

# Susceptibility Patterns of Escherichia coli: Prevalence of Multidrug-resistant Isolates and Extended Spectrum Beta-Lactamase Phenotype

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## Abstract

**Objective:** To study Escherichia coli (E. coli) susceptibility, prevalence of multidrug-resistant isolates and possible role of extended spectrum beta-lactamases (ESBL) in E. coli resistance.

**Setting:** Department of Medicine, Shifa College of Medicine, Islamabad.

**Methods:** Three hundred and seventy eight E. coli isolates from different sources were identified during six-month period. Susceptibility to various antibiotics was checked using standard methods. Multidrug-resistant isolates were separated. Isolates resistant to cefpodoxime and aztreonam were considered as ESBL phenotype, indicating ESBL production.

**Results:** Most of isolates were resistant to ampicillin and trimethoprim/sulfamethoxazole. Overall resistance to ciprofloxacin, ceftazidime, cefotaxime and cefpodoxime was 49%, 34%, 36% and 38% respectively. Resistance to fourth generation cephalosporins was lower. Almost all Isolates were sensitive to amikacin, imipenem and tazobactam. Aztreonam resistance was found in 25% isolates. Similar pattern was observed for urinary E. coli isolates; 45% E. coli isolates were found to be multidrug-resistant. Nitrofurantoin showed low-level resistance both to multidrug-resistant as well as urinary isolates. Resistance to both cefpodoxime and aztreonam was found in 25% isolates suggesting ESBL production.

**Conclusion:** E. coli resistance in Pakistan is much higher than reported from western literature. Multidrug-resistant isolates, including third generation cephalosporins and quinolones, are very common. ESBL production may contribute to this high level resistance against beta-lactams (JPMA 52:407; 2002).

## Introduction

Escherichia coli (E. coli) resistance has escalated over the past many years. Acute urinary tract infections are common, occurring in 10% to 20% of otherwise healthy women during their lifetimes<sup>1</sup>. These infections are usually limited to the bladder and urethra and are most commonly caused by E. coli<sup>2</sup>. Previous studies suggest that ampicillin should no longer be used since at least 30% of causative E. coli strains are ampicillin-resistant<sup>3-5</sup>. Similarly increasing rates of trimethoprim-sulfamethoxazole (TM-SM) resistance have been reported among urinary E. coli isolates<sup>6-9</sup>. A recent study reported that about 18% of E. coli strains were resistant to trimethoprim/sulfamethoxazole<sup>2</sup>. In Spain, the proportion of E. coli resistant to ampicillin in

urine specimens reaches 50% and that resistance to TM-SM ranges from 27 to 49%<sup>10</sup>. In addition, the emergence of E. coli with beta-lactamase overproduction in up to 34% of isolates has decreased the usefulness of amoxicillin plus clavulanic acid combinations for urinary tract infections<sup>11</sup>. Data from Pakistan is even more concerning with very high level of E. coli resistance to ampicillin and TM-SM. Not only that but resistance to quinolones has also increased to alarming levels<sup>12,13</sup>.

With this background, we conducted this retrospective analysis to see susceptibility patterns of E. coli in our setting and compare this with national as well as international data. Besides overall susceptibility, we also studied susceptibility pattern of urinary E. coli. Furthermore, we wanted to see the prevalence of multidrugresistant isolates and the possible role of extended spectrum beta-lactamases (ESBL) in conferring resistance to betalactam antibiotics.

## **Material and Methods**

This study was conducted at Shifa International Hospital affiliated with Shifa College of Medicine, Islamabad. This is a 230-bedded tertiary care hospital. Three hundred and seventy eight different samples, which grew E. coli, were studied retrospectively over a period of six months.

