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*Full Length Research Paper*

# Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae*

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**Emerging antibiotic resistance due to extended spectrum β-lactamase (ESBL) production limited the use of β-lactam antibiotics against *Escherichia coli* and *Klebsiella pneumoniae.* This observational study was conducted at the Microbiology department of the Children's Hospital, Lahore Pakistan, from June, 2009 to November, 2010 to determine the frequency and antimicrobial resistance of ESBL producing *E. coli* and *K. pneumoniae*. A total of 13638 urine samples were processed for culture and antimicrobial sensitivity testing. *E. coli* and *K. pneumoniae* were identified using API 20E. A double disk synergy test (DDST) was performed to determine ESBL production. ESBL production was detected in 312 (57.4%) *E. coli* and 386 (71.7%) *K. pneumoniae*. A multidrug resistance pattern was seen in ESBL producing *E. coli* and *Klebsiella pneumoniae.* ESBL producing *E. coli* showed maximum resistance to cefotaxime (100%), ceftazidime (99.4%) and cefuroxime (93.3%) while minimum resistance was seen with meropenem (1.3%), piperacillin/tazobactam (10.3%) and nitrofurantoin (27.6%). ESBL producing *K. pneumoniae* showed maximum resistance to ceftazidime (100%), cefotaxime (98.7%) and cefuroxime (98.1%) while minimum resistance was seen with meropenem (3.6%), piperacillin/tazobactam (17.6%), and nitrofurantoin (28.5%). In ESBL producing bacteria, high prevalence of antibacterial resistance of non-β-lactam antibiotics is a serious matter of concern. Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with urinary tract infection (UTI).**

**Key words:** Extended spectrum, beta-lactamases, urinary tract infections, *Escherichia coli*, *Klebsiella pneumoniae*.

## INTRODUCTION

Urinary tract infection (UTI) is a general term referring to the infection anywhere in the urinary tract. This is among the most common serious bacterial infections in infants and children (Wald, 2004). UTI is a common cause of morbidity in children both in community and hospitalized patients (Peterson, 2004). If UTI is not diagnosed early and treated adequately, it may result into chronic illness and long term renal damage (Adjei and Opoku, 2004).Extended spectrum β-lactamases (ESBLs) were first described in 1983. They are able to hydrolyse oxyimino-cephalosporins (for example, cefotaxime, ceftazidime and ceftriaxone) and monobactams (for example, aztreonam), but not cephamycins or carbapenems (Patricia, 2001; Bush, 2001).

The β-lactamases produced by bacteria are known to protect against the lethal effect of penicillins, cephalosporins and monobactams on their cell wall synthesis. The production of β-lactamase is the single most prevalent mechanism responsible for resistance to β-lactams

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**Abbreviations: ESBL,** Extended spectrum β-lactamase**; DDST,** double disk synergy test; **UTI,** urinary tract infection; **CLED,** cysteine lactose electrolyte deficient; **CLSI,** Clinical and Laboratory Standards Institute.

among clinical isolates of the family *Enterobacteriaceae* (Sanders and Sanders, 1992). ESBLs have been found most commonly in uropathogens, like *K. pneumoniae* and *E. coli.* Other enterobacteria and non-fermenting Gram negative rods also produce ESBLs but to a lesser extent (Goussard and Courvalin, 1999; Bush and Jacoby, 2010). A variety of β-lactamases have been classified into class A, B, C and D according to their amino acid homology (Bush et al., 1995). ESBLs are Class A enzymes which are inhibited *in vitro* by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam, whereas those belonging to class B, C and D are not affected (Patricia, 2001). Most of the ESBLs are derived from TEM-1 and SHV-1 (sulfhydryl variable) by mutations. ESBL producing organisms have widely spread, and have become a major cause of nosocomial infections associated with high mortality rates, particularly in serious infections such as septicemia (Bjorn et al., 2005).

The ESBL producing bacteria are increasingly causing urinary tract infections both in hospitalized and outpatients. This is making therapy of UTI difficult and promoting greater use of expensive broad spectrum antibiotics, such as carbapenems. Detection of ESBLs using conventional antimicrobial susceptibility methods and delay in the detection and reporting of ESBL production by Gram-negative bacilli are associated with prolonged hospital stay, increase morbidity, mortality and health care costs (Mehrgan and Rahbar, 2008).

The failure of treatment of both complicated and uncomplicated UTI is continuously increasing morbidity and mortality among UTI patients. The aim of our study was to evaluate the antimicrobial resistance of ESBL and non-ESBL producing *E. coli* and *K. pneumoniae* in patients attending a tertiary care children hospital.

