Phenotypic Detection of Extended Spectrum Beta-Lactamases in Uro-pathogens. An Experience from Qazi Hussain Ahmed Medical Complex, Nowshera

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Author’s Contribution

1 Conception of study
2 Experimentation/Study conducton
1,2,3,4 Analysis/Interpretation/Discussion
1,2,3,5,6 Manuscript Writing
2,3,5,6 Critical Review

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Abstract

Objectives: To determine the phenotype and frequency of Extended Spectrum Beta-Lactamase in Uro-pathogens in a tertiary care hospital of Nowshera.

Material and methods: This Prospective cohort study was performed in the clinical pathology laboratory of Qazi Hussain Ahmed Medical Complex (QHAMC) Nowshera from 1st Jan 2019 to 30th May 2019. Relevant information was recorded on a pre-designed proforma prepared as per CLSI Recommendation for data collection.

Results: A total of 192 urine samples were received for Culture and sensitivity. Out of which 56 cases were ESBL phenotypically positive (29.16%), 39(69.9%) were females and 17(30.4%) were males. The age range of the patient was from 3 months to 63 years of age, with a mean age of 30 years with 5.4 SD Frequency of ESBL producing uropathogens was; 51(91%) E-Coli, 4(7.1%) Klebsiella pneumonia and 1(1.8%) Proteus mirabilis. Out of 39 ESBL producing uro-pathogen in Female gender, E Coli-ESBL producing strains were isolated in 35 patients, Klebsiella Pneumonia in 3 patients and one case was of Proteus mirabilis-ESBL. In the male gender, 17 cases with E Coli-ESBL were reported and one case of Klebsiella Pneumonia ESBL.

Conclusion: The prevalence of ESBL producers in the present study was quite alarming and challenging to the clinician in treating urinary tract infections. These types of resistant infections are a challenge to treat and a public health threat that needs accumulative response through advocacy, communication, and social mobilization.

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Introduction

ESBL is short for Extended-Spectrum Beta-Lactamase. ESBL is an enzyme found in some resistant strains of bacteria that are resistant to conventional antibiotics i.e. penicillins, cephalosporins, and monobactam. ESBL producing uro-pathogens causing urinary tract infection (UTI) has reported increased resistance to antimicrobial medications. Urinary tract infections (UTIs) are an important community and acquired infection reported worldwide. Escherichia coli (E. coli) is notorious for causing urinary tract infections (UTIs) and the uropathogenic E.Coli (UPEC) are reported to cause UTI in 90% of community-acquired and up to 50% cases of hospital-acquired UTIs.

UPEC is associated with an increased rate of expression of extended-spectrum β-lactamase (ESBL) gene. ESBLs contain plasmid-mediated derivatives. ESBLs, called CTX-M (i.e., “active on CefoTaXime, first isolated in Munich”) type, have been reported in 2000 globally. Since the treatment of UTI is started with the irrational use of antibiotics without the culture and sensitivity which is essential before attaining the desired objectives from the empirical therapy. This results in the production of ESBL by Micro-organism and the infection proceeds toward resistant type. The prevalence of ESBL-producing E.coli is rapidly increasing in our part of the world because of poor implementation of the rules and regulations about the rational use of antibiotics and increased in the quackery associated with the medical professional, minimal or negligible role of the drug regulatory authority and health care commission.

The use of second and third-generation cephalosporins for the treatment of simple UTI has led to the development of resistant infection and the production of enzymes called the Extended Spectrum Beta Lactamases (ESBLs) that results in the development of multi-drug resistance.

There are many types of ESBLs like TEM, TEM given name, after the name of the patient who developed ESBL for the first time, named Temoniera from Greece, SHV type ESBL is another plasmid-mediated β-lactamase is named after the sulphhydryl variable and CTX for the ESBL that can hydrolyze the cefotaxime. OXA is another β-lactamase and it has hydrolytic activity against the cloxacillin and oxacillin. PER type of ESBL refers to resistance to penicillin’s and cephalosporins, VEB-ESBL is a β-lactamase first derived from a patient of Vietnam, GES-ESBL is β-lactamase isolated from for Guiana patients. ESBL types of infections are strongly associated with high morbidity and mortality than other types of UTIs. Extended-spectrum β-lactamase (ESBL) producing bacteria causing infectious diseases conferring resistance to all β-lactam antibiotics. ESBL-producing organisms frequently show cross-resistance to other antibiotics as well; including aminoglycosides and fluoroquinolones thus making treatment of these infections non-treatable.