### **Specimen Collection and Identification of Isolates**

The common sources of specimens included urine, pus swab from wounds, high vaginal swab, blood, sputum, stool, vascular catheters and bronchial washing. The specimens were inoculated on CLED (Cystine Lactose Electrolyte Deficient agar), blood agar, MacConkey, and chocolate agar, depending on source of specimen. The cultures were incubated at 37° centigrade for 24 hours. If cultures remained negative for 24 hours, they were observed for another 24 hours. When E. coli growth was suspected, colonies were inoculated on triple sugar iron agar and reactions were noted after 24 hours. Biochemical tests as indole and citrate were also performed. Colony count of 10<sup>5</sup> was considered as positive for urine culture.

### **Susceptibility Testing**

Antibiotic susceptibility was performed by using disc diffusion technique. Following antibiotics were applied in E. coli isolates; Ampicillin, TM-SM, cefaclor, ceftriaxone, cefotaxime, cefpodoxime, ceftizoxime, cefepime, cefpirome, imipenem, aztreonam, ciprofloxacin, ofloxacin, norfloxacin, nitrofurantoin, gentamicin, tobramycin, amikacin and tazobactam.

### **Screening for ESBL Production**

Resistance to cefpodoxime and aztreonam has been utilized as a screening method for ESBL production<sup>14-16</sup>. Isolates resistant to both were considered as ESBL phenotype.

### **Multidrug-resistant Isolates**

Isolates resistant to more than two different classes of antibiotics were considered as multidrug-resistant.

### **Data Analysis**

All data were tabulated and analyzed by using Microsoft excel 2000. Data were displayed as numbers and percentages.

## **Results**

Three hundred and seventy eight specimens positive for E. coli were studied over six months. Distribution of specimens according to the site of culture is shown in figure 1.

