

Original Research Article

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Antimicrobial Resistance Pattern of Bacterial Isolates from Urinary Tract Infections at a Tertiary Care Centre

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ABSTRACT

Urinary tract infections (UTIs) are the most common bacterial infections encountered in clinical practice, which cause morbidity in both hospitalized and out patients. Increasing antimicrobial resistance among bacterial uropathogens is a significant health concern. To determine spectrum of microorganisms responsible for UTIs and their antimicrobial resistance pattern. Urine samples received in microbiology laboratory for culture from 17,135 patients clinically suspected of having urinary tract infection during the study period were processed according to standard protocol. Isolates were identified by conventional phenotypic methods and antibiotic resistance determined by Kirby Bauer disc diffusion method and Clinical and Laboratory Standards Institute (CLSI) guidelines. Out of 17,135 urine samples, 2731 (15.9%) were considered as positive growth, which yielded 2760 isolates. The most common isolate was *Escherichia coli* (60.9%) followed by *Klebsiella pneumoniae* (14.6%), *Pseudomonas aeruginosa* (5.6%), *Enterococcus* spp. (4.8%), *Staphylococcus aureus* (4.2%), *Staphylococcus saprophyticus* (3.0%), *Acinetobacter baumannii* (2.9%), *Proteus* spp. (2.6%) and others (1.4%). Most of the Gram-negative isolates showed resistance to fluoroquinolones, cephalosporins and aminoglycosides, whereas most Gram-positive isolates were found to be resistant to penicillins, co-trimoxazole and fluoroquinolones. Nitrofurantoin was the only drug which exhibited good sensitivity against both Gram-negative as well as Gram-positive isolates. *E. coli* and *K. pneumoniae* are the most common uropathogens. Nitrofurantoin can be used for effective treatment as an empirical therapy for UTIs in OPD patients. Carbapenems and colistin should be reserved for complicated UTIs and prescribed only after sensitivity reports are available.

Keywords

Uropathogens ;
Nitrofurantoin ;
carbapenem ;
colistin ;
complicated UTIs.

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Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections, leading to increased morbidity in both hospitalized as well as outpatients (Tankhiwale *et al.*, 2004; Mohajeri *et al.*, 2014). Although, a variety of organisms are responsible for UTI, *Escherichia coli* and other members of the family *Enterobacteriaceae* account for a large majority of UTIs (Banerjee, 2009).

After *Escherichia coli*, the most common pathogens causing UTIs include *Klebsiella pneumoniae*, *Enterococcus* spp., *S. saprophyticus*, *Pseudomonas aeruginosa*, *Candida* spp., *Proteus* spp. and *Enterobacter* spp (Noor *et al.*, 2013).

Increasing prevalence, relapse and ramifications in eradication of UTI make it a

cause for concern and present a real challenge to healthcare professionals. It is also a known fact that women are more vulnerable to UTI due to anatomical and physiological factors. Owing to its position, urogenital tract of women is more susceptible to bacterial infections caused by both internal and external flora.

Increasing drug resistance in bacterial uropathogens is an important and emerging public health problem. The distribution of antimicrobial susceptibility data of UTI-causing microorganisms changes from place to place. Generally, the antimicrobial treatment is initiated before the culture sensitivity results are available which may lead to the frequent misuse of antibiotics (Prakash *et al.*, 2013). The current knowledge of the organisms causing UTI and their antimicrobial susceptibility pattern is a necessity, in order to ensure appropriate empirical therapy. In view of the increasing antimicrobial resistance among bacterial isolates causing UTI, this study was undertaken at a tertiary care centre to determine the spectrum of microorganisms responsible for UTI and their antimicrobial resistance pattern.

Materials and methods

Study population

This cross sectional study was carried out from June 2014 to May 2016 in Microbiology laboratory of a tertiary care centre. A total of 17,135 patients clinically suspected of having urinary tract infections with complaints of fever, frequency or urgency of micturition were involved which included both in-patients and out-patients.