The present study was designed to investigate the occurrence of plasmid-mediated ESBL resistance gene expression in various uro-pathogens using phenotypic.

Materials and Methods

This Prospective cohort study was performed in the Clinical Pathology Laboratory of Qazi Hussain Ahmed Medical Complex Nowshera from 1st Jan 2019 to 30th May 2019. A total of 192 urine samples were received for Culture and sensitivity out of which 56 cases reported being ESBL phenotypically positive (29.16%). The inclusion criteria were midstream urine of all cases irrespective of age and gender received in the laboratory. Exclusion criteria were urine samples received in the laboratory 24 hours after collection, the patient already on the antibiotic therapy and improperly collected urine.

The specimen was received in the pathology section from the respective unit under observance of strict aseptic technique along with the education of patients on a midstream urine sample. Media were prepared as per CLSI (Clinical and laboratory standard institute). Then the urine samples were inoculated on CLED (Cysteine Lactose Electrolyte Deficient) media. Then the specimens were incubated under ambient air 35 ±2 C for 24 hours. In case growth is obtained following inoculation on Mueller Hinton agar for sensitivity to antibiotics. For urine samples the antibiotic desks used were; Ak- Amikacin, Mem-Meropenem, IPM-Imepenum, CAZ-Ceftazadime, CTX-Cefotaxime, TZP-Piperacillin Tazobactum, SCF-Cefperazone Sulbactum, CT-Colistin Sulphate, F-Nitrofruantoin, AMC-Amoxicillin-Clavulanic acid, Fos-Fosfomycin, SXT-Co-Triamaxazole, Levo-Levofloxacin & CIP-Ciproxacin. Plates were incubated for 18-20 hours and then the zone of inhibition was calculated on the caliper, including the size of the desk. Zones were compared with CLSI recommendations for sensitivity to be reported as sensitive, intermediate and resistant.

Desk of AMC-amoxicillin-Clavulanic acid was placed at a distance of 25mm center to center to any of the following antibiotics; CRO-ceftriaxone-CTX-
cefotaxime-CAZ-ceftazidime-ATM-Aztreonam. Phenotypically ESBL was confirmed as enhancement of zone of any of the indicator antibiotics (CRO-ceftiraxone-CTX-cefotaxime-CAZ-ceftazidime-ATM-Aztreonam) towards AMC-amoxicillin Clavulanic acid was considered to be positive for ESBL phenomenon (Figure 1). Finally, the data obtained from the culture and sensitivity was entered into SPSS version 16 for descriptive and correlation analysis of different parameters. Descriptive statistics were used for numerical variables. The categorical variable frequency was shown in percentages likewise for gender and type of ESBL producing organisms. A chi-square test was applied to see the relationship/Preference of ESBL for the type of bacteria and a p-value of 0.05 was taken as significant.

![Figure 1: ESBL phenomenon](image)

