

## Prevalence, antibiotic sensitivity and phenotypic screening of ESBL/MBL producer *E. coli* strains isolated from urine; District Swabi, KP, Pakistan

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### Abstract

The study aimed to record the prevalence of Urinary Tract Infection caused by *E. coli*, its antibiotic sensitivity and phenotypic detection of ESBL and MBL producer *E. coli*. Total of 240 urine samples were collected from Bacha Khan medical complex, Swabi. Antibiotic sensitivity and production of ESBL and MBL according to CLSI guidelines 2017 were performed. Out of 75 *E. coli* strains, only 25 (33.3%) isolates were positive for ESBL. Similarly, 25 were resistant to Imipenem, in which 12 (16%) isolates were positive for MBL production. *E. coli* isolates were found more sensitive to Amikacin 57 (76%) followed by Imipenem 50 (66.67%), Ofloxacin 48 (64%), Ciprofloxacin 44 (58.67%), Norfloxacin 42 (56%) and were 100% resistant to Penicillin, Ceftriaxone, Ceftazidime and Cefotaxime. The current study recorded the prevalence rate of UTI caused by *E. coli* was 75 (66.96%) and occurrence rate of ESBL and MBL producers in the total (n=75) *E. coli* isolates was 25 (33.3%) and 12 (48%) respectively.

**Keywords:** *Escherichia coli*, ESBL, MBL, Urinary tract infection.

### Introduction

Urinary Tract Infection (UTI) remains the most common bacterial infection in human population and is also one of the most frequently occurring nosocomial infection. Among both the gram positive and gram-negative bacteria causing UTI in humans, the most frequent in the community are *Escherichia coli* and other Enterobacteriaceae, which account for approximately 75% of the isolates.<sup>1,2</sup>

Another growing concern is emergence of extended spectrum Beta-Lactamase (ESBL) producing bacteria that hydrolyze the Extended-Spectrum cephalosporins. The persistent exposure of bacterial strains to a multitude of  $\beta$ -lactams has induced dynamic and continuous production and mutation of  $\beta$ -lactamases in these bacteria, expanding their activity even against the newly developed  $\beta$ -lactam

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antibiotics. On the basis of protein homology of enzymes, the beta-lactamase are classified into four types, A, B, C and D classes. The beta-lactamase enzymes shared the ability to hydrolyzed penicillin group and cephalosporin group however inhibited by clavulanic acid.<sup>3</sup> Keeping all the above facts in mind, the main objectives of the study were to determine and evaluate the prevalence of UTI in District Swabi, determine the antibiogram of commonly used antibiotics in UTI and to evaluate the prevalence of ESBL and MBL in *E. coli* isolates from UTI patients.

### Methods and Results

A total of 240 samples were randomly collected at the Department of Pathology, Bacha Khan medical complex, Swabi, KP, Pakistan, from patients visiting the hospital with clinical symptoms of urinary tract infection. The samples were collected From September 2016 to April 2017. The study was ethically approved by the Department of Microbiology, University of Swabi, Anbar, KP, Pakistan. Informed permission was obtained from the patients before including them in the study. Identification, antibiotic susceptibility test and phenotypic detection of ESBL and MBL were done in Microbiology Lab, Department of Microbiology University of Swabi, Anbar Kpk. Urine samples were cultured on CLED agar, Nutrient agar and MacConkey agar (Oxoid). The gram-negative rod was further processed and identified as *E. coli* by performing various biochemical tests. Only those isolates were included in the study which gave positive result for indole production. The positive indole test isolates was considered as *E. coli*.

According to the Clinical Laboratory Standards Institute (CLSI, 2017) guidelines antimicrobial sensitivity test was performed by the Kirby-Bauer disc diffusion method. The antibacterial agents tested for sensitivity were (Penicillin 1U), Ceftriaxone (30 $\mu$ g), Ceftazidime (30 $\mu$ g), Cefotaxime (30 $\mu$ g), Amoxicillin - Clavulanic Acid (20/10 $\mu$ g), Amikacin (30 $\mu$ g), Piperacillin - Tazobactam (100/10 $\mu$ g), Trimethoprim-Sulfamethoxazole (1.25/23.75 $\mu$ g), Imipenem (10 $\mu$ g), Ciprofloxacin (5 $\mu$ g), Norfloxacin (10 $\mu$ g),

Ofloxacin (5µg).

