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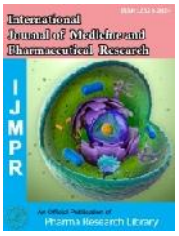

Research Article **Open Access**

Occurrence of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in urinary tract infection (UTI) patients

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A B S T R A C T

A urinary tract infection (UTI) is an infection in any part of the urinary tract system that includes infections of the kidneys, ureters, bladder and urethra; and UTI accounts for several hospital visits around the world – causing morbidity and mortality. A total of 687 urine samples of patients with UTIs were analyzed for the presence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*. The isolation and characterization of these organisms were carried out using microbiology standard techniques. The antibiotic susceptibility test was carried out using disc diffusion method while the phenotypic determination of ESBLs was done using double disc synergy test (DDST). The outcome of this investigation showed that *E. coli* were more prevalent than *K. pneumoniae* in urine samples with their corresponding values of 111 (54.68 %) and 92 (45.32 %) respectively. *E. coli* and *K. pneumoniae* were more prevalent in urine samples of patients between the ages of 11 to 60 years and less common in children (< 11 years) and the elderly (> 60 years) in both females and males. Both *E. coli* and *K. pneumoniae* were significantly higher in female than male patient's urine samples ($P<0.05$). Their prevalence in urine was highest within the age range of 21-30 years. Both *E. coli* and *K. pneumoniae* were found across all the occupational groups of the patients investigated and were most prevalent among students and least among teachers. The occurrences of *E. coli* and *Klebsiella pneumoniae* were generally lower in pupils (0-10 years) and the elderly (71-80 years of age). All the isolates of *E. coli* and *Klebsiella pneumoniae* obtained were most resistant to cefuroxime, ceftazidime and cefotaxime. However, they were most susceptible to imipenem, meropenem and ertapenem. Among the antibiotics evaluated, imipenem was found to be the most active against the isolates, followed by ertapenem and then meropenem. ESBLs were found to be more prevalent in females than males. More so, of the 203 (111 *E. coli* and 92 *K. pneumoniae*) isolates screened for ESBL production, only 13 *E. coli* isolates and 7 *K. Pneumoniae* isolates were ESBL producers. ESBL producing *E. coli* (n=13) and *K. pneumoniae* (n=7) were more prevalent in females than males with their corresponding values of 9 (69.23 %), 6 (85.71 %) and 4 (30.77 %), 1 (14.29 %) respectively. Since UTI is a common bacterial infection that warrant most hospital visits, it is critical to back clinical diagnosis of the infection and/or diseases with proper antimicrobial susceptibility testing especially those that detect the occurrence of multidrug resistant bacteria such as ESBL-producing *E. coli* and *K. pneumoniae* isolates. Conclusively, this study reported the occurrence of ESBL-producing *E. coli* and *K. pneumoniae* isolates with varying rates of susceptibility to some available antibiotics. Concerted efforts are needed to contain the emergence and spread of resistant bacteria in this environment – especially in the hospital were blind-antimicrobial therapy usually abound.

Keywords: *Klebsiella pneumoniae, Escherichia coli,* UTIs, ESBLs, Antibiotics, Resistance

