Drug susceptibility patterns of extended spectrum β-lactamase producing *Escherichia coli* causing urinary tract infections, Khyber Pakhtunkhwa, Pakistan

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**Abstract**

*Escherichia coli* (*E. coli*) are the causative agent of urinary tract infections. Emergence of drug resistance in *E. coli* is serious threat to public health. Production of ESBL is one of the common drug resistance mechanisms which limit the use of β-lactam drugs and affect the disease control strategy. This study was undertaken to isolate and analyze antibiotic susceptibility patterns of extended spectrum β-lactamase (ESBL) producing *E. coli* causing urinary tract infections. A total of 310 urine samples were collected from different wards of tertiary care hospital of Peshawar, Pakistan. *E. coli* was identified in 144 samples, among them 44 (30.55%) were ESBL producing. Among them ESBL was detected 8(18.18%) in male and 36(81.81%) in female. After screening 50 samples from OPD for ESBL producing *E. coli*, only 12 samples were ESBL producing *E. coli*. Furthermore, antibiotic susceptibility patterns of ESBL showed increased sensitivity towards meropenem (97.72%) and imipenem (95.45%) followed by amikacin (86.36%) and piperacillin/tazobactam (72.52%). While increased resistance to nalidixic acid (93.15%) and norfloxacin (86.31%) was shown in ESBL. Antimicrobial sensitivity revealed that ESBL producing *E. coli* from the urine sample possessed increased resistance against commonly used antibiotics. Findings from the current study will be helpful to design appropriate hospital antibiotic policy to minimize the emergence of drug resistance.

Keywords: *E. coli*, ESBL, UTIs, peshawar

I. Introduction

Urinary tracts infections (UTIs) in human are caused by microbial agents including bacterial, fungal and parasitic pathogen. Bacterial agents are the common cause of UTIs. Mostly UTIs samples are diagnosed with members of the family *Enterobacteriaceae* is (84.3) [1, 2]. Production of extended-spectrum β-lactames (ESBL) in family *Enterobacteriaceae* pose a major worldwide threat to public health (Pitout & Laupland, 2008). Uropathogenic *E. coli* (UPEC) is responsible for approximately 90% of urinary tract infection [3]. Only some members of *E. coli*, because frequently originates from the patient’s own intestinal flora can cause infection in persons without local or general predisposing conditions to UTIs. *E. coli* produced two types virulence factors including those produced on the surface of the cell and those produced within the cell which then exported to the site of action [4, 5]. Several beta lactams drugs are employed to treat UTIs while the acquired resistances to these antibiotics in UTIs pathogen are mainly mediated by bacterial β-lactames and the emergence of extended-spectrum β-lactames (ESBLs) is of great clinical importance [6]. Beta-lactames are extra cellular enzymes that cleave the amide bond of beta-lactam ring of the penicillin molecule. The ESBL have serine at their active site and attach the amide bond in the lactam ring of antibiotics causing their hydrolysis [7]. *E. coli* also produces plasmid-mediated ESBL, enzymes capable of hydrolyzing and inactivating a wide variety of β-lactams, including penicillin, cephalosporin and monobactam [8, 9]. These enzymes were first time isolated from *Klebsiella pneumonia* in Germany, while such resistances were reported in the United States following outbreaks of infections caused by these pathogens [10, 11]. The ESBL enzymes are produced by *Enterobacteriaceae* mainly by *Escherichia coli*, *Klebsiella pneumonia* and *Klebsiella oxytoca*, while also have been detected in other Gram-negative bacilli such as *Salmonella* species, *Proteus* species, *Pseudomonas* species etc.
The present study was undertaken to report the prevalence of ESBLs producing isolates of *E. coli* in a tertiary care hospital in Peshawar. ESBLs production was done by using double-disk diffusion test. The same procedure as used for antimicrobial susceptibility testing was utilized but here a disk of AMC (amoxicillin/ clavulanate 20µg /10µg) was placed in between CAZ (ceftazidime 30µg) and CTX (ceftoxime 30µg) at a distance of 20mm from each other on Mueller Hinton agar (MHA) plate and then the plate was incubated at 37 °C for overnight. Furthermore, ESBLs producing isolates were tested for ESBLs production and for the antibiotic susceptibility. The study will be helpful to adopt a judicious hospital antibiotic policy against ESBLs producing pathogens.

2. Materials and Methods

Total 310 clinical samples for this study were collected aseptically, and properly labeled from a tertiary care hospital, Peshawar, Khyber Pakhtoonkhwa, Pakistan, during the period of (2014-15). Out of these samples 119 samples were taken from male patients and 191 samples from female patients, which were collected from different wards included OPD, Medical, Nephrology, Surgical, Children, Guiney, Main OT and Urology, and then inoculated on CLED agar using bacteriuria strips (Medi-Test, UK). These plates were incubated at 35 ± 2 °C for 18 h aerobically. Only those samples were further processed for ESBLs production which showed significant growth and identified as *E. coli* on the basis of culture, Gram staining, and biochemical characteristics.

