ORIGINAL ARTICLE

# Antimicrobial Susceptibility Pattern of Urinary Bacterial Isolates – Local experience at Rawalpindi, Pakistan

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## Abstract

**Background**
Bacteria are the most common cause of urinary tract infections in all age groups. Drug resistance is rising in uropathogens worldwide, highlighting the importance of local antibiotic susceptibility patterns. We have aimed to determine the antimicrobial susceptibility pattern of uropathogens isolates among in and outdoor patients referred to CITI Lab (private sector) Rawalpindi.

**Methods**
A retrospective review of lab records of 3802 urine samples processed from Jan. 2010 to Dec. 2010 was reviewed. Urine culture was performed using conventional microbiological techniques. Biochemical testing was used to identify the organisms and antibiotic sensitivity was done by the modified Kirby Bauer method as per Clinical Laboratory Standards Institute recommendations.

**Results**
Out of total 3802 urine samples 2244 (59%) were culture negative, whereas significant isolates were obtained in 1368 (36%) samples and mixed growth (more than two organisms) was obtained in 190 (5%). Out of these significant isolates, 123 (9%) were *Candida* species and 1245 (91%) were bacterial isolates. *Escherichia coli* (70%) was the most common isolate followed by *Klebsiella pneumoniae* (15%). Imipenem/ meropenem, amikacin, cefoperazon-sulbactam, pipracillin-tazobactam and nitrofurontoin were sensitive to more than 85% of isolates while fluroquinolones, amoxi-clave, cotrimaxazole and ampicillin were sensitive to less than 40% of isolates.

**Conclusion**
More than 60% of uropathogens are resistant to conventional oral antibiotics like fluroquinolones and cotrimaxazole limiting to injectable antibiotics. Nitrofurontoin is the only oral antibiotic left which is affective.

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**Key words**
Antibiotics, Drug resistance, Urinary tract infections, Uropathogens

## Introduction

Clinical infection of urinary tract is said to exist when a significant number of microorganisms, usually greater than $10^5$ CFU/mL of urine, are detected in properly collected mid-stream/clean catch urine.[1] Urinary tract infections (UTIs) are amongst the most common infections in hospitalized patients.[2] It is the second most common infections in community settings. About 150 million people are diagnosed with UTI each year worldwide and it cost the global economy in excess of six billion US dollars.[3] Urinary tract infection can be divided into complicated and uncomplicated infections for the purpose of treatment.[4] Empirical antimicrobial therapy is given to reduce the incidence of postoperative infections including UTI, to prevent development of sepsis, to reduce duration of hospital stay and cost of patient care.[5] There is rising trend of multidrug resistance for the common antibiotics recently all around the globe. Due to this rise in antibiotic resistance among uropathogens, it is important to have local antibiograms. This information would be relevant not only for the local hospitals but would also be a vital for regional database. In view of this, a retrospective analysis was carried out to determine the frequency and resistance pattern of uropathogens in patients of Rawalpindi/Islamabad region referred to Citi lab Rawalpind, Pakistan.

## Material and Methods

This laboratory based retrospective descriptive cross sectional study was carried out from Jan. 2010 to Dec. 2010 in Microbiology department, Citi Lab Rawalpindi, Pakistan. Citi lab is a private lab in which samples from different government and private hospitals and clinics are submitted. Mid stream clean catch urine samples were collected in sterile containers. Non probability consecutive sampling was done. All age groups of both genders who have suspicion of UTI referred to Citi lab were included in the study. Repeated samples from the same patients were excluded.

Bacterial concentration of $\geq 10^5$ CFU/mL was considered as significant obtained after incubating 0.2 ul of urine on cystein lactose electrolyte deficient agar (Oxoid UK) using semi-

quantitative strip method (MAST bacteruritest).[6] These plates were incubated aerobically at 37 °C for 24-48 hours. The colony count was expressed in colony-forming units per mL of urine. Isolations and identifications were performed using biochemical tests. After biochemical identification anti-microbial sensitivity testing was done for the isolates using modified Kirby Bauer disk diffusion methods on Mueller Hinton agar.

Antimicrobial sensitivity was interpreted as per the clinical laboratory standard institute (CLSI) guidelines.[7] The production of ESBL enzyme among gram negative rods was detected employing double disc synergy (DDS) test using 30µg discs of aztreonam, ceftazidime and 10µg cefpodoxime (Oxoid, Basingstoke, UK) placed 15 mm (edge to edge) from amoxycillin-clavulanate; (20/10µg) disc. Inoculated plates were incubated overnight at 35 ± 2 °C. A zone of enhancement between amoxycillin-clavulanate and any one of the above (aztreonam, ceftazidime and cefpodoxime) for an organism was considered as ESBL producer.[8]

*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as control strains. Data was entered in SPSS-15 for statistical evaluation. Descriptive statistics was applied to calculate mean, standard deviation for age, percentages for different variables like gender and antibiograms.