A R T I C L E I N F O

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1. Introduction

Urine, though considered sterile, provides good growth medium for bacteria because of its chemical constituents (Maji *et al.,* 2013; Yabaya *et al.,* 2012). Urinary tract is composed of the bladder, kidneys, ureters and urethra and infection caused by the presence of pathogenic microorganisms in any of these organs is called urinary tract infection (UTI) (Nwosu *et al.,* 2014; Yabaya *et al.,* 2012). Bacterial UTI is the most common type of infection affecting the urinary tract system often causing inflammation of the bladder and kidneys (Maji *et al.,* 2013; Yabaya *et al.,* 2012). UTI has been responsible for a lot of morbidity especially when it is linked with urinary obstruction or renal papillary damage, which leads to serious kidney damage (Lamido *et al.,* 2010). Urinary tract infection can occur in individuals of any sex. However, females are more affected than males because their urethra is naturally shorter, wider and in closer proximity with the anus (Lamido *et al.,* 2010; Dielubanza and Schaeffer, 2011). The bacteria most commonly associated with UTIs are *Escherichia coli, Klebsiella* species and other coliforms, which are part of intestinal normal flora (Niranjan and Malini, 2014; Mbata, 2007).They cause 80-85 % of community acquired UTIs with little involvement of *Staphylococcus saprophyticus* (Niranjan and Malini, 2014). Viruses and fungi species are not often implicated in UTI (Amdekar *et al.,* 2011). *E. coli* and *Klebsiella* species belonging to the same family of *Enterobacteriaceae* have the ability to produce extended spectrum beta-lactamases (ESBLs) – which allow these organisms to be multidrug resistant. The term extended spectrum beta-lactamases (ESBLs) was coined from the ability of the enzymes (ESBLs) to hydrolyze a wider range of beta-lactam antimicrobials than the traditional beta-lactamases they were originally derived from (Bradford, 2001). According to Ma *et al.* (2009), these bacterial enzymes (ESBLs) lead to therapeutic failures of some antimicrobial agents used in clinical medicine. ESBLs are inactivated by clavulanic acid and tazobactam, a property that differentiates extended spectrum beta-lactamases from AmpC- beta-lactamase – which are not inactivated by beta-lactamase inhibitors (Bradford, 2001; Paterson and Bonomo, 2005). The existence of ESBLs has remarkable clinical implication because they are commonly plasmid encoded and most time the plasmids often carry genes encoding resistance to different drug classes thereby restricting antibiotic treatment options of infections caused by ESBL-producing organisms (Paterson and Bonomo, 2005). The selection pressure that drive the evolution of ESBLs has always been associated with the intense use of oxyimino-beta-lactams (mostly the third generation cephalosporins), widespread use of broad spectrum antibiotics, prolonged hospitalization, indwelling devices and severe underlying diseases (Lin *et al.,* 2003; Nathisuwan *et al.,* 2001). The major ESBL producing organisms commonly isolated worldwide are *Klebsiella pneumoniae* and *Escherichia coli* (Bradford, 2001; Jacoby and Munoz-Price, 2005). ESBLs are rapidly increasing among human isolates and over 400 different types of beta-lactamases, having similar resistance mechanisms but differing in substrate specificities and susceptibility to inhibitory substances, have been described, with the CTX-M β-lactamases encoded by the *bla*CTX-M gene being the most prevalent (Pitout, 2010). The increase in the prevalence of ESBLs among clinical isolates therefore threatens the success of the use of majority of the existing beta-lactam antibiotics in the treatment of bacterial infections (Sturm *et al.,* 2010; Babypadmini and Appalaraju, 2004). Numerous reports abound on the increase in the isolation rate of ESBL producing bacteria in several parts of the world (Canton *et al.,* 2008; Bush, 2008). However, it varies greatly across geographical regions and rapidly changes over time (Babypadmini and Appalaraju, 2004). Thus, this study was designed to determine the occurrence of ESBL-producing *E. coli* and *K. pneumoniae* isolates from UTI patients in a teaching hospital in Abakaliki, Nigeria.

2. Materials and Methods

Sample collection: Six hundred and eighty seven (687) urine samples were used for this study. The urine samples were collected from suspected UTI patients who attended the Federal Teaching Hospital, Abakaliki from January to May 2015; and all the samples were transported to the microbiology laboratory of Ebonyi State University, Abakaliki for bacteriological analysis.

Bacteriological analysis: A loopful of each urine sample collected was inoculated ascetically on cystein lactose electrolyte deficient (CLED) medium, MacConkey agar (MAC) and eosin methylene blue (EMB) agar and using sterile wire loop. All the culture plates were incubated

aerobically for 18-24 hr at 37^{o}C. Suspected colonies of *E. coli* and *Klebsiella* species were subcultured onto CLED, EMB and MAC for the isolation of pure cultures of *E. coli* and *K. pneumoniae*. *E. coli* produces non-mucoid pinkish colonies on MAC, yellowish colonies on CLED and colonies with metallic sheen on EMB agar while *K. pneumoniae* produces mucoid colonies without any of these characteristics on the culture media. Pure cultures of the isolated *E. coli* and *K. pneumoniae* isolates were identified based on standard microbiology identification techniques; and these were stored in nutrient agar slants for further use (Cheesbrough, 2000).

Antimicrobial susceptibility testing: Antibiogram was carried out on all the *E. coli* and *K. pneumoniae* isolates as was previously described (Ejikeugwu *et al*., 2013; CLSI, 2006). Single antibiotic disks of ceftriaxone (30 µg), cefuroxime (30 µg), aztreonam (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), imipenem (10 µg), ertapenem (10 µg) and meropenem (10 µg) were used for the susceptibility test as described by Ejikeugwu *et al*. (2013). All the antibiotic disks were procured from Oxoid Limited (Oxoid, UK). Briefly, each of the antibiotic disks was aseptically placed on a Mueller-Hinton (MH) agar plate previously inoculated with the test bacterium (adjusted to 0.5 McFarland turbidity standards). The plates were allowed for about 10 mins for pre-diffusion of the drugs and they were incubated at 37^{o}C for 18-24 hrs. Inhibition zone diameter (IZD) was recorded as per the guidelines of the Clinical Laboratory Standard Institute (CLSI) (CLSI, 2006).