Collection of Sample

The patients were thoroughly instructed on how to collect the urine samples with strict

asepsis. Clean-catch mid-stream samples were collected from each patient. In case of children, the urine sample was collected by suprapubic aspiration. In catheterized patients, the catheter was clamped and the area proximal to the clamp was disinfected. Then, using a 28 gauge needle and syringe, urine was withdrawn. The samples were immediately transported to microbiology laboratory for further processing.

Processing of Sample

A standard calibrated bacteriological loop was used to inoculate 0.01 ml of urine sample on Cysteine Lactose Electrolyte Deficient (CLED) medium. The plates were then incubated at 37°C for 24 hours.

Interpretation of Growth and Identification of Isolates

A sample was considered positive for UTI if the colony count was more than 10^5 CFU/ml by semi-quantitative technique and the direct microscopy revealed pus cells $\geq 8/\text{cu mm}^5$. All the isolates were identified at the species level by standard biochemical tests. Samples growing more than two (02) organisms were not included in the study and were discarded as mixed growth. Urine samples with no growth after 48 hours of incubation were considered as negative.

Antimicrobial susceptibility testing

Antimicrobial sensitivity testing of all Gram-negative and Gram-positive isolates was carried out by Kirby-Bauer disc diffusion method and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Gram-negative isolates which were found to be resistant to all tested antibiotics by disc diffusion method, were tested for Colistin susceptibility by determination of minimum inhibitory concentration (MIC) using E-test

method. Vancomycin susceptibility for Gram-positive isolates was also tested by E-test method using vancomycin E-strips, as there are no disc diffusion guidelines for interpretation.

Results and Discussion

During the two year period of study, a total of 17,135 urine samples were received in microbiology laboratory. Out of these urine samples, only 2731 samples (15.9%) were considered as positive growth which yielded 2760 isolates. This was because 29 samples showed growth of two pathogens.

Out of 2731 samples which yielded bacterial growth and were considered positive for UTI, 1721 samples (63%) were from female patients and 1010 samples (37%) were from male patients. The age of the patients ranged from 3 months to 86 years with a mean of 33 years. The mean ages of male and female patients were 38.3 and 31.1 years respectively. The age and sex-wise distribution of positive urine cultures is shown in Table 1.

Out of 17,135 samples, 1156 were from patients admitted in ICU, 7053 from patients admitted in different wards of the hospital and 8926 were from OPD patients. A total of 199 samples from ICU (17.2%), 1136 from wards (16.1%) and 1396 from OPD (15.6%) yielded positive growth.

Out of 2760 isolated organisms, 1681 (60.9%) were *E. coli*, 403 (14.6%) were *Klebsiella pneumoniae*, 159 (5.6%) were *Pseudomonas aeruginosa*, 132 (4.8%) were *Enterococcus* spp., 115 (4.2%) were *Staphylococcus aureus*, 81 (3.0%) were *Staphylococcus saprophyticus*, 79 (2.9%) were *Acinetobacter baumannii*, 73 (2.6%) were *Proteus* spp. and 37 (1.4%) were other species. A total of 2420 Gram-negative isolates and 340 Gram-positive

isolates were obtained from these urine samples of 2409 and 322 patients respectively.

The antimicrobial resistance profile of 2420 Gram-negative and 340 Gram-positive isolates is shown in Table 2 and Table 3 respectively.

Urinary tract infections (UTIs) are one of the most common infections affecting all age groups and both sexes. They are the most frequent cause of morbidity in patients attending OPD and are also the most common healthcare-associated infection (Sussman, 1998). The most common uropathogens belong to the family *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Acinetobacter baumannii*.

UTI is a common, yet serious ailment in humans due to its recurrence and complexity in eradication. This problem is compounded by the declining number of newer antimicrobial agents entering the clinical practice and increasing antimicrobial resistance.