**Results**

A total of 3802 samples were included in the study. Age range of patients was between 1 and 92 years with a mean of 35.28 years. Most (87%) isolates were from females. Out of total 3802 urine samples 2244 (59%) were culture negative, whereas significant isolates were obtained in 1368 (36%) samples and mixed growth (more than two organisms) was obtained in 190 (5%). Out of these significant isolates, 123 (9%) were *Candida* species and 1245 (91%) were bacterial isolates. *Escherichia coli* 871(70%) was the most common bacterial isolate followed by *Klebsiella pneumonia* 187(15%), *Enterococcus species 100*(8%), *Pseudomonas aeruginosa* 37(3%), *Staphylococcus aureus* 19(1.5%) and others are 31(2.5%) as shown in figure below. The others include *Enterobacter cloacae*, *Citrobacter frendii*, *Proteus mirabilis*, *Morganella morganii* and *Providencia* species. Susceptibility pattern of the isolates is shown in Table 1 and 2.

Among gram negative organisms, extended spectrum β-lactamase (ESBL) production was detected as 41% in *Escherichia coli* and 44% in *Klebsiella pneumoniae*. In Gram positive organisms, three vancomycin resistant enterococci and three methicillin resistant *Staphylococcus saprophyticus* were detected but no MRSA was detected.

**Discussion**

We found gram negative pathogens to be the predominant cause

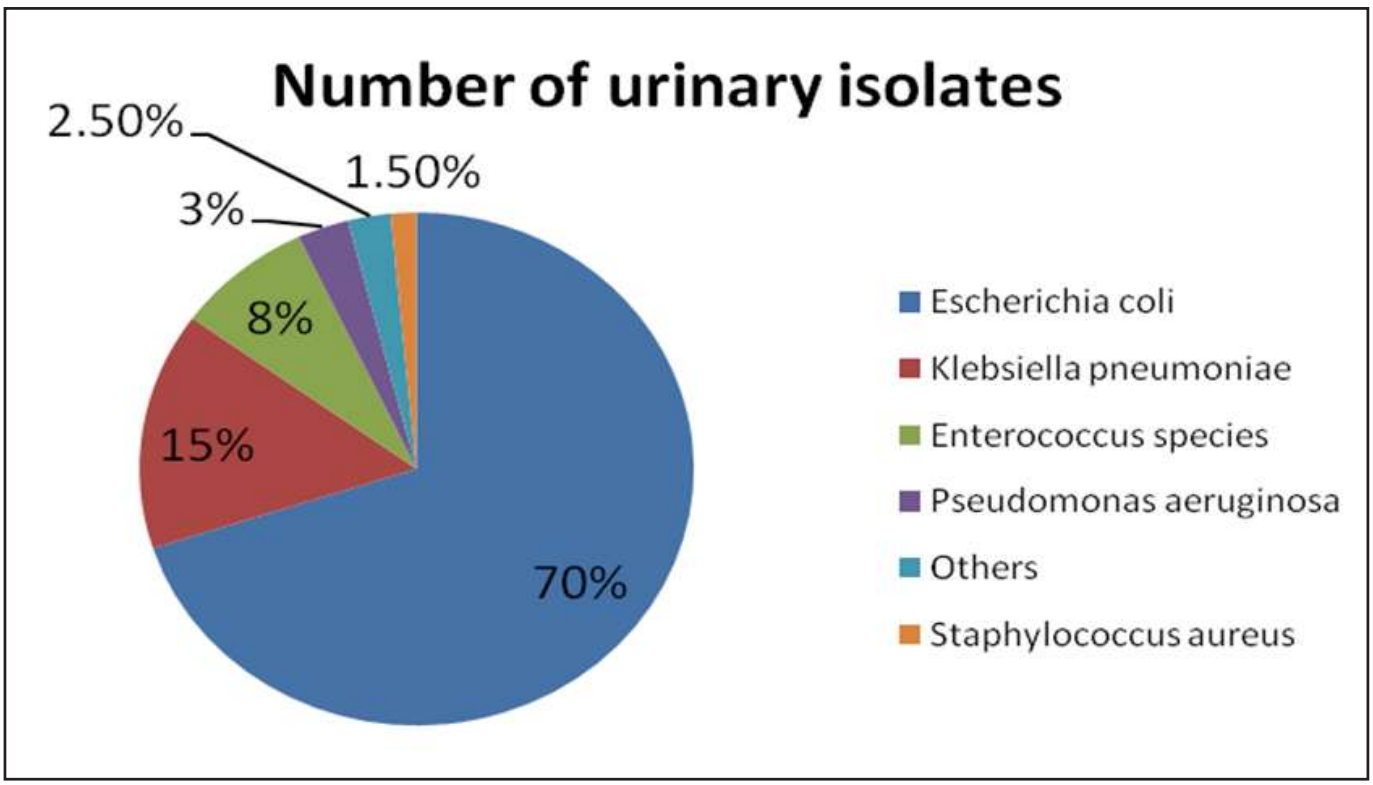


**Fig. 1 Spectrum of urinary pathogens isolated**

of UTI and almost 40% of them were resistant to the commonly used antibiotic, ciprofloxacin. In our community, bacterial urinary tract infection is one of the common causes for seeking medical advice. Isolation of bacteria by appropriate culture methods is one of the diagnostic tools in UTI.[9] In our study we found that UTI was most common in females, which is similar to reports published elsewhere.[10]

*Escherichia coli* were the predominant bacterial pathogens followed by *Klebsiella pneumonia* similar to the reports from other regional and local studies.[2,5,9,10,11] In Gram positive organisms *Enterococcus* species was most common followed by *Staphylococcus aureus*.