**Results**

A total of 192 urine samples were received for Culture and sensitivity out of which 56 cases reported being ESBL phenotypically positive (29.16%). 39(69.9%) were females and 17(30.4%) males (Table 1). The age range of the patient was from 3 months to 63 years of age, with a mean age of 30 years with ±5.4 SD. The mode of age was 25 years (Table 2). The frequency of ESBL producing uro-pathogens was; 51(91%) cases in E-Coli, 4(7.1%) in Klebsiella pneumonia and 1(1.8%) in Proteus mirabilis (Table 3). 29(51%) cases ESBL positive were reported from samples received from the Medical unit, 11(19.6%) from Surgery/Gyne unit and 8(14.3%) from Paeds Medicine and Accident and Emergency departments each (Table 4). Sensitivity pattern to ESBL was; Imepenum(IMP) 56 (100%), Meropenum(Mem) 56 (100%), Amikacin(AK) 56 (100%), (Piperacillin Tazobactum(TZP) 50(89.3%), Nitrofurantoin (F) 44(78.6%), Cefperazone Sulbactum (SCF) 43(76.8%), Fosfomycin (Fos) 42(75%), Cotrimazaxole (SXZ) 15(26.8%) Levofloxacan, ciprofloxacin and colistin sulphate 5.4% each(Table 5). In females, due to many reasons (mainly the short distance between the vagina and anus, because of increased colonization of bacteria in the vagina and increased sexual manipulation), the exposure to ESBLE was more common. Out of 39 ESBL cases in Female; E Coli-ESBL producing strains were found in 35 patients, Klebsiella Pneumonia in 3 patients and in one case was of Proteus mirabilis-ESBL. In the male gender, E Coli-ESBL was 16 and Klebsiella Pneumonia ESBL in one case (Table 6). We received the ESBL cases in three gram-negative strains (E-coli, klebsiella, and proteus) 26,2,1 respectively from the Medical unit. The cases from Surgery and allied including the Gynecology department the ESBL in E-coli and Klebsiella (10,1 respectively). From Paeds medicine and accident and emergency department 16 cases (8 from each unit) with ESBL producers frequency of 7, 1 for E-Coli and Klebsiella respectively (Table 7).

| Table 1. Descriptive Statistics |
|---------------------------------|
| **Gender** | **Frequency** | **Percentage %** |
| **Female** | 39 | 69.6 |
| **Male** | 17 | 30.4 |
| **Total** | 56 | 100.0 |

| Table 2. Age Statistics |
|-------------------------|
| **Age Statistics** | **Values** |
| Mean | 30.23 |
| Median | 28 |
| Mode | 25 |
| Std. Deviation | 5.39 |
| Range | 87 |
| Minimum | 3 |
| Maximum | 90 |

| Table 3. Table Frequency of ESBL Producing Uro-Pathogens |
|----------------------------------------------------------|
| **Frequency** | **Percentage %** |
| E.Coli | 51 | 91.1 |
Table 4. Department wise ESBL Reporting

| Department       | Frequency | Percent |
|------------------|-----------|---------|
| A/E Dept         | 8         | 14.3    |
| Medical          | 29        | 51.8    |
| Paeds Medicine   | 8         | 14.3    |
| Surgery/Gyne     | 11        | 19.6    |
| Total            | 56        | 100.0   |

Table 5: Sensitivity pattern to ESBL

| Sensitivity to Antibiotic | Frequency | Percentage |
|---------------------------|-----------|------------|
| IPM-imipenem              | 56        | 100        |
| Mem-Meropenem             | 56        | 100        |
| TZP- Piperacillin         | 50        | 89.3       |
| SCF- Cefperazone          | 43        | 76.8       |
| sulbactum                 |           |            |
| Ak-Amikacin               | 56        | 100        |
| F-nitrofurantoin          | 44        | 78.6       |
| Fos-Fosfomycin            | 42        | 75         |
| SXT- Co-Triamoxazole      | 15        | 26.8       |
| Levo-Levofoxacin          | 3         | 5.4        |
| CIP-Ciprofoxacin          | 3         | 5.4        |
| CT-Colistin sulphate      | 3         | 5.4        |

Table 6. Gender and ESBL types Crosstabulation

| Gender | E.Coli | Klebsiella | Proteus | Total |
|--------|--------|------------|---------|-------|
| Female | 35     | 3          | 1       | 39    |
| Male   | 16     | 1          | 0       | 17    |
| Total  | 51     | 4          | 1       | 56    |

