The prevalence of extended spectrum beta-lactamases (ESBLs) among *Escherichia coli* and *Klebsiella species* urinary isolates from Abia state university teaching hospital (ABSUTH) aba, Abia State Nigeria

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**Abstract**

The prevalence of extended spectrum β-lactamases among 246 clinical isolates from Abia State University teaching Hospital patients was investigated. The isolates were made up of 134 *Escherichia coli* and 112 *Klebsiella* species. Antimicrobial susceptibility of the isolates was determined by the disc diffusion method. ESBL phenotypes were determined by the double disc synergy method using ceftazidime, cefotaxime, ceftriaxone and co-amoxiclav. Out of the 246 isolates, 125 (50.8%) were ESBL producers, made up of 62 (50.8%) *E. coli* and 63 (50.4%) *Klebsiella* isolates. Seventeen (54.8%) of the ESBL producing *E. coli* isolates were from in-patients while 45 (47.9%) were from out-patients. For the ESBL positive *Klebsiella* spp., 14 (45.2%) and 49 (52.1%) were from in-patients and out-patients respectively. ESBL producing isolates were also found to be more prevalent among the female patients (72.8%) than among the male patients (27.2%). The isolates also expressed high rates of resistance to other classes of antibiotics tested. However, Amikacin was found to have excellent performance against the urinary isolates tested and therefore is recommended for the treatment of infections caused by *Escherichia coli* and *Klebsiella* species. This study shows high prevalence of ESBL producing *E. coli* and *Klebsiella* isolates clinical samples of patients attending the Abia State University Teaching Hospital Aba, Abia State Nigeria.

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Introduction
Extended-spectrum beta-lactamases (ESBLs) are among the important cause of multi-drug resistant infections throughout the world (Livermore et al., 2007). Bacteria carrying such enzymes have long been recognized as the cause of healthcare-associated infections and the incidence of such organisms also appear to be increasing in the community, typically as cause of urinary tract infections (Pitout et al., 2005). Infections due to ESBL-producing organisms such as Escherichia coli, Klebsiella, Pseudomonas, Citrobacter, Enterobacter, Morganella, Serratia, Salmonella, Proteus, and Shigella species can pose major threat to life, and are often difficult and expensive to treat, and can delay discharge from hospital (Lee et al., 2006).

Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Symptoms include frequent feeling and/or need to urinate, pain during urination, and cloudy urine. The main causal agent is Escherichia coli. Although urine contains a variety of fluids, salts, and waste products, it does not usually have bacteria in it. When bacteria get into the bladder or kidney and multiply in the urine, they may cause a UTI, the most common type of which is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious (Nicolle, 2008). There have been significant changes in the antimicrobial resistance patterns of uro-pathogens over the years including resistance due to extended spectrum beta lactamase (ESBL)-producing pathogens (Mohammed et al., 2007). The increasing prevalence of infections caused by antibiotic-resistant bacteria makes empirical treatment of these infections difficult (Steinke et al., 2001).

Antibiotic resistance varies according to geographic locations and is directly proportional to the use and / or misuse of antibiotics. Once selected in one patient, resistant bacteria can spread to other people. Basic infection control measures can help to prevent such spread but these steps may be undermined in ‘high-pressure’ care settings by rapid bed turnover in hospitals, frequent transfers between wards within hospitals, or between care settings, overcrowding, and overstretched of medical and nursing staff (Pitout et al., 2005). Within hospitals, multi-resistance is most prevalent where antimicrobial use is greatest, notably in intensive care units and other high dependency units and especially in immune-compromised, debilitated or elderly patients, or those with underlying diseases such as cancer, diabetes mellitus, chronic liver disease, chronic renal failure or burns (Paterson, 2007). Also, patients discharged from hospital care can carry resistant organisms into the community, with the result that nursing and residential homes provide large reservoirs for the potential spread of resistance and its subsequent reintroduction into hospitals (Pitout et al., 2005). ESBL screening as a routine test has not yet been practiced in Nigeria.