*E. coli* strains were further processed for the screening of ESBL and Non-ESBL isolates by combination disc test (CDT) and MBL and Non-MBL *E. coli* isolates by MBL disc conformation by imipenem -EDTA combined disc synergy test (CDST, 2017).<sup>4</sup>

All the data regarding percentages was analysed by using Microsoft Excel 2010 version .

The chi-square test (SPSS 20 version) was applied to compare the antibiogram of ESBL, MBL, Non-ESBL and Non-MBL *E.coli*; and comparison of antimicrobial Sensitivity and resistance. P-value of < 0.1 was considered to indicate statistically significant differences. P-value of > 0.1 was considered to indicate statistically non-significant differences.

## Results

Out of total 112(44.8%) samples were found positive for bacterial infections while the remaining 128 (53.33%) were negative and thus not processed further. Out of 112, 75(66.96%) samples showed *E. coli* strains followed by *Staphylococcus* (17.85%) isolates, *Streptococcus* (13.39%)

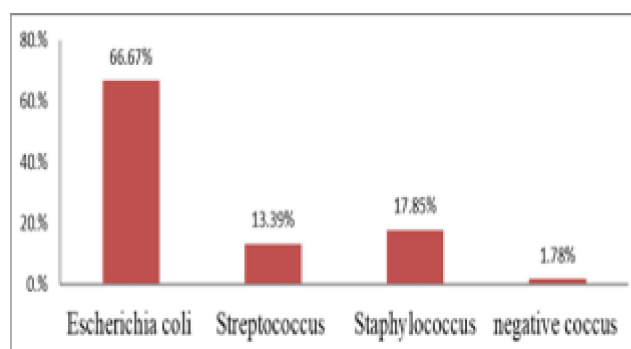
**Table-1:** Age and Gender wise distribution of UTI cases caused by *E.coli*.

Age	Female Cases NO.(%)	Male Cases NO.(%)	Total Cases NO.(%)
10-20 Years	7 (13%)	6 (26%)	13
21-30 Years	19 (36.5 %)	4 (17.3%)	23
31-40 Years	10 (19.2%)	7 (30.4%)	17
41-50 Years	9 (17.3%)	2 (8.6%)	11
51-60 Years	5 (9.6 %)	3 (13%)	8
61-70 Years	2 (3.8%)	1 (4.3%)	3
Total	52 (69.3%)	23 (30.6%)	75

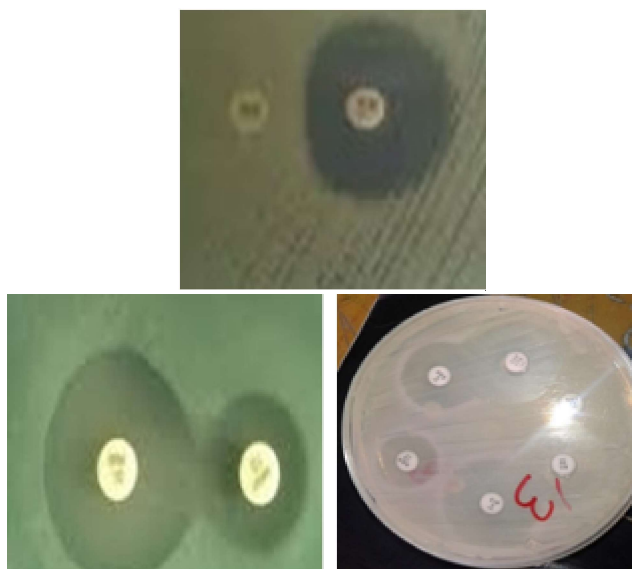
**Table-2:** Number and percentages of Antibiotic Sensitivity of ESBL, Non- ESBL, MBL, and Non-MBL *E. coli* strains (n=75).