This study comprised of a total of 17,135 non-repetitive urine samples received in microbiology laboratory, out of which only 2731 samples were considered as positive growth which yielded 2760 isolates. After detailed review of the literature, it has been found that this study is one of the largest studies on antimicrobial resistance pattern of uropathogens, as far as sample size and number of isolates is concerned (Kibret *et al.*, 2014; Momoh *et al.*, 2011). Our study revealed a positivity rate of 15.9% (2731/17135) which is much lower as compared to other studies of Banerjee (24.5%), Prakash *et al.*, (53.8%), Kibret *et al.*, (22.7%) and Momoh *et al.*, (49%).

In the present study, out of 2731 samples which yielded bacterial growth and were considered positive for UTI, 1721 samples (63%) were from female patients and 1010 samples (37%) were from male patients. Similar findings regarding the sex distribution of positive urine samples have been reported in a study by Banerjee. However, Kibret *et al.*, showed a higher number of female patients being affected by urinary tract infections (Kibret *et al.*, 2014). Although, urinary tract infections affect both sexes, it is a well known fact that it is more common in females as compared to males because of shorter urethra in females which is less effective for preventing bacterial entry and closer proximity of urethral meatus and anal opening (Warren *et al.*, 1999).

In the present study, the age of the patients observed varied from 3 months to 86 years. The mean age of all patients was 33 years and that of male and female patients being 38.3 and 31.1 years respectively. It was observed that in both sexes, the maximum number of uropathogens were isolated from the patients in the age group 31-40 years. Similar findings were seen in a study by Kibret *et al.*, in which the mean age was found to be 32.26 years and that of male and female patients was 35.1 and 30.78 years respectively.

The present study shows that the OPD patients formed the highest sample size followed by samples from wards and ICU. However, the positivity rate was highest from ICU samples followed by wards and OPD samples. This might be attributed to the fact that many of the OPD samples yielded growth of more than two organisms which signifies improper specimen collection techniques. Moreover, it also shows the unwarranted ordering of urine cultures from OPD patients, which are often not required. The causative organisms responsible for UTI are fairly well known and various studies

have not offered significant differences. In our study, *Escherichia coli* (60.9%) was the found to be the predominant isolate followed by *Klebsiella pneumoniae* (14.6%), *Pseudomonas aeruginosa* (5.6%), *Enterococcus* spp. (4.8%), *Staphylococcus aureus* (4.2%), *Staphylococcus saprophyticus* (3.0%), *Acinetobacter baumannii* (2.9%), *Proteus* spp. (2.6%) and other species (1.4%). These findings are consistent with other studies which also reveal that *E. coli* and *K. pneumoniae* are the commonest pathogens responsible for urinary tract infections (Barnett *et al.*, 1997; Moges *et al.*, 2002; Wagenlehner *et al.*, 2004). It was clear from our study that Gram-negative bacteria were more responsible for UTI than Gram-positive bacteria, which is in concordance with the findings of previous studies (Ojumba, 2005).

Empiric antibiotic therapy is the mainstay of treatment for UTIs. However, many previous studies indicate increasing trend of drug resistance among UTI pathogens. As it is clear from Table 2, in our study, 68-82% of the *E.coli* isolates were found to be resistant to ceftazidime, cefepime, ampicillin and gentamicin, 55-60% isolates were resistant to piperacillin + tazobactam, ampicillin+ sulbactam and amoxicillin+clavulanate. The antibiotics with least resistance was found to be imipenem (12%) followed by nitrofurantoin (15%).

Isolates of *K. pneumoniae* were found to be more resistant. In our study, 61-90% of isolates were resistant to amoxicillin + clavulanic acid, ampicillin+ sulbactam, piperacillin + tazobactam, cefepime, ceftazidime and gentamicin. Ninety nine percent (99%) of isolates were sensitive to colistin and 67% were sensitive to nitrofurantoin. The resistance levels are too high when compared to studies done by Kibret *et al.*, (2004).