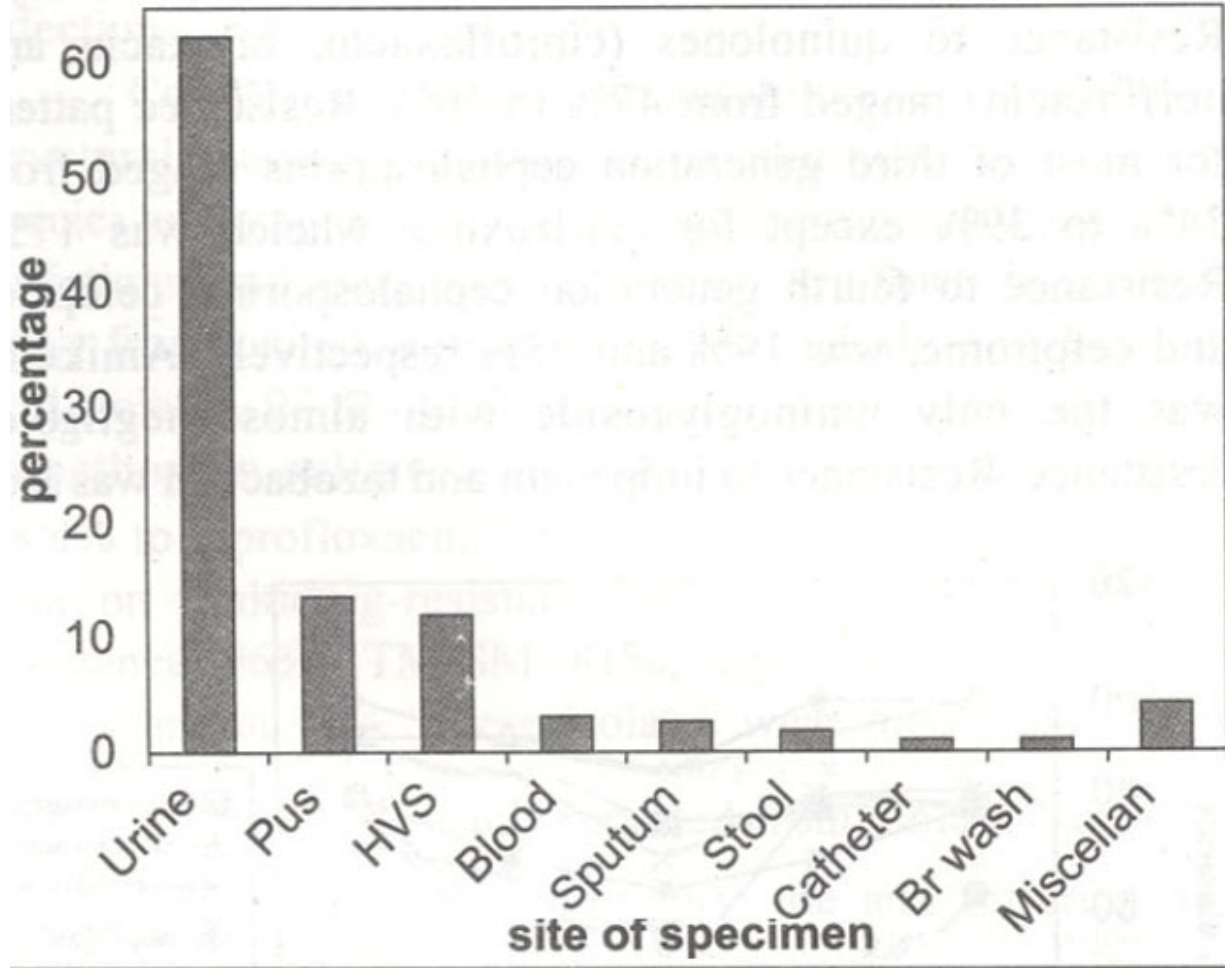


Figure 1: Distribution of sites of E. coli isolates in percent.

There were 227 (60%) females and 151 (40%) males in the study population. Mean age was 46.5 (SD22.3) years. The commonest source of isolation was urine followed by pus from different sites and high vaginal smears (HVS).

#### **Susceptibility Patterns**

Table 1 represents susceptibility to various antibiotics tested.

**Table 1. Overall sensitivity pattern of E. coli isolates.**

Antibiotic	Resistant		Sensitive	
	n	%	n	%
Ampicillin	268	76	84	24
SM-TM*	267	72	106	28
ciprofloxacin	186	49	190	51
ofloxacin	177	47	195	52
Norfloxacin	120	50	119	50
Nitrofurantoin	18	8	213	92
Cefaclor	126	38	200	61
Cefpodoxime	124	38	199	62
Ceftriaxone	127	34	247	66
Cefotaxime	135	36	237	64
Cefixime	126	39	197	61
Ceftizoxime	64	17	307	83
Cefpirome	93	25	282	75
Cefepime	68	19	289	80
Amikacin	3	1	351	99
Gentamicin	141	37	236	63
Tobramicin	118	31	250	67
Imipenem	1	0.3	370	99.7
Aztreonam	86	25	258	74
Tazobactam	2	1	372	99

\*= trimethoprim/sulfamethoxazole

Majority of isolates were resistant to ampicillin and SM-TM (76% and 72% respectively). Resistance to quinolones (ciprofloxacin, ofloxacin and norfloxacin) ranged from 47% to 50%. Resistance pattern for most of third generation cephalosporins ranged from 34% to 39% except for ceftizoxime which was 17%.

Resistance to fourth generation cephalosporins, cefepime and cefpirome, was 19% and 25% respectively. Amikacin was the only aminoglycoside with almost negligible resistance.

Resistance to imipenem and tazobactam was also non-existent, while 25% isolates were found resistant to aztreonam.

Two isolates showed intermediate resistance to Susceptibility of Urinary E. coli isolates only Susceptibility of Urinary E. coli isolates only cefaclor, six to cefepime, seven to tobramycin, five to aztreonam and one each to ceftriaxone, cefotaxime, cefixime and cefpirome.

Urinary E. coli isolates were studied and separated according to different age groups (table 2).