## MATERIALS AND METHODS

This observational study was conducted in the Microbiology Department of the Children's Hospital and Institute of Child Health Lahore, Pakistan, from 1st June, 2009 to 30th November, 2010. Urine samples were collected aseptically by the hospital staff nurses and sent to the laboratory. The samples were immediately processed for urine culture.

The samples received were streaked on cysteine lactose electrolyte deficient (CLED) agar. The plates were incubated overnight at 37°C. Following the appearance of pure bacterial growth, only strains of *E. coli* and *K. pneumoniae* with a clinically significant growth (>$10^5$CFU/ml) were included in the study. The bacterial strains were identified using the API 20E (bioMérieux, France). All the isolates of *E. coli* and *K. pneumoniae* were tested for their antimicrobial resistance to various antibiotics *in vitro* by the Kirby-Baur disk diffusion method. The antibiotic disks of amikacin (30 µg), cefixime (5µg), cefotaxime (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), co-amoxiclav (20/10 µg), co-trimoxazole (1.25/23.75 µg), gentamicin (10 µg), meropenem (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg) and piperacillin/tazobactam (100/10 µg) were placed on the Mueller-Hinton agar (Oxoid) plates and incubated at 37°C overnight. After overnight incubation, the diameter of each zone of inhibition was measured in mm. The susceptibility testing results were noted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009).

The double disk synergy test (DDST) was performed by placing a disk of co-amoxiclav on the inoculated Mueller-Hinton agar plate at a 20 mm distance from the indicator drugs ceftazidime and cefotaxime. ESBL production was considered positive when the clavulanate mediated enhancement of the activity of an indicator drug produced a keyhole effect. The quality assurance was performed weekly using *K. pneumoniae* ATCC (American type culture collection) 700603 (ESBL producing isolate) and *E. coli* ATCC 25922 (susceptible isolate) as positive and negative controls, respectively (Pannika et al., 2008).

## RESULTS

The total numbers of samples sent to the microbiology laboratory for culture during the study period was 13638, out of which 1950 showed bacterial growth. The most common organisms isolated from these cultures were Gram-negative bacilli.

Among the isolated organisms, there were 544 (27.9%) *E.coli,* 538 (27.6%) *K. pneumonia,* and 868 (44.5%) other bacteria (Figure 1).

In the present study, 453 (83.3%) isolates of *E. coli* and 459 (85.3%) *K. pneumoniae* were isolated from hospitalized patients, while 91 (16.7%) *E. coli* and 79 (14.7%) *K. pneumoniae* strains were isolated from out patients (Figure 2).

In this study, ESBL producing *E. coli* and *K. pneumoniae* were 312 (57.4%) and 386 (71.7%), respectively. The non-ESBL producers were 232 (42.6%) *E. coli* and 152 (28.3%) *K. pneumoniae* (Figure 3). Table 1 shows antibacterial resistance of ESBL and non-ESBL producing *E. coli* and *K. pneumoniae.* ESBL producing *E. coli* showed maximum resistance to cefotaxime (100%), ceftazidime (99.4%) and cefuroxime (93.3%) while minimum resistance was seen with meropenem (1.3%), piperacillin/tazobactam (10.3%) and nitrofurantoin (27.6%). The non-ESBL producing *E. coli* showed maximum resistance to co-amoxiclav (45.6%), cefuroxime (41.4%) and norfloxacin (40.9%) while the minimum resistance was seen with meropenem (0%), nitrofurantoin (3.4%), piperacillin/tazobactam (3.9%), gentamicin (5.2%), amikacin (6.0%) and ciprofloxacin (9.9%).ESBL producing *K. pneumoniae* showed high resistance to ceftazidime (100%), cefotaxime (98.7%) and cefuroxime (98.1%) while low resistance was seen with meropenem (3.6%), piperacillin/tazobactam (17.6%), and nitrofurantoin (28.5%).

The non-ESBL producing *K. pneumoniae* showed high resistance to co-amoxiclav (72.4%), cefuroxime (37.5%) and norfloxacin (34.9%) while the low resistance was seen with meropenem (0%), piperacillin/tazobactam (0%), gentamicin (3.9%), amikacin (6.6%), ceftazidime (9.9%), and ciprofloxacin (10.5%).