Table.1 Age and sex-wise distribution of positive urine cultures

| Age group (years) | Female | | Male | |
|----------------------|--------|-------|--------|-------|
| | Number | %age | Number | %age |
| 0-10 | 129 | 7.5% | 88 | 8.7% |
| 11-20 | 289 | 16.8% | 146 | 14.5% |
| 21-30 | 273 | 15.9% | 133 | 13.2% |
| 31-40 | 478 | 27.8% | 266 | 26.3% |
| 41-50 | 245 | 14.2% | 198 | 19.6% |
| 51-60 | 190 | 11.0% | 122 | 12.1% |
| >60 | 117 | 6.8% | 57 | 5.6% |
| Total | 1721 | - | 1010 | - |

Table.2 Antimicrobial resistance profile of Gram-negative urinary isolates

| S. No. | Antimicrobial | <i>E. coli</i> (1681) | <i>K. pneumoniae</i> (403) | <i>P. aeruginosa</i> (159) | <i>A.baumannii</i> (79) | <i>Proteus spp.</i> (73) |
|--------|---|--------------------------|-------------------------------|-------------------------------|----------------------------|-----------------------------|
| 1. | Ampicillin (10µg) | 1378 (82%) | Not tested | Not tested | Not tested | 62 (85%) |
| 2. | Amoxycillin + Clavulanic acid (20 µg + 10 µg) | 1009 (60%) | 322 (80%) | Not tested | Not tested | 57 (78%) |
| 3. | Ampicillin + Sulbactam (10 µg + 10 µg) | 992 (59%) | 314 (78%) | Not tested | 26 (33%) | 48 (66%) |
| 4. | Piperacillin + Tazobactam (100 µg + 10 µg) | 924 (55%) | 314 (78%) | 125 (79%) | 71 (90%) | 44 (60%) |
| 5. | Cefepime (30 µg) | 1294 (77%) | 363 (90%) | 146 (92%) | 71 (90%) | 62 (85%) |
| 6. | Ceftazidime (30 µg) | 1176 (70%) | 342 (85%) | 137 (86%) | 64 (81%) | 61 (84%) |
| 7. | Imipenem (10 µg) | 201 (12%) | 197 (49%) | 73 (46%) | 53 (67%) | 16 (22%) |
| 8. | Gentamicin (10 µg) | 571 (34%) | 246 (61%) | 86 (54%) | 60 (76%) | 39 (53%) |
| 9. | Ciprofloxacin (5 µg) | 1143 (68%) | 314 (78%) | 87 (55%) | 64 (81%) | 51 (70%) |
| 10. | Co-trimoxazole (1.25 µg +23.75 µg) | 689 (41%) | 173 (43%) | Not tested | 42 (53%) | 32 (44%) |
| 11. | Nitrofurantoin (300 µg) | 252 (15%) | 133 (33%) | Not tested | Not tested | 13 (18%) |
| 12. | Colistin (MIC) | Nil | 4 (1%) | 3 (2%) | Nil | Not tested |

Table.3 Antimicrobial resistance profile of Gram-positive urinary isolates

| S. No. | Antimicrobial | <i>Enterococcus spp.</i> (115) | <i>S. aureus</i> (81) | <i>S. saprophyticus</i> (79) |
|--------|---------------------------------------|-----------------------------------|--------------------------|---------------------------------|
| 1. | Penicillin (10 units) | 61 (53%) | 77 (95%) | 75 (95%) |
| 2. | Cefoxitin (30 µg) | Not tested | 42 (52%) | 26 (33%) |
| 3. | Co-trimoxazole (1.25 µg +23.75 µg) | Not tested | 38 (47%) | 34 (43%) |
| 4. | Tetracycline (10 µg) | 70 (61%) | 13 (16%) | 15 (19%) |
| 5. | Ciprofloxacin (5 µg) | 85 (74%) | 43 (53%) | 38 (48%) |
| 6. | Amikacin (30 µg) | Not tested | 17 (21%) | 15 (19%) |
| 7. | High-level Gentamicin (120 µg) | 77 (67%) | Not tested | Not tested |
| 8. | Vancomycin (MIC) | 22 (19%) | Nil | Nil |
| 9. | Linezolid (30 µg) | 8 (7%) | Nil | Nil |
| 10. | Nitrofurantoin (300 µg) | 30 (26%) | 13 (16%) | 11 (14%) |

