16]. A 5 mm increase in a zone of inhibition of growth for cefotaxime plus clavulanic acid as compared with the zone around the cefotaxime disc, and a 5 mm increase in the zone diameter for ceftazidime plus clavulanic acid as compared with the zone formed by the ceftazidime disc, were confirmatory for the result of ESBL-producing strains.

Interpretation of results for other antibacterial agents was as per the guidelines of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) for enterobacteriaceae [16].

2.3. Statistical Analysis

Chi square was used to analyze data on gender distribution of the isolates, site distribution of the isolates and frequency of ESBL production by *K. pneumoniae* and *E. coli* using the software Smith's Statistical Package (SSP) version 2.80 copyright© 1995-2005 Gary Smith.

3. Results

A total of 215 isolates were collected from the University of Abuja Teaching Hospital and the National Hospital Abuja to determine ESBL production. Out of 215 isolates 128 were *E. coli* (60%) and 87 were *K. pneumoniae* (40%). The age range of these patients was between 2 - 78 years.

Figure 1 shows that more females were infected by these pathogens than men with values of 40.63% (52) for *E. coli* infections in men which was less than the 59.37% (76) for women. Similarly in *K. pneumoniae* infections the frequency was higher in females n = 52, (55.170%) compared to males n = 39, (44.83%). The observed differences in male and female proportions of the two pathogens were not significant with a p value of 0.74 χ^2 , 1 df, (p > 0.5).

The distribution of *K. pneumoniae* and *E. coli* in the different specimens examined is shown in **Figure 2**. The number of isolates obtained from different sites was not uniform. The highest numbers of both *E. coli* as well as *K. pneumoniae* were obtained from urine at 60 and 36 respectively. For *E. coli* diarrheic fecal samples, pus, HVS wound swabs and sputum in decreasing order accounted for the remainder of the isolates at 37, 17, 10 and 0 respectively. *K. pneumoniae* was isolated in decreasing

order from sputum (30), pus (9), HVS (8), wound swabs (3) and stool (0). The distribution of *E. coli* and *K. pneumoniae* isolates was significantly different at these sites p value of 0.00000 χ^2 , 5 df, ($p < 0.05$).

Table 1 shows the antimicrobial susceptibilities of *K. pneumoniae* isolates to different classes of antibiotics namely third generation cephalosporins (ceftriaxone and ceftazidime), fourth generation cephalosporins (cefepime), aminoglycosides (gentamicin and amikacin),

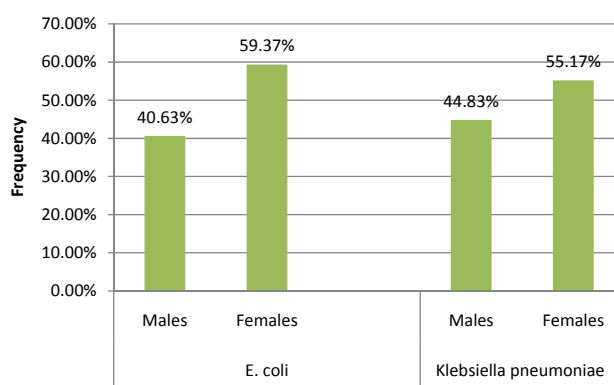


Figure 1. Gender Distribution of the isolates.

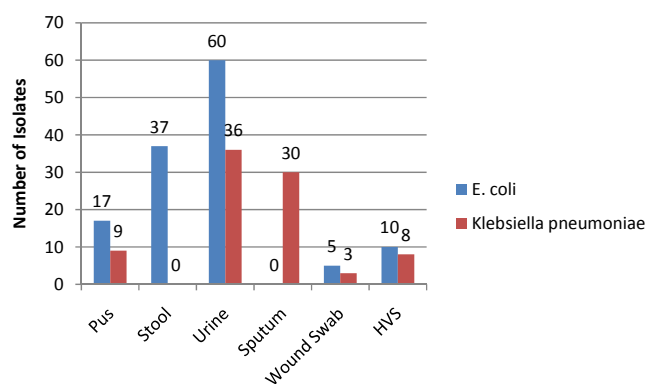


Figure 2. Site Distribution of the isolates.

