Causative agents of urinary tract infections and their antimicrobial susceptibility patterns at a referral center in Western India: An audit to help clinicians prevent antibiotic misuse

Harshkumar B. Patel1, Sumeeta T. Soni2, Aroor Bhagyalaxmi3, Neev M. Patel1

1B J. Medical College and Civil Hospital, Departments of 2Microbiology and 3Community Medicine, B. J. Medical College and Civil Hospital, Ahmedabad, Gujarat, India

Background: Urinary tract infections (UTIs) remain one of the most common infections in community and susceptibility of uropathogens to commonly used antimicrobials has declined over years. It is important to periodically study susceptibility patterns of uropathogens, so that empiric treatment can be determined using recent data, helping improve patient outcomes. Methods: Urine samples received by the laboratory for culture and susceptibility testing over a period of 3 months were analyzed and included in this study. Antimicrobial susceptibility testing was done on cultured isolates. Results: Of total 3,151 urine samples received, 3,066 were processed, and organisms were isolated from 1,401 (45.69%) samples. Isolation rate from male and female urine samples was 45.29% and 46.32%, respectively. The most commonly isolated organism was Escherichia coli (36.11%), followed by Candida spp. (18.56%), and Klebsiella spp. (18.06%). E. coli was most susceptible to imipenem (91.69%) and meropenem (91.49%). Klebsiella spp. was most susceptible to imipenem (75.89%) and meropenem (75.49%). Susceptibility of E. coli and Klebsiella spp. to nitrofurantoin, cotrimoxazole, and ciprofloxacin was 72.33%, 32.02%, and 18.97%, and 51.77%, 27.27%, and 22.13%, respectively. Candida spp. was most susceptible to amphotericin B (97.30%). Conclusion: Treatment for UTIs should be determined based on current local antimicrobial susceptibility patterns of uropathogens to minimise therapeutic failures and prevent antibiotic misuse.

Keywords: Antimicrobial susceptibility, Escherichia coli, urinary tract infections, uropathogens

Introduction

Urinary tract infection (UTI) describes microbial colonization and infection of structures of the urinary tract. UTI is categorized by infection site as pyelonephritis (kidney), cystitis (urinary bladder), and urethritis (urethra), and can also be classified as uncomplicated or complicated.[1] UTIs are among the most prevailing infectious diseases in the community with substantial clinical and financial burden.[2] Almost 95% of all UTIs are caused by bacteria, most of them by Escherichia coli (30%–90%, depending on clinical setting).[3] Klebsiella, Enterobacter, Proteus, Pseudomonas, Enterococcus, Staphylococcus, and others can also cause UTI.[4] Antibiotic resistance among bacteria causing common infections is increasing in all regions of the world.[4] It is interesting that pattern of resistance observed varies from hospital to community, large hospital to small hospital, state to state, and even vary from country to country.[5] Emergence of resistance to antibiotics illustrates importance of using evidence-based strategies for

Address for correspondence: Harshkumar B. Patel, A/1, Hardwar Society, Thalota Road, Visnagar − 384 315, Gujarat, India. E-mail: harshpatel02704@gmail.com

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The isolation rate from samples received from OPD (30.23%) was significantly or insignificant bacteriuria. A growth of ≥10^5 colonies/ml was considered as significant bacteriuria, suggestive of UTI. Identification was done based on standard biochemical and other laboratory tests.

Materials and Methods

Ethics

This study was approved by Institutional Ethics Committee.

Study area and population

A cross-sectional study was conducted in a teaching and public-sector referral hospital and its affiliated Bacteriology Laboratory, located in urban area of Ahmedabad, Gujarat. The hospital has around 2,000 beds and provides treatment to over 9 lac OPD (outdoor/ambulatory) patients and 1 lac IPD (indoor/hospitalized) patients annually. This hospital caters needs of patients from nearby urban and rural areas and also of patients referred from various districts of Gujarat as well as from Madhya Pradesh and Rajasthan. Patients whose urine samples were received by the Bacteriology Laboratory during 3-month period – July, August, and September of 2016 – were included in the study. Culture and susceptibility reports were obtained directly from the Bacteriology Laboratory.

Sample collection and processing

Clean catch mid-stream urine samples or those obtained by aspiration from catheter tube or suprapubic aspirate collected in sterile, wide mouth universal container, from all the suspected UTI patients (outdoor/ambulatory patients and indoor/hospitalized patients) were received and processed by the Bacteriology Laboratory. Contaminated/non-sterile samples were discarded and not processed.