Seventy three (73) – 92% of isolates of *P. aeruginosa* were resistant to most commonly used antibiotics with anti-pseudomonal activity like piperacillin+tazobactam, cefepime and ceftazidime. The reserve drug Colistin being the only drug which is sensitive against *P. aeruginosa* isolates, which is in agreement with the studies done by Shanti *et al.*, Shah *et al.*, and Somashekara *et al.*, (2014).

Considering the fact that, *E.coli* and *K. pneumoniae* constitute around 75% of the etiological agents, nitrofurantoin can be judiciously used as a first line antibiotic in treating community acquired UTIs. Strikingly, most of the gram negative isolates were resistant to third and fourth generation cephalosporins and the commonly used antibiotic β -lactam and β -lactamase inhibitor combinations, which

portrays the inappropriate and injudicious use of these antibiotics as empirical therapy. Hence necessitates the need for an antibiotic policy in every hospital as per Chennai Declaration.

Among the gram positive isolates, 61-85% isolates of *Enterococcus spp.*, which are the most common Gram-positive uropathogens, were resistant to most of antibiotics including penicillin, tetracycline, high-level gentamicin and ciprofloxacin. It was also seen that 22% of the isolates were resistant to vancomycin, 30% to nitrofurantoin. Fortunately, only 8% isolates were resistant to linezolid, which is the last line of defense against *Enterococcus spp.* Among *S. aureus*, 52% isolates were methicillin resistant, which signifies resistance to all β -lactam antibiotics except the 5th generation cephalosporins with anti-MRSA activity.

Around 43% isolates were also resistant ciprofloxacin which is higher as compared to study by Onanuga *et al.* Nitrofurantoin was the only antibiotic which showed acceptable sensitivity and only 13% isolates were resistant to it which is much

lesser as compared to study by Onanuga A *et al.*, (2012).

Staphylococcus saprophyticus was the 3rd most common Gram-positive uropathogen. Around 95% of these isolates were found to be resistant to penicillin, 38% to ciprofloxacin, 34% to co-trimoxazole and 26% of isolates were resistant to ceftiofuran, which signifies methicillin-resistance. This resistance pattern is much higher as compared to the study of Jhora *et al.*, who reported only 9% resistance to ciprofloxacin and 17% to co-trimoxazole²⁸. In our study, only 14% isolates were found to be resistant to nitrofurantoin, which was comparable to study of Jhora *et al.*, who reported 15% resistance. The major limitation of the study is that it did not distinguish the distribution of organisms in the community acquired UTI and nosocomial UTI. As a consequence, the prevalence of microorganisms and their resistance pattern in both types of UTI could not be ascertained.

In conclusion, this study provides the details about common uropathogens and their antimicrobial resistance pattern. It is clear that, *E. coli* and *K. pneumoniae* are still the most common uropathogens. Antibiotics such as β -lactams, co-trimoxazole, and fluoroquinolones have limited value for the treatment of UTI. Sensitivity to imipenem and colistin are still retained and may be prescribed for complicated UTI. Nitrofurantoin has good sensitivity against Gram-negative as well as Gram-positive bacteria and can be used as an effective

treatment especially for OPD patients. Routine monitoring of resistance patterns is necessary, which will help the clinicians in the empirical treatment of UTI and also for preparation of an antibiotic policy.

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