The present study is undertaken to investigate the prevalence of Extended Spectrum Beta Lactamases (ESBL) by phenotypic methods, among isolates of Escherichia coli and Klebsiella species from Urinary Tract Infection (UTI) patients at Abia State University Teaching Materials and Method

Collection of clinical isolates
A total of two hundred and forty six (246) clinical bacterial isolates comprising of Escherichia coli (134) and klebsiella spp. (112) were collected from Abia State University Teaching Hospital Aba (ABSUTH), Nigeria. All clinical isolates were urinary isolates, from confirmed urinary tract infection (UTI) patients. The isolates were identified by colonial appearance on MacConkey agar, gram staining reactions and standard biochemical tests (Iroha et al., 2009).
Antimicrobial susceptibility test
Modified Kirby-Bauer sensitivity testing method was used for this purpose. Muller Hinton agar media (pH 7.2), was poured into 90 mm diameter sterile Petri dishes and allowed to solidify at ambient temperature (28 ± 2)°C. The surface of the medium was uniformly inoculated with sterile cotton swab stick in three directions rotating the plate approximately 60° to ensure even distribution. Prior to inoculation, the swab stick was dipped into bacterial suspension (having visual equivalent turbidity to 0.5 McFarland standards). The swab stick was then removed and squeezed on the wall of the test tube to discard excess suspension. Inoculated plates were incubated at 37°C for 24 hours, after which the plates were read by measuring the zones of inhibition. Antimicrobial discs used included amoxycillin-clavulanic acid (20/10 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30µg), Gentamicin (10 µg), Amikacin (30 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Cephalexin (30 µg), Ofloxacin (5 µg), Perflaxcin (5 µg) (Oxoid, UK).

Confirmation by double disc synergy test
The isolated colonies were inoculated in peptone water at 37°C for 2–6 hours. The turbidity was adjusted to 0.5 McFarland standard and lawn culture was made on Mueller-Hinton agar medium using sterile swab stick. Amoxicillin/clavulanic acid disc (20/10µg) was placed in the center of the plate, a disc of cefotaxime (30µg), ceftriaxone (30µ) and ceftazidime (30µg), were placed centre to center at a distance of 15 mm to the centrally placed Amoxicillin/clavulanic acid disc. The plate was incubated overnight at 37°C. Enhanced zone of inhibition between any of the beta-lactam discs and the centre disc was recorded. In this study an enhanced zone of inhibition between any of the third generation cephalosporin antibiotic discs and the co-amoxiclav disc was confirmation of ESBL production according to clinical and laboratory standards institute (CLSI, 2010) criteria. E. coli ATCC 25922 was used as control strain for the study.

Results
The 246 isolates as identified were made up of 134(54.5%) of Escherichia coli and 112(45.5%) of Klebsiella species. The urinary pathogens were isolated more from females, 180 (73.2%) than males, 66 (26.8%) patients. There was significantly higher proportion of bacteria isolated from outpatients 191 (77.6%) than inpatients 55(22.4%). The isolates were also characterized for their antibiogram. Table 1, shows the rate of resistance to the different antimicrobial tested on isolates from both in-patients and out-patients. The isolates showed least resistance to amikacin, Escherichia coli (6.5%) among inpatients and (7.8%) among outpatients as well as Klebsiella species (12.3%) among the in patients and (13.6%) among the outpatients.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>E.coli isolated from inpatients (n=31)</th>
<th>E.coli isolated from outpatients (n=103)</th>
<th>Klebsiella sp. from inpatients (n=24)</th>
<th>Klebsiella sp. from outpatients (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>20(64.5)</td>
<td>55(53.4)</td>
<td>18(75.0)</td>
<td>60(68.2)</td>
</tr>
<tr>
<td>Cephaexin</td>
<td>22(71.0)</td>
<td>65(63.1)</td>
<td>19(79.2)</td>
<td>56(63.6)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>18(58.1)</td>
<td>52(50.5)</td>
<td>10(41.2)</td>
<td>40(45.5)</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>28(90.3)</td>
<td>70(68.0)</td>
<td>15(62.5)</td>
<td>75(85.0)</td>
</tr>
<tr>
<td>Ciprofloxain</td>
<td>08(25.8)</td>
<td>30(29.1)</td>
<td>05(20.8)</td>
<td>30(34.1)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>27(87.1)</td>
<td>70(68.0)</td>
<td>20(83.3)</td>
<td>71(80.7)</td>
</tr>
<tr>
<td>Ceftaxione</td>
<td>30(96.8)</td>
<td>68(66.0)</td>
<td>21(87.5)</td>
<td>71(80.7)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>22(71.0)</td>
<td>67(65.1)</td>
<td>18(75.0)</td>
<td>63(71.6)</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>14(45.2)</td>
<td>56(54.4)</td>
<td>10(41.7)</td>
<td>43(48.9)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>02(06.5)</td>
<td>08(07.8)</td>
<td>03(12.5)</td>
<td>12(13.6)</td>
</tr>
</tbody>
</table>