## DISCUSSION

The most common bacteria responsible for UTI in the

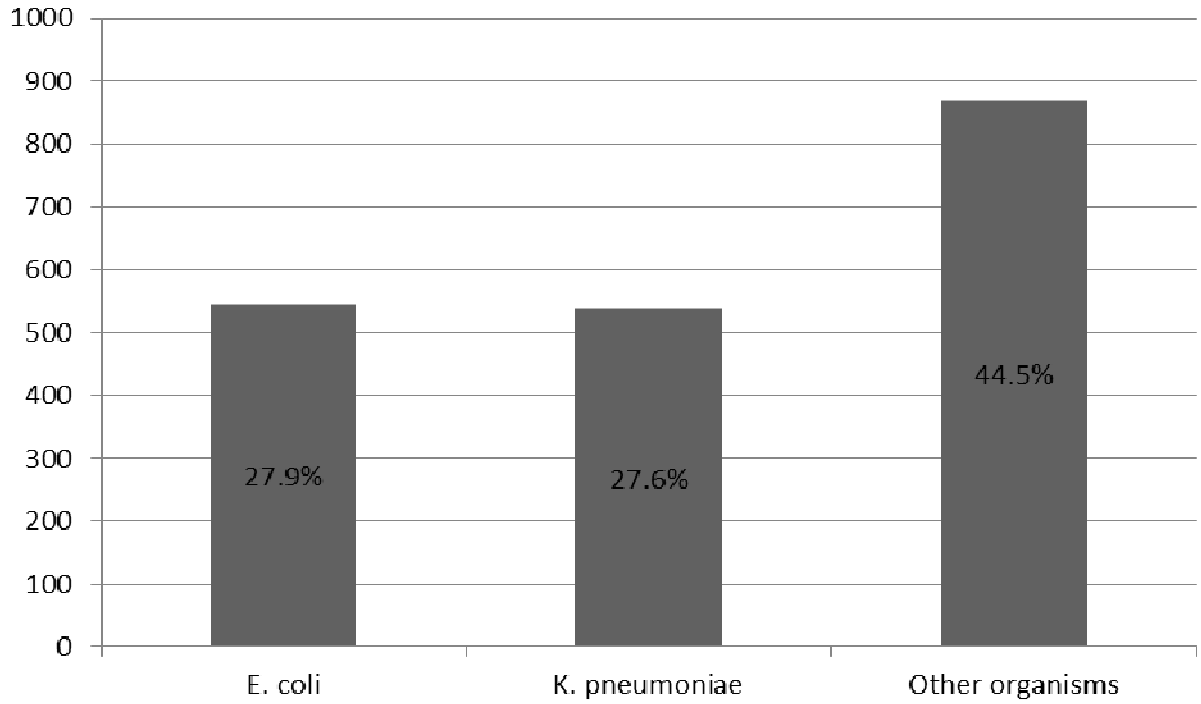


**Figure 1.** Frequency of uropathogens isolated in the study (n=1950).

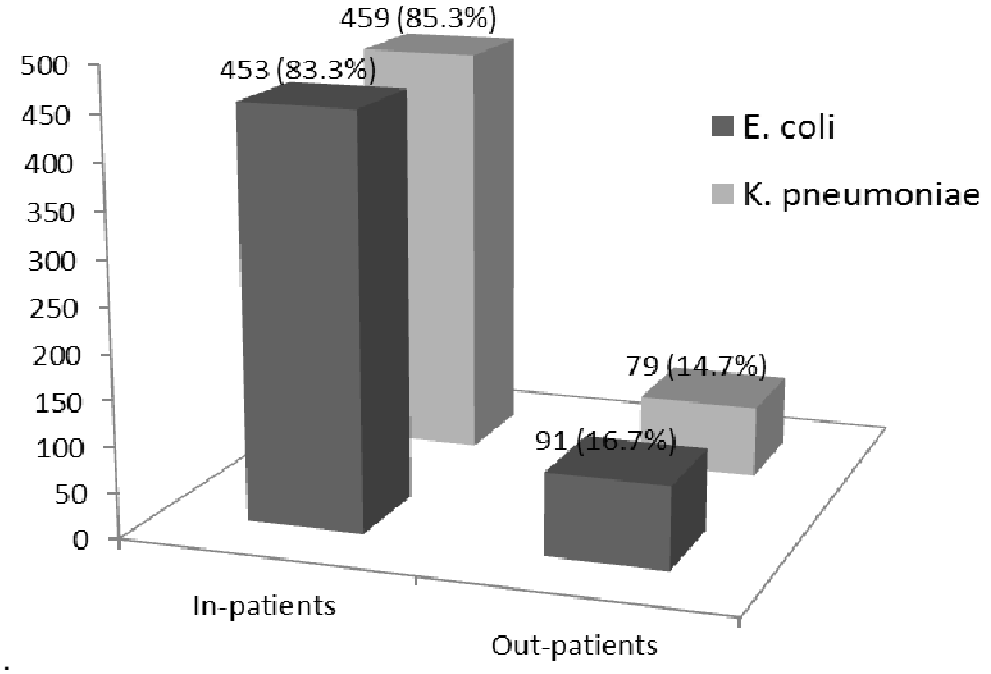


**Figure 2.** Distribution of *E. coli* and *K. pneumoniae* among inpatients and outpatients.

present study are *E. coli* and *K. pneumoniae.* The findings of the present study were supported by another study where *E. coli* and *K. pneumoniae* were found as main culprits responsible for the UTI among children in Pakistan (Qureshi, 2005).