Screening for ESBL production: The screening for ESBL production was carried out according to a previously described method (Ejikeugwu *et al*., 2013). To screen the *E. coli* and *K. pneumoniae* isolates for ESBL production, single antibiotic disks containing 30 µg of cefotaxime and 30 µg of ceftazidime were placed aseptically at a distance of 30 mm apart on Mueller-Hinton (MH) agar (Oxoid, UK) plate(s) that was previously inoculated with standardized inoculums(s) of the test bacterium (adjusted to 0.5 McFarland turbidity standards). The plates were allowed for about 30 mins for pre-diffusion of the antibiotics; and these were incubated for 18-24 hrs at 37^{o}C. The zones of inhibition were measured as per the Clinical Laboratory Standard Institute (CLSI) guidelines; and ESBL production was inferred or suspected if any of the test bacteria showed reduced susceptibility or is resistant to any one of the third generation cephalosporins used for the screening studies (Ejikeugwu *et al*., 2013; CLSI, 2006; Iroha *et al*., 2008).

Double disk synergy test (DDST): ESBL production was phenotypically confirmed in the *E. coli* and *K. pneumoniae* isolates by the DDST method as was previously described (Ejikeugwu *et al*., 2013; CLSI, 2006; Iroha *et al*., 2008). Briefly, antibiotic disks of amoxycillin-clavulanic acid (20/10 µg) was placed at the center of MH agar plate(s) previously inoculated with the test bacteria, and cefotaxime (30 µg) and ceftazidime (30 µg) disks were each placed adjacently at a distance of 15 mm from amoxycillin-clavulanic acid (AMC). The plates were incubated at 37^{o}C for 18-24 hrs; and ESBL production was inferred phenotypically when the zones of inhibition of any of the cephalosporins (cefotaxime and ceftazidime) were expanded by the amoxycillin-clavulanic acid disk (20/10 µg) disk. A ≥ 5 mm increase in the inhibition zone diameter for either of the cephalosporins tested in combination with AMC versus its zone when tested alone confirms ESBL production phenotypically (Ejikeugwu *et al*., 2013; CLSI, 2006; Iroha *et al*., 2008).

3. Results and Discussion

Results

A total of six hundred and eighty seven (687) urine samples were used for this study. Table 1 show the prevalence of *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine samples. A total of 203 bacterial isolates of *E. coli* and *K. pneumoniae* were obtained from the 687 urine samples analyzed in this study. While 111 (54.68 %) isolates were *E. coli*, 92 (45.32 %) of the isolates were *Klebsiella pneumoniae* (Table 1).

Table 2 and Table 3 show the distribution of the isolated bacteria based on the age and sex of the patients. The highest number of *E. coli* isolates was recovered from the age bracket of 21-30 years, and this was followed by the age bracket of 61-70 and 71-80 from which *E. coli* was least isolated (Table 2). Similarly, highest number of *K. pneumoniae* isolates was isolated from the age brackets of 21-30, followed by age brackets of 71-80 – from which *K. pneumoniae* was least isolated from (Table 3). The number of *E. coli* and *K. pneumoniae* isolated from the urine samples was extrapolated based on the occupation of the patients (Table 4). A higher percentage of the *E. coli* and *K. pneumoniae* isolates was recovered from students; and this was followed by housewives, traders and farmers.

Figure 1 and Figure 2 show the percentage susceptibility of the *E. coli* and *K. pneumoniae* isolates to some beta-lactam antibiotics. The result shows that imipenem and meropenem was the most active agent against the *E. coli* and *K. pneumoniae* isolates. Meropenem was also active against the *E. coli* and *K. pneumoniae* at a percentage susceptibility of 95.5 % and 94.57 % respectively. However, the *E. coli* and *K. pneumoniae* isolates showed least susceptibility to ceftriaxone, cefuroxime, aztreonam, cefotaxime, and cefoxitin. The frequency of ESBL-positive *E. coli* and *K. pneumoniae* isolates is shown in Table 5. Only 13 isolates of *E. coli* and 7 isolates of *Klebsiella pneumoniae* were phenotypically confirmed to produce ESBL by the DDST method.