Culture and identification of isolates

Urine samples were inoculated on appropriate culture media by using semiquantitative methods and inoculated media was incubated for 48 hours aerobically at 37°C. Cultures were then examined for growth and colonies counted for determination of significant or insignificant bacteriuria. A growth of ≥10^5 colony forming units/ml was considered as significant bacteriuria, suggestive of UTI. Identification was done based on standard biochemical and other laboratory tests.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by Modified Kirby Bauer Disc Diffusion method on Muller Hinton Agar as per the CLSI Standards. The antibiotics tested were amikacin (30 μg), ampicillin (10 μg), aztreonam (30 μg), cefepime (30 μg), cefazidime (30 μg), cefuroxime (30 μg), cefotaxime (30 μg), ceftazidime (75 μg), ciprofloxacin (5 μg), clindamycin (2 μg), colistin (10 μg), gentamicin (10 μg), imipenem (10 μg), levofloxacin (5 μg), linezolid (6 μg), meropenem (10 μg), nitrofurantoin (300 μg), piperacillin + tazobactam (100 + 10 μg), polymyxin-B (300 μg), piperacillin (100 μg), tetracycline (30 μg), ticarcillin + clavulanic acid (75 + 10 μg), and vancomycin (30 μg). For Candida spp., amphotericin B, clotrimazole, fluconazole, itraconazole, ketoconazole, miconazole and nystatin were used in antifungal susceptibility testing.

Statistical analysis

Calculations were done using Microsoft Excel. Results were presented as frequencies and percentages, and Z-test was applied where necessary and result considered significant at P ≤ 0.05.

Definition of terms

1. Urinary tract infection: Infection and microbial colonization of urinary tract.
2. Cystitis: Infection limited to urinary bladder/lower urinary tract. It often presents with dysuria, urinary urgency, frequency, and/or suprapubic pain.
3. Pyelonephritis: Infection of the kidney/upper urinary tract. It often presents with fever, tachycardia, chills or rigors, costovertebral tenderness, and/or flank pain with or without symptoms of cystitis.
4. Uncomplicated UTI: Acute, sporadic or recurrent lower (uncomplicated cystitis) and/or upper (uncomplicated pyelonephritis) UTI, limited to nonpregnant, premenopausal women with no known relevant anatomical and functional abnormalities within the urinary tract or comorbidities.
5. Complicated UTIs: UTIs in a patient with an increased chance of a complicated course, that is, all men, pregnant women, patients with relevant anatomical or functional abnormalities of the urinary tract, indwelling urinary catheters, renal diseases, and/or with other concomitant immunocompromising diseases.

Results

Of total 3,151 urine samples received, 1,901 from males and 1,250 from females, 85 contaminated/non-sterile samples were discarded. Of 3,066 samples processed, organisms were isolated from 1,401 samples yielding positive culture rate or isolation rate of 45.69%.

The isolation rate from samples received from OPD (30.23%) was lower than from samples received from IPD (51.62%) and the difference was statistically very significant (Z-value = 10.64, P < 0.0001) [Table 1]. Isolation rates in samples from males
and females were 45.29% and 46.32% respectively, without statistically significant difference (Z-value = 0.55, P = 0.58). Isolation rate from IPD + OPD (overall) and IPD samples was lower in males than in females. Isolation rate from OPD samples was higher in males than in females [Table 2].

The most common isolated organism was E. coli with 506 isolations (36.11%), followed by Candida spp. (18.56%), Klebsiella spp. (18.06%); K. pneumoniae = 17.15% and other Klebsiella spp. = 0.91%), Pseudomonas spp. (14.65%; P. aeruginosa = 13.19% and other Pseudomonas spp. = 1.46%), Acinetobacter spp. (5.06%; A. baumannii = 4.25% and other Acinetobacter spp. = 0.81%), Enterococcus spp. (4.14%; E. faecalis = 2.76% and E. faecium = 1.38%), Proteus spp. (1.78%; P. mirabilis = 1.07% and other Proteus spp. = 0.71%), Staphylococcus spp. (0.78%; S. aureus = 0.67% and coagulase-negative staphylococci = 0.11%), Providencia spp. (0.43%), and Morganella morganii (0.43%). Due to low number of Providencia and Morganella isolates, their antibiotic susceptibility results were excluded from the study. In males, followed by E. coli (33.29%), Klebsiella spp. (19.12%) was the second and Candida spp. (17.59%) was the third most isolated organism. In females, however, after E. coli (40.43%), Candida spp. (20.03%) was the second and Klebsiella spp. (16.42%) was the third most isolated organism [Table 3].

E. coli was most susceptible to meropenem (91.89%) closely followed by imipenem (91.69%). E. coli had least susceptibility to cefuroxime (18.18%) and ciprofloxacin (18.97%). Klebsiella was most susceptible to imipenem (75.89%) closely followed by meropenem (75.49%). Klebsiella spp. was least susceptible to ampicillin (2.37%). Pseudomonas spp. was most susceptible to polymyxin B (92.19%) followed by aztreonam (68.29%). Pseudomonas spp. was least susceptible to ciprofloxacin (25.85%). Gram-positive bacteria Entereococcus spp. and Staphylococcus spp. were most susceptible to linezolid at 96.55% and 100%, respectively followed by vancomycin at 87.93% and 100%, respectively [Table 4].

