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Prevalence of Multiple Drug Resistant *Escherichia coli* in Patients of Urinary Tract Infection Registering at a Diagnostic Laboratory in Lahore, Pakistan

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Abstract.- The prevalence and antibiotic susceptibility pattern of *E. coli* was analyzed in urine samples of patients with acute and recurrent urinary tract infection visiting a diagnostic laboratory in Lahore. The prevalence of MDR strains of *E. coli* among the isolates was also determined. A total of 310 isolates (73.1%) of *E. coli* were obtained from 424 positive culture isolates. The highest percentage of the isolates was present in the age group of 51-75 years in both males and females (30.9% and 9.6% respectively). Females were more frequently affected (77.4%) as compared to males (22.5%, $P < 0.02$). *E. coli* showed highest sensitivity towards Imipenem (100%), Meropenem (99.3%), Piperacillin/Tazobactum (96.8%), Amikacin (89.7%), Cefoperazone/Sulbactum (86.8%), Ceftazidime (77.2%), Gentamicin (73.2%), and Aztreonam (67.1%). 65.5% of isolates were resistant to more than 8 drugs, belonging to 3 or more than 3 different classes of drugs.

Key Words: Urinary tract infection, multi-drug resistant, *Escherichia coli.*

INTRODUCTION

Urinary tract infection (UTI) has become the most common disease encountered in clinical practice (Gobernado *et al*., 2007) with an estimated 150 million UTI's occurring annually. In the United States alone it is responsible for 8 million physician visits and approximately 100,000 admissions to the hospital per year (Memon, 2007). Although UTI's are encountered in both sexes and all ages, but it is commoner in females. Out of every two female one has suffered from a urinary infection at least once in her life: 12% with an initial infection and 48% with a repeat episode (Hummers-Pradier and Kochen, 2002).

Although the etiology of UTI is diverse encompassing both Gram positive and negative organisms (Memon, 2007), *E. coli* remains the leading cause in 75% to 90% of the cases (Gupta *et al*., 2001). In those patients who are hospitalized with a complicated UTI, those with history of indwelling catheters and/or previous antibiotic therapy, 40% of the isolates are *E. coli* (Gobernado *et al*., 2007).

Antibiotics are the main weapon against infections (Erb *et al*., 2007). All the pathogenic bacteria are developing resistance to the commonly prescribed antibiotics. This problem is more marked in uropathogens especially *E. coli* causing difficulties in treatment. Community strains of *E. coli* are gradually showing increased resistance towards commonly used drugs like ampicillin/ amoxicillin (60%) and co-trimoxazole (10-30%) (French, 2005).

As resistance to these drugs developed flouroquinolones emerged as the alternative therapy. Currently the majority of urinary isolates of *E. coli* remain susceptible although resistance is on the rise since first reported in 1990s (Sabir *et al*., 2004). As the problem of antibiotic resistance increases, the number of drugs available for cure decreases.

The aim of the present study was to determine the prevalence of MDR *E. coli* in patients of UTI with both acute and recurrent visiting Chughtai's Medical Laboratory, Lahore.

MATERIALS AND METHODS

Acquisition of samples

A total of 1000 clean catch midstream urine samples were collected from patients coming to

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Chaughtai's Medical laboratory (Lahore, Pakistan) during the period of Nov, 2007 to July, 2008. The samples were collected in sterile plastic bottles and the bacteria from the samples were immediately processed for culture and antibiotic susceptibility testing. Further studies were conducted on 310 *E. coli* strains isolated from these urine samples. Microscopic testing of the samples was also done and the presence of pus cells were noted.

Patient history

Patient history was taken and the samples were collected from only those patients who had complaints of acute uncomplicated UTI as well as those that had a history of recurrent UTI. A history of usage of antimicrobials prescribed for UTI was also noted such as cephradine, ciprofloxacin, trimethoprim, sulfamethoxazole, norfloxacin. The history of dosage and duration of these antimicrobials was also taken, in case of cephradine for acute uncomplicated UTI 250mg every12 hours for 3-7 days was taken and for recurrent UTI 500mg every 6 hours for 7-14 days was taken. For ciprofloxacin in cases of acute uncomplicated UTI 250mg every 12 hours for 3-7 days and in case of recurrent UTI 250mg every 12 hours for 7-14 days was taken. For trimethoprim the dosage for acute uncomplicated UTI was 100mg every 12 hours for 10 days and for recurrent cases 100mg every 12 hours for 7-14 days. For sulphamethoxazole/ trimethoprim combination the dosage for acute uncomplicated UTI was 800/160mg every 12 hours for 5-7 days and for recurrent cases 800/160mg every 12 hours for 7-14 days. For norfloxacin the dosage taken for acute uncomplicated UTI cases caused by *E. coli, K. pneumoniae* or *P. mirabilis* was 400mg every 12 hours for 3 days, in cases of acute uncomplicated infections caused by organisms other than above the dosage was 400mg every 12 hours for 7-10 days and in cases of recurrent UTI 400mg every 12 hours for 10-21 days was taken. The patients had received the antimicrobial treatment at least 6months prior to urine sample collection.