**Table 2. % resistance in urinary E. coli isolates.**

Age (years)	Number/sex	Ampi cillin	TM-SM*	Cipro+	Norflo xacin	Nitrofu rantoin	Cefpodo xime	Ceftri axone	Cefpi rome	Genta micin	Aztreo- nam
1-20	12M, 13F	86	76	44	42	14	27	28	24	40	32
21-40	11M, 38F	66	69	25	24	2	17	8	6	20	9
41-60	21M, 35F	70	64	41	47	4	26	24	15	18	15
61-80	27M, 42F	77	82	74	72	12	58	49	38	55	39
>81	7M, 11F	93	94	66	72	12	62	53	50	67	50
Overall	78M, 139F	78	77	50	51	11	38	32	27	40	27

\*= trimethoprim/sulfamethoxazole

+ = ciprofloxacin

When divided for age groups, most E. coli resistance was seen in older population (age more than 61 years). The highest resistance was seen to ampicillin (93%) and TM-SM (94%) in age group more than 81 years. Similar pattern was seen with quinolones, cephalosporins and aminoglycosides. Imipenem, tazobactam and amikacin were again sensitive to almost all isolates. Nitrofurantoin showed low-level resistance at all ages ranging from 2% (age 21 to 40 years) to 14% (age 1 to 20 years). Another finding was a relatively high resistance to most antibiotics in age group 1 to 20 years.

#### **Susceptibility patterns of multidrug-resistant isolates**

Forty five percent E. coli isolates were found to be multidrug resistant.