Antibiotics	Sensitivity NO.(%)	ESBL Producer NO.(%)	NonESBL Producer NO.(%)	MBL Producer NO.(%)	Non MBL Producer NO.(%)
P (10 U)	0(0)	0(0)	0(0)	0(0)	0(0)
CRO (30 µg)	0(0)	0(0)	0(0)	0(0)	0(0)
CAZ (30 µg)	0(0)	0(0)	0(0)	0(0)	0(0)
CTX (30 µg)	0(0)	0(0)	0(0)	0(0)	0(0)
AMP(20/10 µg)	24 (32)	6 (24)	18(36)	6(50)	3 (23.07)
AK (30 µg)	57(76)	21(84)	36(72)	9(75)	12(92.31)
TZP (100/10 µg)	29(38.67)	12(48)	17(34)	6(50)	5(38.46)
SXT(1.25/23.75 µg)	26(34.67)	8(32)	18(36)	3(25)	3(23.07)
IMP (10 µg)	50(66.67)	16(64)	34(68)	0(0)	0(0)
CIP (5 µg)	44(58.67)	17(68)	27(54)	8(66.67)	8(61.53)
NOR (10 µg)	42(56)	13(52)	29(58)	8(66.67)	9(69.23)
OF (5 µg)	48(64)	17(68)	34(68)	7(58.33)	9(69.23)

P= Penicillin, CRO= Ceftriaxone, CAZ= Ceftazidime, CTX= Cefotaxime, AMP=Amoxicillin - Clavulanic Acid, AK= Amikacin.



**Figure-1:** Percentage of isolated gram positive and negative bacteria.



**Figure-2:** Antibiotic sensitivity pattern of a) *E. coli* b) MBL Producer c) MBL producer.

strains and gram-negative coccus (1.78%) isolates (Figure-1). The prevalence of *E. coli* was more in female than male. Out of 75 *E. coli* isolates, 52(69.33%) and 23(30.67%) strains were isolated from females and males respectively.

In this study, urinary tract infections were found in all age groups. In total of 75 *E. coli* isolates, most of the patients 23(30.67%) reporting UTIs were between 21-30 years age. Of the remaining 17 (22.67%) were 31-40 years old, 13(17.33%) were 10-20 years, 11 (14.67%) between 41-50 years, 8(10.66%) were 51-60 years and 3(4%) patients were 61-70 years old (Table-1).

The antibiotic susceptibility testing against *E. coli* isolates showed more sensitivity to Amikacin 57(76%) followed by Imipenem 57(66.67%), Ofloxacin 48(64%), Ciprofloxacin 44(58.67%), Norfloxacin 42(56%) and 75(100%) were resistant to Penicillin, Ceftriaxone, Ceftazidime and Cefotaxime (Table-2 and Figure-2).

**Table-3:** Statistical comparison of antibiotic Susceptibility pattern of resistant and sensitive ESBL and Non- ESBL E. colistrains.

Antibiotics	Percentage of sensitive ESBL E. Coli Isolates	Percentage of sensitive Non-ESBL E. Coli Isolates	Non-significant Value	Percentage of sensitive MBL E. coli Isolates	Percentage of sensitive NON-MBL E. coli Isolates	Non-significant Value
Amoxycillin - Clavulanic Acid 20/10 µg	24%	32%	0.294	50%	23.08%	0.161
Amikacin 30 µg	84%	72%	0.251	75%	92.08%	0.238
Piperacillin - Tazobactam 100/10 µg	48%	34%	0.241	50%	38.46%	0.561
Trimethoprim- Sulfamethoxazole 1.25/23.75 µg	32%	36%	0.731	25%	23.08%	0.910
Imipenem 10 µg	64%	68%	0.729	0%	0%	
Ciprofloxacin 5 µg	68%	54%	0.246	67%	61.54%	0.790
Norfloxacin 10 µg	52%	58%	0.632	67%	69.23%	0.891
Ofloxacin 5 µg	56%	68%	0.307	67%	69.23%	0.571

**Table-4:** Number and percentage of E.coli isolates in male and female UTI patients (n=75).

Name	Male number and %	Female number and %
E.COLI isolates	23(30.67%)	52(69.33%)

Out of 75 strains, 25 (33.33%) were ESBL producers while 50(66.67%) were Non-ESBL strains. Similarly, 25(33.33%) strains were resistant to Imipenem (10µg) antibiotic, in which 12(48%) were MBL producers while 13 (52%) were Non-MBL producer (Table-2 and 3). Non- significant differences were found in Susceptibility pattern of various antibiotics between ESBL, Non-ESBL, MBL and Non-MBL (Table-4).