Isolation and identification of isolates

The samples were inoculated on cysteine-lactose-electrolyte-deficient (CLED) media (Oxoid, England) and incubated at 37°C for 24 hours. A sterile calibrated wire loop procedure was performed for colony counts and 0.01ml of loopful of urine was used. The growth as per Kass count (more than 10^5 organism single species count per ml of urine) was considered significant (Kass, 1956). Colonies were characterized biochemically using the recommended guide lines of Barrow and Feltham (1993) and later on through Gram staining (Gram, 1884). Pure strains were obtained by sub-culturing on MacConkey agar (Oxoid, England) and incubating at 37°C for 24 hours. The colonies were again Gram stained to confirm the presence of Gram negative rods. API 20 E standardized identification system (Biomerieux, France) was used to confirm the identification of the isolates as *E. coli*.

Antibiotic susceptibility testing

Antibiotic susceptibility pattern of *E. coli* was established using Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). The 310 *E. coli* isolates were tested for their sensitivity against 23 different antibiotics (Oxoid, England) that are routinely administered in cases of infections in the city *viz.*, amikacin (30µg), amoxycillin (25µg), amoxycillin/ clavulanic acid (30µg), ampicillin (25µg), aztreonam (30µg), cefaclor (30µg), cefoperazone/ sulbactum (105µg), ceftazidime (30µg), ceftriaxone (30µg), cephalexin (30µg), cephradine (30µg), ciprofloxacin (5µg), doxycycline hydrochloride (30µg), enoxacin (10µg), gentamicin (10µg), imipenem (10µg), meropenem (10µg), nalidixic acid (30µg), norfloxacin (10µg), ofloxacin (5µg), pipemidic acid (20µg), piperacillin/tazobactum (110µg) and sulphamethoxazole/trimethiprim (25µg). Mueller Hinton agar (Oxoid, England) was used for sensitivity testing. The plates were incubated at 37 °C for 24 hours. After incubation was completed, zones of inhibition were measured for each antibiotic and were compared with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2004) for sensitivity and resistance. *E. coli* ATCC 25922 was the reference strain used according to CLSI protocols.

MDR was defined as resistance to more than 8 antimicrobials (belonging to 3 or < 3 different classes) among the antimicrobials tested in this study.

Statistical analysis

The data was expressed in percentages. Pearson's Chi-square test was applied and a value of $P < 0.05$ was considered as significant. Analysis was done using statistical package SPSS version 16.0 (SPSS, USA).

RESULTS

Culture positivity

For the 1000 urine samples, 424 samples were positive for the isolation of organisms, out of which the culture positivity results revealed 310 (73%) samples to be positive for *E. coli*, 114 (27%) samples containing organisms other than *E. coli* whereas 576 (57.6%) samples gave a negative result after culturing. Table I shows presence of uropathogen other than *E. coli viz.*, *Acinetobacter* spp. (12%) and *Klebsiella* spp. (7%). *Candida* spp., *Staphylococcus* spp., and *Citrobacter* spp. were also isolated but relatively fewer in number. The number of recurrent UTI were quite significant *i.e.*, 235 cases (75.8%) as compared to acute cases that were only 75 (24.1%).

Table I.- Type of Uropathogens isolated from UTI patients (+N= 424)

Organism isolated	No. of isolates	Percentage (%)
Escherichia coli	310	73.1
Acinetobacter spp.	50	11.7
Klebsiella spp.	28	6.6
Candida spp.	22	5.1
Staphylococcus spp.	7	1.6
Citrobacter spp.	7	1.6

+N, total number of positive isolates

Prevalence of E. coli

Out of the 1000 urine samples, 310 were found positive for *E. coli*. The highest isolation rate in females (n = 96, 31%) were from the age group of 51-75 years. This was followed by the age group of 26-50 years (n = 78, 25%). Only (n = 50, 16%) of the isolates fell in the age group of 0-25 years. The lowest isolation rate (5%) was from the age group of 76-100 years (n = 16) (Table II).