Discussion

In this study, a total of 687 urine samples of patients in Federal Teaching Hospital, Abakaliki were analyzed for the presence of *E. coli* and *Klebsiella pneumoniae*. And out of this, a total of 203 isolates were obtained of which 111 (54.68 %) isolates were *E. coli* while 92 (45.32 %) isolates were *K. pneumoniae*. The values obtained from this result showed that *E. coli* was more prevalent in urine samples of the patients than *K. pneumoniae*. This is similar with a report by Inabo and Obanibi (2006) in Kaduna, Nigeria, who reported *E. coli* as the predominant bacteria followed

by *Staphylococcus aureus* and then *Klebsiella* species from the urine samples of patients analyzed in their study. In agreement with this finding also was a report by Zaria *et al.* (2010) in Maiduguri, Nigeria, who recorded 54 (47.4 %) isolates *E. coli* out of 114 *E. coli* isolated from pregnant and non-pregnant women. The observation of higher prevalence of *E. coli* species in patients' urine samples (as obtainable in this study) is also in accordance with earlier reports by Maji *et al.* (2013) and Niranjan and Malini (2014).

The presence of these organisms in urine is not a surprise since they constitute part of the normal flora of the human gastrointestinal tract (GIT). In contrast however, Noor *et al.,* (2004) recorded *Klebsiella* and *Proteus* species as the most prevalent isolates in patients' urine samples in their own study. The effect of gender on uropathogens prevalence is frequently observed in most urinary tract infection studies and a number of factors including sex, age, disease, hospitalization and obstruction of urine flow have been known to influence human susceptibility to UTIs (Epoke *et al.,* 2000; Oladeinde *et al.,* 2011). Inappropriate and irrational use of drugs by physicians in clinical practice, unskilled practitioners and the end-users have been recognized as major contributing factors to the development and spread of bacteria strains that are resistant to antimicrobial agents especially in the developing countries (Mincey and Parkulo, 2001). The result of this study showed that *E. coli* from the patient's urine were susceptible only to imipenem and meropenem.

The *E. coli* isolates exhibited varying degrees of resistance ranging from 34.23 – 95.5 % to the other antibiotics tested. However, *K. pneumoniae* isolates also showed higher susceptibility to imipenem, meropenem and ertapenem. The isolated *K. pneumoniae* were also consistently resistant to cefuroxime (73.91 %) and cefotaxime (78.26 %). The observed high resistance of the *E. coli* and *K. pneumoniae* isolates to the cephalosporins in this study is worrisome because this class of antibiotics is mostly used for treatment of bacterial infections when other first line antibiotics fail. Similar high levels of resistance to the cephalosporins have also been reported in other parts of the world including the UK (Farrell *et al.,* 2003), India (Sasirekha *et al.,* 2010), and Nigeria (Iroha *et al*., 2008; Nwosu *et al*., 2014; Ejikeugwu *et al*., 2013). Of the total *E. coli* species isolated (n=111) from patients urine sample, only 13 isolates of *E. coli* were ESBL positive while 7 isolates of *K. pneumoniae* were also confirmed ESBL positive phenotypically. This result is similar to what was obtained in Iran by Behroozi *et al* (2010), who recorded ESBL producing *E. coli* (21 %) to be more prevalent than *Klebsiella* species (12 %). Several other reports also indicated that ESBL positive *E. coli* are usually more prevalent that *K. pneumoniae* in urine samples (Nwosu *et al.,* 2014; Babypadmini and Appalaraju, 2004). However, our result is contrary to findings by other researchers who recorded *Klebsiella* species as the leading ESBL producers followed by *Escherichia coli* (Akanbi *et al.,* 2013).

Conclusively, this study show that *E. coli* and *K. pneumoniae* from urine samples are resistant to some available antibiotics in the beta-lactam class because they express extended spectrum beta-lactamase (ESBL) enzymes. The prompt and accurate detection of ESBL-producing *E. coli* and *K. pneumoniae* isolates from urine samples in our hospitals across Nigeria is critical to the proper care of UTI patients, because such measures will help to guide therapy and forestall the emergence and spread of ESBL-positive bacteria in the hospital environment.