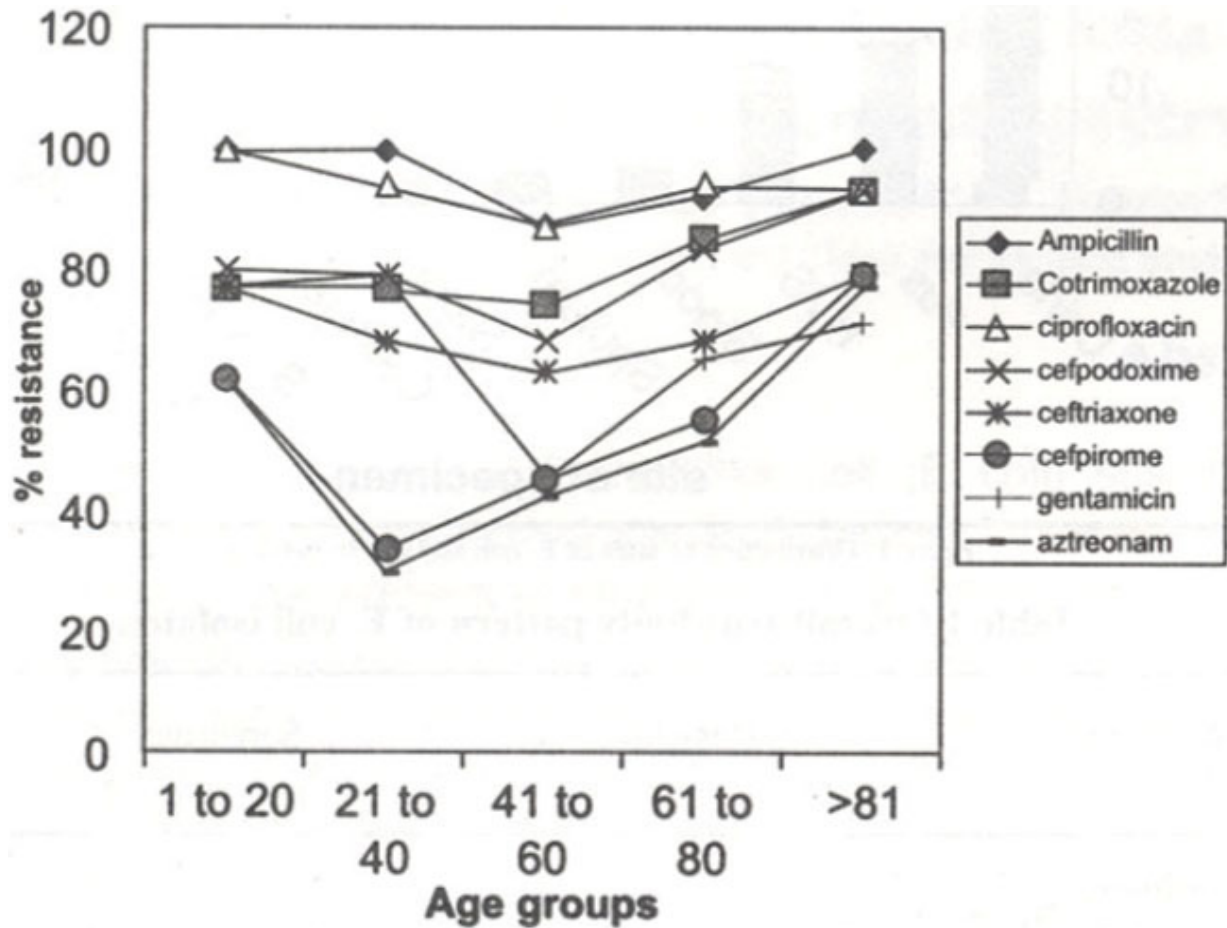


Figure 2: Percent resistance of multidrug-resistant *E. coli* isolates to various antibiotics.

Figure 2 depicts resistance patterns of multidrug resistant isolates in different age groups. Among these, most of them were resistant to ampicillin (87% to 100%), TM-SM (74% to 93%) and ciprofloxacin (87% to 100%). Maximum resistance to these multi resistant isolates was seen at extremes of age, pattern consistent with urinary isolates.