In males the highest isolation rate (10% n = 30) was also from the age group of 51-75 years. This was followed by the age group of 26-50 years (n = 21, 7%). Only 4% of the isolates fell in the age group of 0-25 years (n = 12). The lowest isolation rate (2%) was from the age group of 76-100 years (n = 7) (Table III). UTI was commoner in females (77.4%) as compared to males (22.5%) ($\chi^2 = 4.8$, $P < 0.02$).

Table II.- Age wise distribution of Multidrug resistant (MDR) *E. coli* strains in females with urinary tract infection (+n=310)

-Age group(s)	Females	No. of *MDR *E. coli* isolates	Percentage of patient(s)
0-25	50	29	16.1
26-50	78	49	25.1
51-75	96	64	30.9
76-100	16	10	5.1

+N, total number of *E. coli* isolates.
-Ages were taken from 0-100 years.
*Isolates resistant to more than 8 antimicrobials (belonging to 3 or < 3 different classes of antimicrobials) were considered as MDR

Table III.- Age wise distribution of Multidrug resistant (MDR) *E .coli* strains in males with urinary tract infection (+n=310)

-Age group(s)	Males	No. of *MDR *E. coli* isolates	Percentage of patient(s)
0-25	12	08	3.8
26-50	21	12	6.7
51-75	30	22	9.6
76-100	07	06	2.2

+N, total number of *E. coli* isolates.
-Ages were taken from 0-100 years.
*Isolates resistant to more than 8 antimicrobials (belonging to 3 or < 3 different classes of antimicrobials) were considered as MDR

Antibiotic susceptibility pattern

The antibiotic susceptibility pattern of urinary tract *E. coli* isolates was checked against 23 different antibiotics normally prescribed in cases of infections in area of Lahore, Pakistan. The concentrations of antibiotics in the disks were according to the MIC of the *E. coli* ATCC 25922 reference strain. Detailed analysis of susceptibility pattern of *E. coli* strains showed 99%, 100% and 67% sensitivity to β- lactam antibiotics

(Meropenem, Imipenem and Aztreonam respectively), 97% to Penicillin+ β-Lactamase antibiotics (Piperacillin/Tazobactum), and 90% to Aminoglycoside antibiotics (Amikacin). Towards Cephalosporins the sensitivity was Cefoperazone/Sulbactum 87%, Gentamicin 73%, Ceftazidime 72%. Rest of the *E. coli* strains showed a susceptibility pattern in the range of 49% to 6% (Fig. 1).

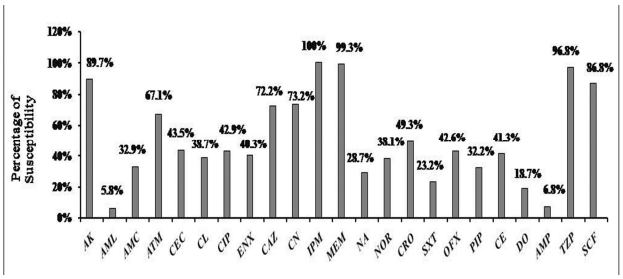

Fig 1. Antibiotic susceptibility pattern of urinary tract isolates of *E. coli* against commonly used antibiotics

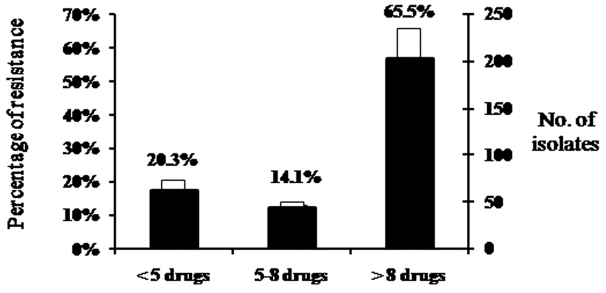

Fig 2. Multidrug resistance in urinary tract isolates of *E. coli*

Multidrug resistant (MDR) E. coli

For the study the *E coli* isolates resistance to more than 8 antimicrobials (belonging to 3 or < 3 different classes of antimicrobials) were referred to as MDR *E. coli*. Hence of the 310 isolates tested, 65.5% (203) were resistant to more than 8 drugs whereas 14.1% (44) isolates were resistant to 5 to 8 drugs. 20.3% (63) were resistant to less than 5 drugs (Fig. 2). The MDR *E. coli* obtained in acute and recurrent cases was also noteworthy, in acute cases only 35 and in recurrent cases 123 isolates were obtained.