Key: Values in parenthesis are in percentage.
The rates of resistance to 10 antibiotics tested on the ESBL producing isolates among the inpatients and outpatients are shown in Table 2. The isolates from the urine samples collected from the in-patients, expressed the highest rates of resistance to ceftazidime (90.3%). The isolates from the out-patients also followed a similar trend at (95.7%) for ceftazidime.

Table 2. The rate of ESBLs producing isolates among the inpatients and outpatients.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Rate of resistant to ESBL positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>inpatient (n=31)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>23(74.2)</td>
</tr>
<tr>
<td>Cephalixin</td>
<td>25(80.6)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>20(64.5)</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>24(77.4)</td>
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<tr>
<td>Ciprofoxacin</td>
<td>12(38.7)</td>
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<tr>
<td>Ceftazidime</td>
<td>28(90.3)</td>
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<tr>
<td>Ceftaxone</td>
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</tr>
<tr>
<td>Amikacin</td>
<td>02(06.4)</td>
</tr>
</tbody>
</table>

Key: Values in parenthesis are in percentage

Fig. 1 shows the prevalence of ESBL producing *Escherichia coli* and *Klebsiella* species among inpatients and outpatients from ABSUTH. Among *Escherichia coli* isolates 62(49.6%) were ESBL-producers, 17(54.8%) from inpatients and 45(47.9%) from outpatients, while among the *Klebsiella* species 63(50.4%) were ESBL-producers, 14(45.2%) from inpatients and 49(52.1%) from outpatients.

Fig. 2 shows the pattern of distribution of ESBL producing *Escherichia coli* and *Klebsiella* species among the male and female patients. ESBL producing isolates were found to be more prevalent among the female patients 91(72.8%) than among the male patients 34(27.2%). *Escherichia coli* had a prevalence rate of 41.2% for the males and a prevalence rate of 52.7% for the female patients. *Klebsiella* species on the other hand, had a prevalence rate of (58.8%) and (47.3%) for the male and female patients respectively.

**Discussion**

An extensive use of β-lactam antibiotics in hospitals and communities has created major problems leading to increased morbidity, mortality and health care costs (Blomberg et al., 2005). Knowledge on local antimicrobial resistance trends among urinary isolates is important not only in guiding clinicians to prescribe appropriate antibiotics but also for evidence based recommendations in empirical antibiotic treatment of Urinary Tract Infection (UTI) (Blomberg et al., 2005). This study described the antimicrobial resistance rates, including the phenotypic detection of ESBL among *Escherichia coli* and *Klebsiella* spp urinary isolates.