**ESBL phenotype**

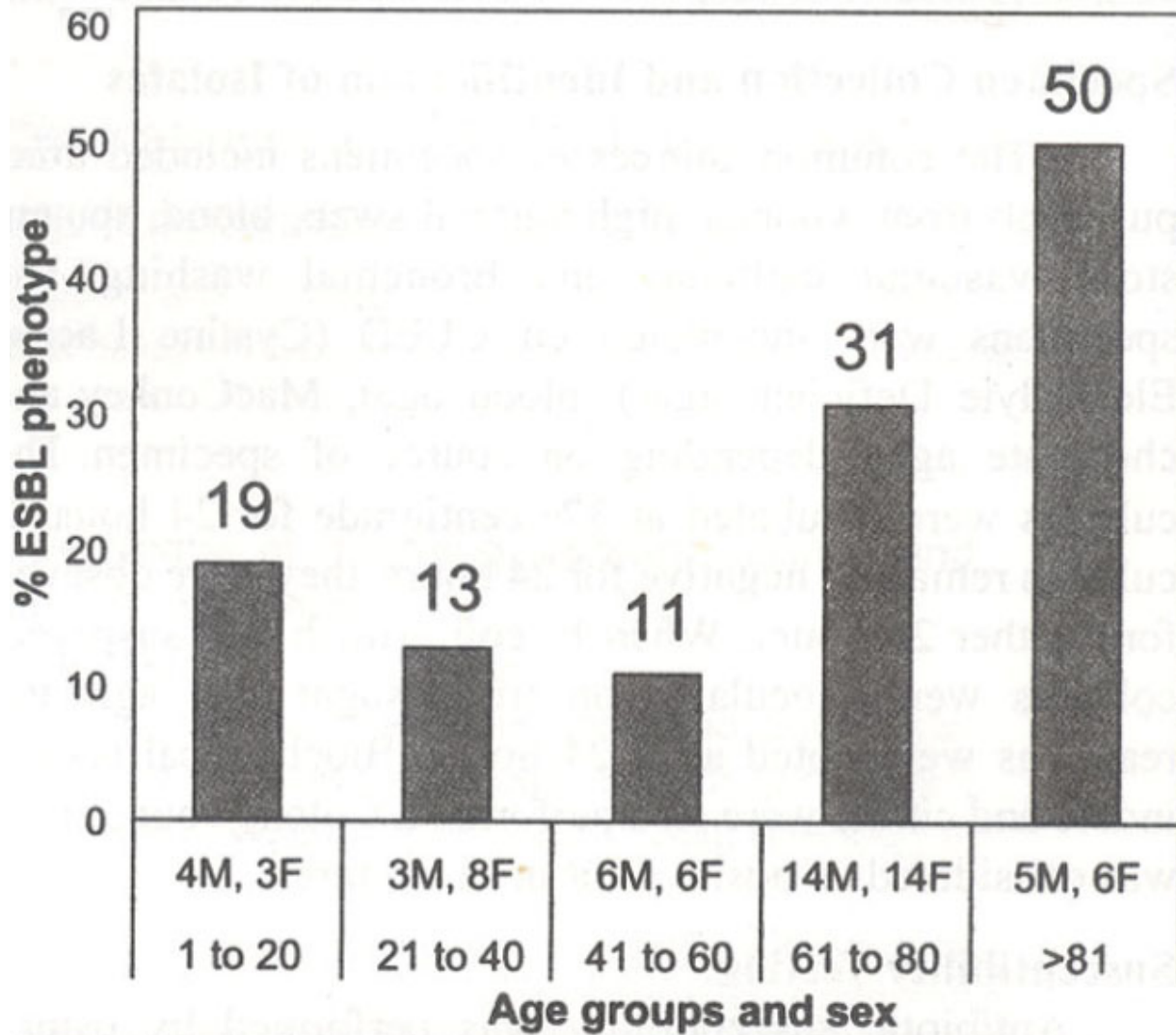


Figure 3: Frequency of Extended spectrum beta lactamases (ESBL) in different age groups.

Figure 3 shows percentage of such isolates in different age groups along with sex distribution. Over all 25% isolates were resistant to both cefpodoxime and aztreonam, suggesting ESBL production. Highest degree of ESBL production was observed in age group over 61 years of age.

## Discussion

This retrospective data revealed high degree of *E. coli* resistance to various antibiotics. Similar results were seen for urinary *E. coli* isolates. The data regarding urinary *E. coli* resistance to ampicillin is consistent with other studies reported from Pakistan (78.4% in the current study), showing high degree of resistance to *E. coli* ranging from 58% in 1989 to 74% in 2001<sup>12,14</sup>. Resistance to TM-SM was very high in our study (77%). Previously reported studies showed 60% TM-SM resistance in one study<sup>13</sup> and more recently 25% from a Medical University<sup>12</sup>. Resistance to quinolones was comparable to the study by Khan and Ahmed (50% vs 46%)<sup>12</sup>, which is much higher than reported by Farooqi et al (25% in 1997)<sup>13</sup>. Gentamicin and Tobramycin resistance has also increased as compared to other studies (Gentamicin 1% in 1989 vs 40% in our study)<sup>17</sup> but Amikacin has remained sensitive so far. Resistance to third generation

cephalosporins was also much higher in our study, which was negligible in 1989<sup>17</sup>. An interesting finding was escalating resistance to fourth generation cephalosporins, which has not been previously reported from Pakistan, suggesting cross-resistance with third generation cephalosporins.

Results of the current study are even more alarming when compared with international data. In 1994 (Dutch study), resistance rates to TM-SM among uropathogens associated with acute uncomplicated pyelonephritis were generally less than 10% at investigative sites<sup>7</sup>. However, increasing rates of TM-SM resistance have been reported among urinary *E. coli* isolates<sup>6,9</sup>. In another study, resistance to quinolones was virtually non-existent but 6% *E. coli* strains were resistant to trimethoprim /sulfamethoxazole. In 1996, a survey from United States has found a resistance rate of approximately 17% to trimethoprim /sulfamethoxazole among more than 42,500 urinary isolates of *E. coli*<sup>2</sup>. A recent study of 378 women with acute uncomplicated pyelonephritis reported that about 18% of *E. coli* strains were resistant to trimethoprim /sulfamethoxazole, compared with none for ciprofloxacin<sup>2</sup>. Compared to these data resistance reported from Pakistan is extremely high making trimethoprim/sulfamethoxazole virtually a useless first line therapy in urinary tract infections.