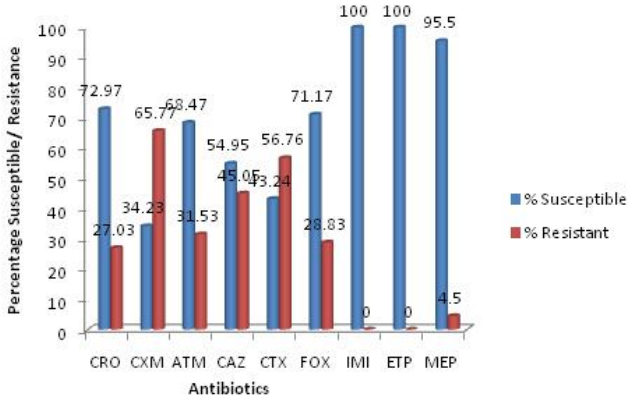

Figure 1: Percentage Susceptibility and Resistance pattern of *E. coli* Isolates

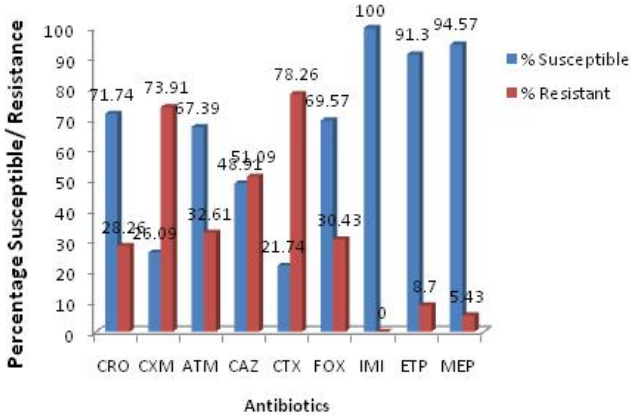

Figure 2: Percentage Susceptibility and Resistance pattern of *Klebsiella pneumoniae* Isolates

Table 1: Percentage occurrence of *E. coli* and *Klebsiella Pneumoniae* isolated from urine samples of patients

Organisms	Total No. of isolates tested	No (%) of isolates from males	No (%) of isolates from females
E. coli	111 (54.68)	35 (31.53)	76 (68.47)
Klebsiella Pneumoniae	92 (45.32)	22 (23.91)	70 (76.09)

Table 2: Age and sex distribution of *E. coli* isolated from urine samples

Age range	No isolated	No. of isolates from males	No. of isolates from females
0-10	6	2 (5.71)	4 (5.26)
11-20	14	1 (2.86)	13 (17.11)
21-30	30	5 (14.29)	25 (32.89)
31-40	25	6 (17.14)	19 (25.00)
41-50	15	8 (22.86)	7 (9.21)
51-60	16	9 (25.71)	7 (9.21)
61-70	3	2 (5.71)	1 (1.32)
71-80	2	2 (5.71)	0 (0.00)
Total	**111**	**35**	**76**

Table 3: Age and sex distribution of *Klebsiella Pneumoniae* isolated from urine samples

Age range	No isolated	No. of isolates from males	No. of isolates from females
0-10	6	0(0.00)	6(8.57)
11-20.	15	3(13.64)	12(17.14)
21-30	25	3(13.64)	22(31.43)
31-40	22	5(22.73)	17(24.29)
41-50	9	1(4.55)	8(11.43)
51-60	6	3(13.64)	3(4.29)
61-70	6	4(18.18)	2(2.86)
71-80	3	3(13.64)	0(0.00)
Total	**92**	**22**	**70**

Table 4: Occupational Distribution of *E. coli* and *Klebsiella pneumoniae* Isolated from Urine Samples

Occupation	Number (%) of *E. coli*	Number (%) of *K. pneumoniae*
Pupils	6 (5.41)	6 (6.52)
Farmers	15 (13.51)	6 (6.52)
Civil servants	6 (5.41)	14 (15.23)
Housewives	16 (14.41)	12 (13.04)
Traders	13 (11.71)	10 (10.87)
Students	34 (30.63)	21 (22.83)
Dependents	5 (4.50)	8 (8.70)
Bankers	6 (5.41)	7 (7.61)
Teachers	3 (2.70)	2 (2.18)
Drivers	7 (6.31)	6 (6.52)
Total	**111 (100)**	**92 (100)**

Table 5: Occurrences of ESBL producing *E. coli* and *Klebsiella pneumoniae* from Urine Samples of Patients with UTIs

Organism	Total number screened	ESBL positive isolates	ESBL negative isolates	ESBL positive from males	ESBL positive from females
E. coli	111	13	98	4(30.77 %)	9(69.23 %)
Klebsiella pneumoniae	92	7	85	1(14.29 %)	6(85.71 %)

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