**Table 1: Percentage of antimicrobial susceptibility in Gram negative isolates**

| Antibiotics | Escherichia coli n=631 | Klebsiella pneumoniae n=135 | Pseudomonas aeruginosa n=26 |
|---|---|---|---|
| Ampicillin | 16% | 00% | -- |
| Amoxi-clav | 40% | 07% | -- |
| Gentamicin | 63% | 66% | 65% |
| Amikacin | 94% | 92% | 96% |
| Ciprofloxacin | 39% | 36% | 61% |
| Cotrimoxazole | 21% | 25% | -- |
| Ceftriaxone | 56% | 54% | -- |
| Ceftazidime | 56% | 54% | 81% |
| Cefoperazone | 56% | 54% | 81% |
| Aztreonam | 58% | 56% | 77% |
| Pipera-tazo | 85% | 74% | 92% |
| Imipenem/ meropenem | 99% | 98% | 96% |
| Cefoperazon-sulbactam | 87% | 83% | 92% |
| Norfloxacin | 35% | 28% | 50% |
| Nitrofurantoin | 85% | 60% | -- |

**Table 2: Percentage of antimicrobial susceptibility in Gram positive isolates**

| Antibiotics | *nterococcus* spp. *n=71* | *Staphylococcus aureus n=13* |
|---|---|---|
| Ampicillin/penicillin | 77% | 15% |
| Amoxi-clav | 77% | 100% |
| Gentamicin | -- | 92% |
| Amikacin | -- | 92% |
| Ciprofloxacin | 27% | 69% |
| Cotrimoxazole | -- | 46% |
| Pipera-tazo | 83% | -- |
| Imipenem/meropenem | 86% | -- |
| Norfloxacin | 20% | 61% |
| Nitrofurantoin | 86% | 100% |
| Vancomycin | 96% | 100% |
| Linezolid | 100% | 100% |

This study shows *Escherich coli* was 99% sensitive to imipenem/meropenem, similar results (96%) were shown by Khan et al at AFIP Rawalpindi.[12] But results were contrary to a similar study conducted in Lahore by Sabir *et al* which showed only 66% sensitivity to carbapenems.[13] These differences may be due to isolates from hospital settings where carbapenems may be used more frequently. Among third generation cephalosporins, ceftriaxone was 54% sensitive and similar pattern was noted by Khan et al in 2014.[12]

Antimicrobial susceptibility pattern of commonly used oral antibiotic ciprofloxacin was 39% for *E coli* and 36% for *Klebsiella pneumoniae* that somewhat more than 15% reported from Rawalpindi.[12]This has serious implications for treatment of UTI in the community setting and physicians should be alerted to these findings. A similar susceptibility pattern has been reported earlier from Pakistan and India.[14]

Fluoroquinolones are common antibiotics which are prescribed empirically in community settings and could be underlying reason for the high resistance rates.[5,15] Piperacillin-tazobactam, cefoperazone-sulbactam and nitrofuratoin were >80% effective against *E. coli* isolates but it was less effective in *Klebsiella pneumoniae* isolates. Reason may be that they are not routinely used by general practitioners for UTI, so uropathogens may have lower exposure.[5,9,15] Extended spectrum β-lactamase production is also common as described by Jain *et al*.[15,16]

From this study, it is obvious that cotrimoxazole is no more useful against uropathogens as only one fourth of the isolates were susceptible to it. Previously this antibiotic was used as the drug of choice for empirical treatment of UTI.[17] It is shown in our study that nitrofurantoin has tremendous effect against *Enterococcus* spp., *E coli* and *Klebsiella pneumoniae*, which are responsible for UTI in community setup. Hence our study recommends nitrofurantoin as the drug of choice for empirical treatment in community acquired UTI. This is laboratory based review of record this may not show the true picture of whole community but can be used for local antibiotic policies. Another limitation of this study that hospital and community acquired isolates were not separated and fosfomycin disc was not available in our lab at that time.

**Conclusion & Suggestions**

In view of the above findings it is concluded that uropathogens are becoming resistant to commonly used oral antibiotics limiting to injectables which are more costly. We suggest that inappropriate and empiric antimicrobial therapy should be avoided to prevent emergence of resistance.

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