Table 1. Antimicrobial susceptibilities of *Klebsiella pneumoniae* isolates.

Antibiotics	ESBL negative (n = 54) Isolates		ESBL Positive (n = 33) Isolates	
	Resistant n (%)	Susceptible n (%)	Resistant n (%)	Susceptible n (%)
Ceftriaxone	17 (31.5)	37 (68.5)	33 (100)	0 (00)
Ceftazidime	13 (24.1)	41 (75.9)	33 (100)	0 (00)
Cefepime	16 (29.6)	38 (70.4)	13 (39.4)	20 (60.6)
Gentamicin	20 (37.0)	34 (63.0)	18 (54.5)	15 (45.5)
Amikacin	19 (35.2)	35 (64.8)	9 (27.3)	24 (72.7)
Ciprofloxacin	14 (26.0)	40 (74.0)	6 (18.1)	27 (81.9)
Chloramphenicol	12 (22.2)	42 (77.8)	8 (24.2)	25 (75.8)
Trimethoprim/sulfamethoxazole	21 (38.9)	33 (61.1)	10 (30.3)	23 (69.7)
Multiple resistance	7 (33.3)		5 (38.5)	

Key: Multiple Resistance: Strains that were resistant to two or more antimicrobials were regarded as multiresistant.

fluoroquinolone (ciprofloxacin), sulfonamide/trimethoprim combination (trimethoprim/sulfamethoxazole) and chloramphenicol.

A large number of ESBL-producing *Klebsiella pneumoniae* strains isolated also showed resistance to other classes of antimicrobials tested. Out of the tested isolates, 54.5% were resistant to gentamicin whereas resistance was lower to other antibacterial agents with values of 27.3%, 18.1%, 24.2% and 30.3% resistance to amikacin, ciprofloxacin, chloramphenicol and trimethoprim/sulfamethoxazole respectively. Resistance of a strain to 2 or more antimicrobials was considered multiple resistance and in this case 38.5% were multiple resistant. Comparatively non-ESBL producing strains were less resistant with resistant values of 37.0%, 35.2%, 26.0%, 22.2% and 38.9% to gentamicin, amikacin, ciprofloxacin, chloramphenicol and trimethoprim/sulfamethoxazole respectively. Seven of the non-ESBL (33.3%) were multiple resistant which is slightly lower than that of ESBL producing strains.

Table 2 shows the antimicrobial susceptibilities of *E. coli* isolates to different classes of antibiotics. Of the ESBL-producing *E. coli* strains isolated, 32.5%, 20.0%, 25.0%, 27.5% and 35.0% were resistant to gentamicin, amikacin, ciprofloxacin, chloramphenicol, and trimethoprim/sulfamethoxazole respectively. In addition 42.9% were multiple resistant to the test antibiotics as previously defined. Resistance to these antimicrobials by non-ESBL producing strains was variable being higher with amikacin (29.5%) but lower with respect to gentamicin (23.9%), ciprofloxacin (22.7%), chloramphenicol (23.9%) and, trimethoprim/sulfamethoxazole (31.8%).

Comparatively more isolates of *K. pneumoniae* (37.9% of all *K. pneumoniae* isolates) were ESBL producing when compared to ESBL producing *E. coli* (31.25% of all *E. coli* isolates). These values were not however statistically different ($p > 0.05$). χ^2 , 1 df, $p = 0.31$

4. Discussion

K. pneumoniae and *E. coli* infections are some of the most commonly encountered ones in clinical medicine, causing a wide range of clinical conditions from relatively mild to serious, sometimes life-threatening conditions that can lead to death. In the present study, both organisms were isolated from different age groups and equally from both male and female genders. No differences were apparent which is similar to observations in a study that found no relationship between ESBL-producing *E. coli* or *K. pneumoniae* infection with age or sex [17]. However in another study that assessed risk factor for mortality, most patients infected with ESBL producers were elderly and with a slight male predominance (59%) in the group studied [18].