During the past few years, ESBL producing *E. coli* and

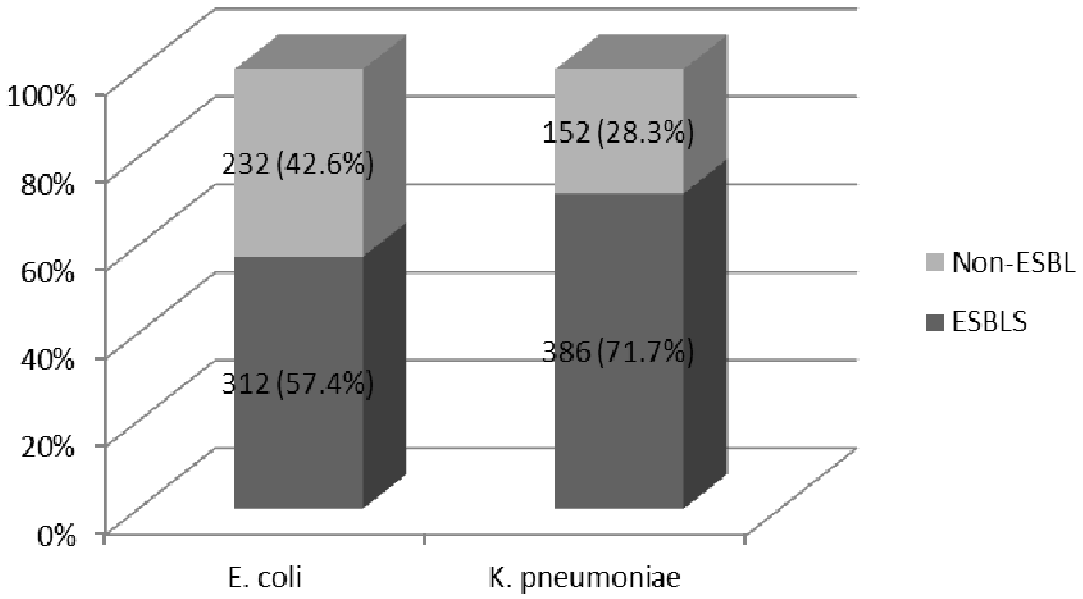


**Figure 3.** Frequency of ESBL and non-ESBL producing bacteria.

**Table 1.** Antibacterial resistance of ESBL and non-ESBL producing *Escherichia coli* and *Klebsiella pneumonia.*

| Antibiotic | *Escherichia coli* | | *Klebsiella pneumoniae* | |
|---|---|---|---|---|
| | **ESBL** | **non-ESBL** | **ESBL** | **non-ESBL** |
| Amikacin | 145 (46.5%) | 14 (6.0%) | 205 (53.1%) | 10 (6.6%) |
| Cefixime | 286 (91.7%) | 68 (29.3%) | 377 (97.7%) | 37 (24.3%) |
| Cefotaxime | 312 (100.0%) | 82 (35.3%) | 381 (98.7%) | 37 (24.3%) |
| Ceftazidime | 310 (99.4%) | 32 (13.8%) | 386 (100.0%) | 15 (9.9%) |
| Cefuroxime | 291 (93.3%) | 96 (41.4%) | 379 (98.1%) | 57 (37.5%) |
| Ciprofloxacin | 248 (79.5%) | 23 (9.9%) | 317 (82.1%) | 16 (10.5%) |
| Co-amoxiclav | 267 (85.6%) | 106 (45.6%) | 302 (78.2%) | 110 (72.4%) |
| Co-trimoxazole | 244 (78.2%) | 55 (23.7%) | 340 (88.1%) | 36 (23.7%) |
| Gentamicin | 156 (50%) | 12 (5.2%) | 290 (75.1%) | 6 (3.9%) |
| Meropenem | 4 (1.3%) | 0 (0%) | 14 (3.6%) | 0 (%0) |
| Nalidixic acid | 278 (89.1%) | 77 (33.2%) | 336 (87.0%) | 37 (24.3%) |
| Nitrofurantoin | 86 (27.6%) | 8 (3.4%) | 110 (28.5%) | 41 (27.0%) |
| Norfloxacin | 259 (83.0%) | 95 (40.9%) | 311 (80.6%) | 53 (34.9%) |
| Piperacillin/tazobactam | 32 (10.3%) | 9 (3.9%) | 68 (17.6%) | 0 (0%) |