## Discussion

E. coli is the major cause of UTIs. In the developing countries like Pakistan, this type of infection is very common and its eradication is a challenge, as the etiological agents of UTIs have become resistant to various antibiotics.<sup>5</sup>

In the current study the prevalence rate of E. coli was 75(66.96%). Similar results were observed in the previous study.<sup>6</sup> Another study was conducted in Pakistan where the prevalence rate of E. coli was 76.3%.<sup>7</sup> The difference in prevalence rate of E. coli may be due to social, and environmental conditions of different geographical regions within the same country.

In the present study, the prevalence rate of UTIs caused by E. coli was found more in females 52(66.33%) than males 23(30.67%) in all age groups. Two studies were conducted in 2014, and they observed the prevalence of urinary tract infection in females as 66.5%, 53.3%, respectively and in males as 33.6%, 30.2%, respectively.<sup>7,8</sup>

Particularly, the prevalence rate of E. coli was found more frequent in age group between 21-40 years. A research was conducted in 2014, where prevalence of urinary tract infections was found between 21-30 years.<sup>7</sup> Another study conducted in 2015, found prevalence of urinary tract infections between 21-30 years.<sup>9</sup>

The sensitivity of E. coli isolates in our study showed more sensitivity to Amikacin (76%) followed by Imipenem (66.67%), Ofloxacin (64%), Ciprofloxacin (58.67%), Norfloxacin (56%) which can be compared to a study by Ahmed et al.<sup>7</sup> The least effective antimicrobial agents to E. coli strains were Trimethoprim-Sulfamethoxazole (38.67%), Piperacillin - Tazobactam (34.47%) and Amoxycillin - Clavulanic Acid (32%) and (100%) resistant to Penicillin, Ceftriaxone, Ceftazidime and Cefotaxime. In the current study, all the E. coli isolates were found 100% resistant to Ceftriaxone, Ceftazidime which is contrary to the findings of a study by Haddadi et al.<sup>10</sup> Another study was conducted in 2016, where E. coli strains were 83% and 41.5% resistant to Amoxycillin-clavulanic acid and Piperacillin - Tazobactam respectively.<sup>11</sup> A study by Sabir et al in 2014 where E. coli was 89.04% resistant to Cefotaxime.<sup>12</sup>

In our study E. coli isolates were found 100% resistant to Penicillin which is similar to a study conducted in 2016.<sup>11</sup> In the current study 65.54% E. coli isolates were found resistant to Trimethoprim-Sulfamethoxazole, which can be compared to a study conducted in 2013.<sup>13</sup> In Pakistan Trimethoprim-Sulfamethoxazole is the useless first line drug used in E. coli UTIs.

In the current study the prevalence of ESBL E. coli isolates

were (33.3%) and Non-ESBL *E. coli* isolates were (66.6%) which is similar to a study conducted in 2013.<sup>14</sup> Similar to this study some other studies were conducted in 2015 where the prevalence rate of ESBL *E. coli* isolates was recorded as (46.6%) respectively and prevalence of Non-ESBL *E. coli* isolates as (53.4%) respectively.<sup>7</sup> In our study all the ESBL *E. coli* isolates were found 100% resistant to ceftriaxone and cefotaxime which is similar to a study conducted in 2014 and 2016.<sup>8,15</sup>

In our study, the prevalence rate of MBL producer *E. coli* isolates were 48%. A study conducted in Pakistan in which prevalence rate of MBL *E. coli* isolates was 71%,<sup>16</sup> which is more than the findings of the current study. A study conducted in India, in which prevalence rate of MBL *E. coli* isolates was 7.03%.<sup>17</sup> The difference may be due the different geographical regions within the same and neighbouring country.

## Conclusions

The current study recorded the prevalence rate of UTI caused by *E. coli* in District Swabi which was 66.96%. While the occurrence rates of ESBL and MBL producers *E. coli* strains were 33.3% and 48% respectively. Females were found more prone to UTI. Amikacin, Imipenem, Ofloxacin, Ciprofloxacin and Norfloxacin were found the most effective drugs for the treatment of UTI which was caused by *E. coli*.

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**Conflict of interest:** Not declare.

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