Concurrent resistance to antimicrobials of different structural classes has arisen in a multitude of bacterial species and may complicate the therapeutic management of infections, including those of the urinary tract. In a recent study from United States, among the multidrug-resistant *E. coli* isolates, 97.8% were resistant to ampicillin, 92.8% to trimethoprim-sulfamethoxazole, 86.6% to cephalothin, 38.8% to ciprofloxacin, and 7.7% to nitrofurantoin<sup>18</sup>. Our data on multidrug-resistant isolates revealed, ampicillin resistance 96%, TM-SM 81%, ciprofloxacin 94% and nitrofurantoin 11%. These isolates were more common in females than males (27% vs 22%), which is contrary to what was seen in the study reported from United States.

When separated for urinary site and different age groups, interestingly, *E. coli* resistance was much higher in younger (to 20 years) and older population (>61 years). In previous studies from Pakistan, susceptibility patterns were not characterized according to age, so no local data is available for comparison. However, in a study reported from Spain, age more than 64 was found to be a risk factor, along with other factors, for higher antibiotic resistance against *E. coli*<sup>10</sup>. Though we did not look at the reasons for such high resistance in these two populations but sex, previous antibiotic treatment, nosocomial vs community acquired infections, urinary catheterization and urinary tract abnormalities may contribute to this resistance but further studies are required to look at these variables acting as independent risk factors.

Many bacteria including *E. coli* produce beta Lactamases. Beta Lactamases are classified according to their amino-acid sequences and by their functional characteristics<sup>19</sup>. Plasmid mediated enzyme TEM plays an important role in the generation of ESBL variants in *E. coli*. Even minor amino-acid substitutions in TEM can dramatically alter their ability to confer resistance to betalactam antibiotics<sup>19</sup>. Increased incidence of ESBL phenotype was also seen in our study, which might be one of the reasons for increasing beta lactam resistance in Pakistan. Though cefpodoxime and aztreonam resistance may not be a very specific indicator of ESBL phenotype, but it is very much sensitive and has been used extensively as a screening tool<sup>14-16-20</sup>. ESBL strains show high level of co-resistance to aminoglycosides, tetracycline, TM-SM, and ciprofloxacin<sup>20</sup>. In a study reported from Hong Kong 11% *E. coli* and 13% *Klebsiella* spp. were found to produce ESBL<sup>21</sup>. In our study 25% of the isolates were identified as ESBL phenotype. Percentage resistance seen in beta-lactam antibiotics among ESBL phenotypes was: ceftriaxone (96%), cefotaxime (97%), ceftiofime (77%), cefepime (77%). ESBL strains also showed high

levels of coresistance to aminoglycosides, TM-SM, and ciprofloxacin. Imipenem remains highly effective against ESBL strains. Increased frequency of ESBL phenotype in our study, compared with above data, is extremely disturbing and may contribute to treatment failures and longer hospital stay. Further studies are needed to confirm ESBL production by detecting type of TEM mutation involved in its overproduction.

An important fact in our study is quinolone resistance, which is much higher than reported in contemporary literature. Mechanism of quinolone production may include; reduced drug permeation and altered regulation of active efflux mechanisms (which also confers resistance to other classes of antibacterials including beta-lactams, imipenem, and tetracycline)<sup>22</sup>. Emergence of resistance while the patient is receiving treatment seems to occur more frequently with the quinolones (about 4%) than with the cephalosporins (about 2%)<sup>22</sup>. Although a relationship between the emergence of quinolone resistant *E. coli* and the extensive use of quinolones has been shown<sup>23</sup>, however, we did not study this aspect and further studies would be needed to sort this. In conclusion, increasing *B. coli* resistance to various antibiotics needs continued local and national surveillance. Antimicrobial-resistance patterns are essential to provide optimal care for patients with urinary as well as other *E. coli* infections in an era of increasing antimicrobial resistance. Though *In vitro* resistance correlates with achievable serum antimicrobial levels, it may not predict clinical outcome in urinary tract infections as urine antimicrobial levels are many times greater than serum levels for most antimicrobial agents. But such information can still be used for proper selection of empiric treatment and actually may decrease resistance to various antibiotics as inappropriate antibiotic therapy unnecessarily extends morbidity and is never cost effective.

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