*K. pneumoniae* have emerged as serious pathogens both in hospital and community acquired infections. Recent studies revealed that patients with ESBL producing organisms had significantly higher fatality rate than those with non-ESBL isolates (Mehrgan and Rahbar, 2008). In the present study the ESBL producing *E. coli* and *K. pneumoniae* were 57.4 and 71.7%, respectively. In another study conducted in Pakistan, 56.9% isolates of *E. coli* were ESBL positive (Ullah et al., 2009 ) and in a study from India, nearly 40% urinary isolates of *E. coli* and *K. pneumoniae* were ESBL positive (Babypadmini and Appalaraju, 2004). ESBL producing *K. pneumoniae* were 54.4% in a study from Latin America (Aminzadeh et al., 2008). Mekki et al. (2010) reported ESBL producing 53% *E. coli* and *K. pneumoniae* from the patients suffering from urinary tract infections. The findings of our study are similar to other studies in case of ESBL producing *E. coli* while the number of ESBL producing *K. pneumoniae* was higher in our study as compared to others. In our study, 83.3 and 85.3%, respectively of ESBL producing *E. coli and K. pneumoniae* were isolated from hospitalized patients, while 16.7 and 14.7%,

respectively were isolated from outpatients. So, the results of the present study showed a higher prevalence of ESBL producing *E. coli* and *K. pneumoniae* among the hospitalized patients. These results are different from those of a study conducted in Iran, where lower numbers of ESBL producing *E. coli* and *K. pneumoniae* (33% each) had been isolated from inpatients and a higher frequency of ESBL producing *E. coli* and *K. pneumoniae* (66% each) detected from the outpatients (Behroozi et al., 2010).ESBL producing *E. coli* showed lower resistance to meropenem (1.3%), piperacillin/tazobactam (10.3%) and nitrofurantoin (27.6) and higher resistance was seen against cefotaxime (100%), ceftazidime (99.4%) and cefuroxime (93.3%).

ESBL producing *K. pneumoniae* showed higher resistance to ceftazidime (100%), cefotaxime (98.7%) and cefuroxime (98.1%) while lower resistance was seen with meropenem (3.6%), piperacillin/tazobactam (17.6%), and nitrofurantoin (28.5%). In a study conducted in Saudi Arabia, ESBL producing *E. coli* and *K. pneumoniae* showed higher resistance to ceftriaxone while lower resistance was seen with meropenem (4.2%), amikacin (6.3%) and imipenem (8.3%). ESBL producing *K. pneumoniae* were resistant to meropenem (5.6%), gentamicin and piperacillin/tazobactam (11.1%) and amikacin, ciprofloxacin (6.7%) (Zahrani and Akhtar, 2005). Kadar and Angamathu (2005) reported 11% resistance of meropenem which is more than seen in the present study. Mekki et al. (2010) reported the higher antimicrobial resistance of *E. coli* with nalidixic acid, nitrofurantoin, co-trimoxazole, gentamicin (100% each), ciprofloxacin (97.96%), cefuroxime (95.92%) and amikacin (69.39%). In the same study, ESBL producing *K. pneumoniae* showed maximum resistance to nalidixic acid, cefuroxime, ciprofloxacin, co-trimoxazole, gentamicin (100% each), nitrofurantoin (97.37%) and amikacin (39.47%). Most of these results are in accordance with our study except that the nitrofurantoin showed minimum resistance in our study.

In the present study, ESBL producing *E. coli* and *K. pneumoniae* were found to be multidrug resistant. In ESBL producing bacteria, high percentage of antibacterial resistance of non-β-lactam antibiotics is a serious matter of concern. Monitoring of ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI. ESBL producers are associated with increased morbidity and mortality. The majority of ESBL producing *E. coli* and *K. pneumoniae* were resistant to the common antibiotics used in the treatment of urinary tract infections. The early detection and reporting of suitable antibiotics can reduce the treatment failure in ESBL UTI. The molecular characterization of ESBL producing bacteria in our country is required to understand the mechanism of ESBL resistance.

This study is important for strict antibiotic policy implementation in hospitals, to estimate the impact of increased drug resistance in bacteria and to take steps for reducing their resistance.

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