Among the test organisms, *Escherichia coli* were 134(54.5%) while *Klebsiella* species recorded
112(45.5%). The results of the antibiotic resistance profile of *Escherichia coli* and *Klebsiella* species among outpatients and inpatients from Abia State University Teaching Hospital (ABSUTH) by disc diffusion method (Table 1) showed that *Escherichia coli* and *Klebsiella* species exhibited highest resistance rates to ceftriaxone at 96.8% and 87.5% respectively while the least resistance for both isolates were observed with amikacin. The observed resistance rate to amikacin by *Escherichia coli* (6.5%) among inpatients and (7.8%) among outpatients as well as *Klebsiella* species (12.5%) among the in patients and (13.6%) among the outpatients (Tables 1). The *Escherichia coli* and *Klebsiella* isolates expressed resistance rates of 41.2; 58.1, 41.7; 54.4 and 20.8; 34.1% respectively for the quinolones (Ofloxacin, Pefloxacin and Ciprofloxacin). This is a cause for concern as many clinicians fall back on the quinolones for the treatment of gram-negative pathogens in the face of multi-drug resistance (Paterson, 2007). The observed resistance to ciprofloxacin by *Escherichia coli* (25.8%) among inpatients and (29.1%) among outpatients as well as *Klebsiella* species (20.8%) among the in patients and (34.1%) among the outpatients (Tables 1) respectively where in accordance with the study by Sabrina et al., (2010) where 30.4% resistance was reported for *Escherichia coli* in Tanzania. Akujobi and Ewuru (2010) reported 37.6% resistance to Ciprofloxacin among ESBL producers in which is similar to the findings of this study where all the isolates recorded resistance rates of 28.4-31.3%. Also Sasirekha *et al.*, (2010) in their work observed a higher resistance value of 68% by *Escherichia coli* to ciprofloxacin.

Aminoglycosides have good activity against clinically important gram negative bacilli (Gonzalez and Spencer 1998). In the present study, *Escherichia coli* (92.5%) and *Klebsiella* species (86.6%) isolates were susceptible to amikacin, followed by 44.0% *Escherichia coli* and 30.4% *Klebsiella* species to gentamicin. These findings are similar to that of Sasirekha *et al.*, (2010), where 82.1% of *Escherichia coli* isolates were susceptible to amikacin and 41.8% to gentamicin. Several studies show that amikacin is more effective against *Escherichia coli* than gentamicin but if over used, the organisms may also develop resistance to it. The isolates were most sensitive to amikacin in this study (Tables 1 and 2). This is similar to the study of Al-Zarouni *et al.*, (2008), where the isolates expressed susceptibility rates of 100% and over 90% of ESBL isolates showed resistance to aztreonam and cephalosporin. According to Haque and Salam, 2010; Sasirekha *et al.*, 2010 and Ullah *et al.*, 2009, similar isolates expressed resistance rates of 59% and 55.5% to gentamicin in India and Bangladesh. These variations may be due the selection pressure on aminoglycosides brought on due to increased use of gentamicin, in different regions. (Miller and Sabatelli, 1997).

In Bangladesh, Chowdhury *et al.*, (1994) reported that 65-92% of commensal *Enterobacteriaceae* and other organisms isolated from urine were resistant to commonly used antibiotics like ampicillin, tetracycline, cotrimoxazole. These days, organism encoding multiple antibiotic resistance genes have become increasingly prevalent (Perez *et al.*, 2007). In this study, resistance rates to tetracycline, was found to be 70-95% among the *Escherichia coli* and *Klebsiella species*. (Tables 1 and 2). This result is in agreement with other studies (Sasirekha *et al.*, 2010; Ullah *et al.*, 2009), where resistance rate to tetracycline was found to be 75-98% among the *Enterobacteriaceae*. Cephalosporins especially the third generation versions have been used for gram negative bacterial treatment (Samah-Kfouri and Araj, 2003). In the present study resistance rates to ceftriaxone, ceftazidime and cefotaxime were found to be 96.8%, 87.1%, 71% among hospital acquired *Escherichia coli* and 66.0%, 68.0%, 65.1% among community acquired *Escherichia coli* respectively (Table 1). It
correlates with the study done by Sasirekha et al., (2010) in India where they found resistance rates of 84% to cefotaxime and 75%, 85% resistance rates for ceftriaxone and ceftazidime respectively. *Klebsiella species* were found resistant to ceftriaxone, ceftazidime and cefotaxime with rates of 87.5%, 83.3%, and 75.0% in hospital acquired isolates and 80.7%, 80.7%, 71.6% in community acquired isolates respectively (Table 2).

In this study, the ESBL producing isolates (50.8%) were more prevalent among the outpatients (75.2%) than the inpatients (24.8%). This correlates with the findings of Okesola and Fowotade, (2012) in Western Nigeria, where 70% of the positive cases were from the outpatients and 30% from the inpatients. However, in a study conducted in Spain between January 2001 and May 2002, 51% of ESBL producing *Escherichia coli* strains were isolated from outpatients and 49% from inpatients (Rodriguez-Bono et al., 2004). This is slightly in contrast to the findings in this study (Fig. 1).