2.1 Determination of ESBLs production:
ESBLs production was done by using double-disk diffusion test. The same procedure as used for antimicrobial susceptibility testing was utilized but here a disk of AMC (amoxicillin/ clavulanate 20µg /10µg) was placed in between CAZ (ceftazidime 30µg) and CTX (ceftoxime 30µg) at a distance of 20mm from each other on Mueller Hinton agar (MHA) plate and then the plate was incubate at 37 °C for overnight. Clear extension of the edge of the inhibition zone of cephalosporin toward the amoxicillin/clavulanic acid AMC disk was interpreted as ESBL producer.

3. Results

A total of 144 (46.4%) of 310 cultured samples showed significant growth of suspected pathogens and were then processed for staining and biochemical identification. On the basis of culture, Gram staining and biochemical characteristics, about 34 (23.61%) were male and remaining 110 (76.38%) were female. On the basis of number of samples, the highest ratio were from out-door patients 50(34.72%) followed by Medical ward (25.00%) and Children (12.5%) having 36 and 18 respectively. About 17 (11.8%) samples from Nephrology showed the *E. coli* growth, while 14 (9.72%) isolates obtained from Guiney. 5 (3.4%) samples were from Surgical and Main OT and Urology showed less number of isolates, which were 2 (1.3%), 2 (1.3%) samples, respectively (Figure 1).

![Fig 1: Occurrence of *E. coli* in urin samples](image1)

From total samples, the samples containing *E.coli* were categorized as positive samples. These positive samples were subjected to check its resistance pattern against β-lactums and the ESBL production was detected in 44(30.55%), in which 8(18.18%) were from male and the remaining 36(81.81%) samples were from female. Highest percentage 12(27.30%) was recorded in OPD samples and the lowest percentage in Main OT 1(2.27%), and Urology 1(2.27%). Incidence of ESBL in other samples is shown in (Figure 2).

![Fig 2: Ward wise distribution of ESBL producing *E. coli*](image2)

Age wise distribution showed that incidence of ESBL producing *E. coli* has the highest percentage of 9(20.45%) in patients of (51-60) years age. Followed by 8(18.18%) in age of (21-30) patients and of age (70-80) have the lowest percentage of 1(2.27%). Figure 3 show other results.

![Fig 3: Age wise distribution of ESBL producing *E. coli*](image3)
Figure 4 shows antibiotics susceptibility patterns of ESBL isolates to different antibiotics in urine samples, where ESBL producing E. coli showed high resistance to various antibiotics used. The most sensitive antibiotics were meropenem (97.72%) and imipenem (95.45%), followed by amikacin and pipracillin/tazobactum, (86.36%), (72.52%), respectively. While the most resistance showed to nalidixic acid (93.15%) and norfloxacin (86.31%). Antibiotics susceptibility patterns on petri plates are shown in (Figure 4).

4. Discussion
Escherichia coli (E. coli) is the causative agent of urinary tract infections. About 150 million people worldwide are diagnosed with UTIs each year, costing the global economy in excess of 6 billion US dollars. UTIs provide an excellent model to study how the host recognizes and deals with mucosal pathogens.

Emergence of drug resistance in E. coli is serious threat to public health. There have been significant changes in the antimicrobial resistance patterns of uropathogens over the years including resistance due to extended spectrum β-lactamase (ESBL) producing pathogens. ESBLs production in E. coli is one of the common drug resistance mechanisms which limit the use of beta lactam drugs and affect the disease control strategy. A growing body of literature is available on ESBL producing E. coli from Pakistan; however limited data are available from Peshawar, Pakistan. This study was undertaken to isolate and analyze antibiotic susceptibility patterns of extended spectrum β-lactamase (ESBL) producing E. coli causing urinary tract infections. Antibiotic susceptibility patterns of ESBL were detected by using the disk diffusion method. In present study E. coli was isolated from various wards samples collected from a tertiary care hospital in Peshawar, Khyber Pakhtoonkhwa, Pakistan. Out of 310 urine samples 144 samples were positive for E. coli in which 34 (23.61%) were male and remaining 110 (76.38%) were female. Our results also showed a ratio of ESBL producing E. coli in outpatient and inpatients, which were 13(29.54%) and 31(70.45%) respectively. Similarly in Gandhinagar [18, 19] conducted a study, overall E. coli was (70.96%) among the all Gram negative bacilli of UTIs patients. The majority (67%) of the isolates were from female. ESBL prevalence was 16.67% and 30% among community acquired and hospital acquired E. coli respectively. And in East London, the study revealed extended spectrum β-lactamase production, was observed in 5.7% of community and 21.6% of nosocomial isolates [20, 21].

ESBL positive isolates were found 48% in Pakistan reported from patients between 50-60 years of age. Current study finding are almost similar to previous studies in which ESBL producing E. coli have highest percentage of (9(20.45%)) in patients of (51-60) years age in current study. Followed by 8(18.18%) in age of (21-30) patients and of age (70-80) have lowest percentage of 1(2.27%) [22, 23].

5. Conclusion
In conclusion, our data indicate the spread of extended spectrum β-lactamase (ESBL) producing E. coli in tertiary care hospital at Peshawar. The common bacterial isolate was E. coli, and ESBL were detected in 44(30.55%) E. coli isolates out of 144 urine samples. Further clinical study is required to monitor the molecular epidemiology and transmission of ESBL types in order to control the spread of drug resistance among Gram negative bacteria in the community and hospitals setup. Pathogens producing ESBL limits the treatment option even further.

6. References