The distribution of isolates and sites of infection were significantly different in this study and supports in a similar report were out of a total of 33 patients with ESBL-producing *E. coli* or *K. pneumoniae* infection. 25 (75.8%) of them had infections due to *K. pneumoniae* and 8 (24.2%) had infections due to *E. coli* [17].

Moreover, the distribution of the two pathogens studied reflects differences in their pathogenicity and associated disease conditions. *K. pneumoniae* is known to cause suppurative infections, bacteremia, and a substantial percentage of nosocomial infections. However, urinary tract infections and infections of the respiratory tract predominate [19]. *E. coli* strains are known to cause a wide variety of diseases. The isolates from this study were not, however, further characterized into patho groups.

About 38% of *K. pneumoniae* were ESBL-producing compared to 31% of *E. coli* isolates; this may indicate that ESBL production is more common among *K. pneumoniae* isolates as reported in other studies such as Lautenbach *et al.* (2001) with *K. pneumoniae* accounting for 75.8% of ESBL production compared to 24.2% for *E. coli* [17]. Also Serefhanoglu *et al.* (2009) reported 60.6% of ESBL producing isolates to be *K. pneumoniae* whereas 39.4% were *E. coli* in a total of 94 bloodstream infections examined [20].

A large number of ESBL-producing *K. pneumoniae* isolates also showed resistance to other classes of antimicrobials tested particularly gentamicin (54.5%) and trimethoprim/sulfamethoxazole (30.3%). This is consistent with observations that ESBL-producing bacteria are associated with the transfer of conjugative plasmids, which also carry genes of resistance to aminoglycosides and sulfonamides, giving the bacteria multiresistance attributes [21].

Importantly, conjugative plasmids can be easily transferred across species as demonstrated in transfer from clinical isolates of *Klebsiella pneumoniae* to *Escherichia coli* involving transfer of resistance to ceftazidime, cefotaxime, ceftriaxone, gentamicin, amikacin, ciprofloxacin, aztreonam, ceftiofur and ticarcillin/CA and intermediate resistance to piperacillin/tazobactam, cefoperazone/sulbactam and cefepime [22].

Similarly, a substantial number of ESBL-producing *E. coli* strains isolated were also resistant to other antibiotics although the rates appeared lower than those of *Klebsiella pneumoniae* and the same mechanisms of resistance have been noted earlier. Comparatively, resistance to these antimicrobials by non-ESBL producing strains was lower probably reflecting lower rates of transfer of multiple transfers of resistance genes in non ESBL producers.

Our results show a very high incidence of ESBL producing clinical isolates. The implications of which are the necessity for circumspection in prescription of anti-

Table 2. Antimicrobial susceptibilities of *E. coli* isolates

Antibiotics	ESBL negative (n = 88) Isolates		ESBL Positive (n = 40) Isolates	
	Resistant n (%)	Susceptible n (%)	Resistant n (%)	Susceptible n (%)
Ceftriaxone	36 (41.0)	52 (59.0)	40 (100)	0 (00)
Ceftazidime	28 (31.8)	60 (68.2)	40 (100)	0 (00)
Cefepime	18 (20.5)	70 (79.5)	10 (25.0)	30 (75.0)
Gentamicin	21 (23.9)	67 (76.1)	13 (32.5)	27 (67.5)
Amikacin	26 (29.5)	62 (70.5)	8 (20.0)	32 (80.0)
Ciprofloxacin	20 (22.7)	68 (77.3)	10 (25.0)	30 (75.0)
Chloramphenicol	21 (23.9)	67 (76.1)	11 (27.5)	29 (72.5)
Trimethoprim/sulfamethoxazole	28 (31.8)	60 (68.2)	14 (35.0)	26 (65.0)
Multiple resistance	10 (27.8)		6 (42.9)	

Key: Multiple Resistance: Strains that were resistant to two or more antimicrobials were regarded as multiresistant.

biotics, appropriate hospital waste disposal and efficient hospital sewage treatment to prevent and limit environmental spread. Furthermore, regular surveillance is necessary including more detailed molecular studies as well as research in the prevalence studies on carbapenemase producing *Klebsiella pneumoniae* in this region to get a fuller picture of the extent of the problem.

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