Isolates from the inpatients (Hospital acquired isolates) were more resistant than the isolates from the outpatients (community acquired isolates). This may be due to lack of antibiotic policy, irrational use of third generation cephalosprins (3GC), mainly ceftriaxone in the hospital (Shobha et al., 2007) and the emergence of antibiotic-resistant organisms in hospitals. It was also noted that *Klebsiella* species were more resistant than *Escherichia coli* which may be due to the fact that *Klebsiellaspecies* have some virulence factors like hyperviscosity, polysaccharide capsules and production of endotoxin, carbapenemases, which make it more resistant than *Escherichia coli* (Lin et al., 2011). Thomson, (2001) found that *Klebsiella* species were more resistant to cefotaxime and ceftazidime. This probably indicates over use and irrational use of third-generation cephalosprins.

The females (73.2%) were infected more than the males (26.8%) in the case of *Escherichia coli* (Fig. 2). This finding correlates with that of Harding and Ronald (1994) who showed that the prevalence and incidence of urinary tract infections is higher in women than in men, which may be as a result of several clinical factors; including hormonal effects, behavioral patterns or their having a short urethra and vestibule which can easily be contaminated (Harding and Ronald, 1994). The patients with ESBL producing strains of *Escherichia coli* consisted of 41.2% male and 52.7% female (Fig. 2). This finding is in accordance with the result obtained in the study of Rodriguez-Bono et al., (2004) where the male/female ratio was 1:1.3. That is 41.4% male and 53.6% female.

In the present study *Klebsiella species* 56.3% (63/112) was the leading ESBL producers followed by *Escherichia coli* 46.3% (62/134), which correlates with studies done by Livrelli et al. (1996) and Lal et al. (2007) where they found *Klebsiella species* was the leading bacteria that produces ESBLs. The high occurrence of ESBLs in *Klebsiella species* is of great concern since infections caused by this bacterium is very common and resistance of the organism may be due to the presence of the capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, ability to acquire and disseminate resistance plasmid (Chaudhary and Aggarwal 2004; Yusha et al., 2010). In the present study most of the isolates were from the community (outpatients). *Escherichia coli* was more prevalent in hospital acquired isolates (Sheryll et al., 2004). Originally ESBLs were most commonly reported to be a hospital based problem but it is now common among community acquired isolates, especially *Escherichia coli* (Heffernan and Woodhouse, 2006). In this present study *Escherichia coli* and *Klebsiella species* were found in the community (Fig.1). Denholm et al., (2009) found *Escherichia*
coli the most common community acquired isolates among the ESBLs producers.

In Nigeria, β-lactam antibiotics are the most frequently prescribed antibiotics against aerobic gram negative bacilli infections and selective pressure exerted by the extensive use of these β-lactam drugs, especially in treating some life threatening infections most likely result in strains developing ESBL enzymes (Iroha et al., 2009). High proportions of resistance detected against second and third generation cephalosporins indicate that it may not be long before these antibiotics will no longer be effective in the treatment of infections caused by these Enterobacteria at ABSUTH. Occurrence and distribution of ESBLs differ from country to country and from hospital to hospital (Ali, 2009).

In Eastern Nigeria, ESUTH (Enugu State University Teaching Hospital) had the greater number of ESBL occurrence from Escherichia coli and Klebsiella pneumoniae isolates (55.9%) than UNTH (University of Nigeria Teaching Hospital) (31.0%) (Iroha et al., 2009). In the present study also, the number of ESBL occurrence from Escherichia coli and Klebsiella species isolates was 50.8%. These type of discrepancies between susceptibility data and disc diffusion results has increased the need for an improved method of ESBL detection and its incorporation into routine susceptibility procedures (Iroha et al., 2009). Further studies are required to investigate multi-drug resistant (MDR) bacteria and ESBL from other sample sources using more isolates. Studies of molecular epidemiology of these resistant genes can also be used for comparison with genes already isolated from other parts of Nigeria and the world at large.

References