Pearson Chi-Square = 0.515, P-value = 0.773

Discussion

Bacterial resistance to commonly used antibiotics in clinical practice has resulted in the evolution of a wide range of Extended-spectrum β-lactamase (ESBL) producing strains that is a global public health concern resulting in wastage of huge loss of economy on simple infections that have now become resistant due to ESBL production by the microbial. ESBL has failed in treatment strategies normally practiced up to secondary care treatment facilities where there are no facilities for culture and sensitivity. There is an increase in the reporting ratio of ESBL, producing gram-negative bacteria from our set-ups especially by Enterobacteriaceae with diverse types of ESBL genes as mentioned above are alarming. ESBL producing bacteria can hydrolyze the 3rd and 4th generation of cephalosporins, all types of penicillins and monobactams. These resistant strains are inhibited by β-lactamase inhibitors i.e. (clavulanic acid, sulbactam, and tazobactam). In the present study, the ESBL production rate was 29.16% lower as compared to other studies that have reported to be 52%. However various other studies have reported the range of ESBL production rate from 17% to 70%. We detected the ESBL in uro-pathogens in the present study, many other studies have reported urine as the major source of ESBL producing microbial. One of the authors has, however, reported blood as a major source for isolating ESBL producing organisms.

In our study, the frequency of ESBL producing uro-pathogens was 51(91%) in E-Coli, followed by Klebsiella pneumonia 4(7.1%) and Proteus mirabilis 1(1.8%). Studies have reported E. coli was the major ESBL producer that coincides with our findings. Similarly, one study reported the highest ESBL production in Klebsiella spp. One author reported the prevalence of the CTX-M encoding gene in the majority of E. coli strains(96%) followed by K. pneumonia(71%) that phenotypically, however, matches our findings. In this study, we found that in females due to the short distance between the vagina and anus, increased colonization of bacteria in the vagina lead to exposure to ESBL in the female gender. Out of 39 ESBL cases in the female gender, E Coli-ESBL producing strains were found in 35 patients, Klebsiella Pneumonia in 3 patients and one case was of Proteus mirabilis-ESBL. A study from Saudi Arabia reported that The ESBL phenotype was detected in 351 of 1151 isolates (30.5%). Their phenotypic detection rate...
coincides with our findings. Escherichia coli (E. coli) being the most common pathogen producing the ESBL (62.7%) followed by Klebsiella pneumoniae (K. pneumoniae) (23.6%) that again matched our findings. They reported that the highest proportion of ESBL producing microbials were isolated from the urine samples (62.5%), and further that their majority of the target samples were reported from the female medical ward (20.2%) as seen in our findings.

In present study the sensitivity pattern to ESBL was; Imipenem (IMP) (100%), Meropenem (Mem) 56(100%), Amikacin (AK) 56(100%), (Piperacillin Tazobactum (TZP) 50(89.3%), Nitrofurantoain (F) 44(78.6%), Cefperazone Sulbactum (SCF) 43(76.8%), Fosfomycin (Fos) 42(75%), Co-trimoxazole (5XZ) 15(26.8%) Levofloxacin, ciprofloxacin and colistin sulphate 5.4% each. Many other studies have reported that the sensitivity pattern to E. coli ESBL isolates was Imipenem (99.54%), Ampicillin-Sulbactam (97.48%), Piperacillin-Tazobactam (96.86%), Fosfomycin (94.51%), Amikacin (92.26%) and Nitrofurantoain (90.68%).

While antibiotic sensitivity to K. pneumoniae ESBL isolates was imipenem (97.62%), Piperacillin-Tazobactam (95.35%), Ampicillin-Sulbactam (90.48%) and Amikacin (88.37%). Another study reported that the sensitivity pattern to E. coli-ESBL was amikacin (50.7%), SCF (51.4%), TZP (52.7%), and MXF (54.1%) that matched our findings.

## Conclusion

The prevalence and incidence of ESBL-producing Enterobacteriaceae are high in Pakistan. Very little is reported from the Khyber Pakhtunkhwa province to know about the prevalence of ESBL in uro-pathogens. There is need for regular surveillance to address this antimicrobial resistance issue and to take remedial actions at different forums including the legislative reforms to ban quackery by strengthening Health Care Commission (HCC) Role and to develop proper protocols for prescribing antibiotics in Medical Teaching Institutions (MTI) and tertiary care treatment facilities and need for the clinician and surgeon to reduce their ego and to value the recommendations of the Pathologists in treatment of such resistant cases of UTI.

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