DISCUSSION

In the present study the prevalence of *E. coli* was 73% and organisms other than *E. coli* were only 27%. Mortazavi and Shahin (2009) have reported 63% prevalence of *E. coli* in Iran; 88% prevalence has been reported in Bangladesh (Lina *et al*., 2007). Of these 310 *E. coli* strains in the present study the highest percentage in both females and males samples was in the age group of 51-75 years. A study done in Khyber Pakhtunkhwa and Punjab, Pakistan showed comparable results with percentage of urinary tract infection prevailing also found to be higher in the age group of 41years and above (Ramzan *et al*., 2004). In western Nepal, the highest percentage of patients in the population was present in the age group of 41-60 years (Das *et al.*, 2006). Both these studies show results consistent with ours that might refer to a number of factors including the fact that majority are recurrent cases. It also suggests the facts that the female patients in this age group are in the postmenopausal condition. The study conducted by Raz *et al.* (2000) concluded that the factors that highly influence the occurrence of recurrent UTI among postmenopausal women include nonsecretor status, a history of UTIs, along with other inherited inclination, as well as urodynamic factors, especially incontinence, presence of a cystocele and residual urine volume. However in the age group of 26-50 years there was a considerably less isolation rate *i.e.*, 25% in females although especially in female patients in Lahore area this is the age involving frequent coitus, pregnancy, and antibiotic usage as well as spermicide exposure which also plays a key role in acute as well as recurrent UTI.

The susceptibility pattern for *E. coli* strains was checked to determine the effectiveness of prescribed drugs for treatment of infections in Lahore. The results obtained are similar to those observed in a study done in Turkey where imipenem and meropenem were the most effective antibiotics with 100% sensitivity for *E. coli* (Savas *et al*., 2006). Our study further revealed that 90% of *E. coli* isolates were sensitive to amikacin. A study done in Nepal showed a similar susceptibility pattern (87%) for amikacin (Das *et al*., 2006). Amikacin is an aminoglycoside antibiotic which can

only be administered through intravenous injections. Since this drug is administered in cases of severe infections only, perhaps the reason behind its not so frequent usage has made *E. coli* more sensitive against it as compared to cefoperazone/sulbactum (86.8%) a cephalosporin antibiotic, gentamicin (73.2%) an aminoglycoside antibiotic, ceftazidime (72.2%) another cephalosporin antibiotic, and aztreonam (67.1%) a β-Lactam antibiotic that are frequently prescribed.

Emergence of multidrug resistant organism poses a great public health and therapeutic challenge to clinicians all over the world. Commonly encountered Gram negative bacteria have the potential to acquire cross resistance to several antimicrobial agents leading to treatment failures. In our study 65.5% of the isolates showed resistance to more than 8 drugs (belonging to 3 or < 3 different classes of drugs) (Fig. 2). These results are comparable to those found in a study done in Sudan by Ahmed *et al*. (2000). Also 20.3% of the isolates from our study showed resistance to less than 5 drugs. Moniri *et al* from the neighboring country Iran also reports many of the isolates to be resistant to 3 antimicrobials (Moniri *et al*., 2003). However in a study by Saeed *et al*. (2009) in Karachi, Pakistan 92% of Gram negative clinical isolates including species of *E. coli* were resistant to one or more antibiotics (Saeed *et al*., 2009). Similarly Al-Mardeni *et al*. (2009) in Jordan reports 59.9% of the *E. coli* isolates to be resistant to three or more antimicrobials. Mathai *et al*. (2008) in a study in Tamil Nadu, India reports 42% of commensal isolates of *E. coli* to be resistant to one or more drugs. Another study done in Saudi Arabia by Al-Tawafiq (2006) reports 2-29% *E. coli* isolates to be resistant to more than two antibiotics. All these results from countries neighboring to Pakistan as well as those countries that are far off report more or less the same results. The surprising finding in this study is that MDR *E. coli* were most resistant to β-Lactam antibiotics with amoxycillin, clavulanic acid, aztreonam and ampicillin being the most ineffective whereas the most effective antibiotics in the study also belonged to the same class. Cephalosporins and flouroquinolone being the second class of antibiotic against which the MDR *E. coli* was most resistant. Narchi and Al-Hamdani in a recent study in our neighboring state of United Arab Emirates also reported cephalosporins and co-trimoxazole to be the most common antibiotics to be related to multidrug resistance (Narchi and Al-Hamdani, 2010).

Keeping in mind that our isolates were from UTI patients with a majority cases having a history of recurrent UTI (235, 75.8%) and a low number of acute cases (75, 24.1%) such multidrug resistance may provide new insight. It suggests the fact that the patients with a history of recurrent UTI harbor *E. coli* isolates (123) that are resistant to less active drugs such as cephradine, ciprofloxacin, trimethiprim, sulfamethoxazole, norfloxacin which are prescribed routinely for UTI treatment.

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