Susceptibility of Candida spp. was maximum to amphotericin B (97.30%), followed by nystatin (89.61%). Candida had minimal susceptibility to clotrimazole (50.38%) [Table 5].

Discussion

Positive culture rate or isolation rate of 45.69% obtained in this study was close to that obtained by similar studies conducted across India.[14‑17] The data obtained from this study shows that spectrum of organisms causing UTI is also similar to that reported by other studies across India. However, isolation rates of various organisms varied from study to study.[14‑17] Isolation rates in male and female samples were not statistically different which was in stark contrast to other studies conducted across India, in which the isolation rates were found to be higher in females.[14,15,17] This discrepancy could in part be because of higher number of complicated UTIs in males resulting in higher number of requests for culture and susceptibility reports and because treatment of most uncomplicated UTIs is usually done empirically without requesting culture and susceptibility reports.

E. coli was the most common isolated organism responsible for causing UTIs in our setup, in trend with other studies across India.[15‑17] High E. coli isolation rate of 69.8% was seen in study conducted by George et al. in Karnataka compared to 36.11% seen in our study.[14] High susceptibility of E. coli to meropenem (91.89%) and imipenem (91.69%) was similar to that seen in other studies across India.[14‑17] However, a study done in Lahore, Pakistan by Sabit et al. reported low E. coli...
Table 4: Susceptibility rates of isolated bacteria to various tested antibiotics

| Antibiotic drugs | Escherichia coli (N=506), n (%) | Klebsiella spp. (N=253), n (%) | Pseudomonas spp. (N=205), n (%) | Acinetobacter spp. (N=71), n (%) | Proteus spp. (N=25), n (%) | Enterococcus spp. (N=58), n (%) | Staphylococcus spp. (N=11), n (%) |
|------------------|--------------------------------|-------------------------------|---------------------------------|---------------------------------|---------------------------|-------------------------------|---------------------------------|
| Amikacin         | 311 (61.46)                   | 113 (44.66)                   | 113 (55.12)                     | 35 (49.29)                      | 11 (44)                   | 6 (54.54)                     |                                  |
| Ampicillin       | 137 (27.07)                   | 6 (2.37)                      |                                 |                                 | 4 (16)                    | 12 (20.69)                   | 6 (54.54)                       |
| Aztreonam        | 246 (48.61)                   | 73 (28.85)                    | 140 (68.29)                     | 6 (8.45)                        | 7 (28)                    |                               |                                 |
| Cefepime         | 173 (34.19)                   | 65 (25.69)                    | 82 (40.00)                      | 25 (35.21)                      | 14 (56)                   |                               |                                 |
| Cefoperazone     | 131 (25.89)                   | 50 (19.76)                    | 69 (28.78)                      | 18 (25.35)                      | 11 (44)                   |                               |                                 |
| Cefazidime       | 131 (25.89)                   | 50 (19.76)                    | 83 (40.48)                      | 20 (28.17)                      | 9 (36)                    |                               |                                 |
| Ceftoxamine      | 92 (18.18)                    | 37 (14.62)                    |                                 | 3 (12)                          |                         |                               |                                 |
| Ciprofloxacin    | 96 (18.97)                    | 56 (22.13)                    | 53 (25.85)                      | 22 (30.98)                      | 6 (24)                    | 10 (17.24)                   | 7 (63.63)                       |
| Cotrimoxazole    | 162 (32.02)                   | 69 (27.27)                    |                                 | 6 (24)                          |                         | 10 (90.90)                   |                                 |
| Gentamicin       | 269 (53.16)                   | 97 (38.34)                    | 96 (46.83)                      | 26 (36.62)                      | 8 (32)                    | 10 (90.90)                   |                                 |
| Imipenem         | 464 (91.69)                   | 192 (75.89)                   | 138 (67.31)                     | 45 (63.38)                      | 23 (92)                   |                               |                                 |
| Levofloxacin     | 109 (21.54)                   | 63 (24.90)                    | 67 (32.68)                      | 26 (36.62)                      | 9 (36)                    | 8 (13.79)                    | 9 (81.81)                       |
| Linezolid        | 192 (38.36)                   | 89 (35.17)                    | 111 (54.14)                     | 27 (38.02)                      | 17 (68)                   |                               |                                 |
| Meropenem        | 465 (91.89)                   | 191 (75.49)                   | 137 (66.83)                     | 45 (63.38)                      | 23 (92)                   | 32 (55.17)                   |                                 |
| Nitrofurantoin   | 366 (72.33)                   | 131 (51.77)                   |                                 | 4 (16)                          |                         | 48 (82.75)                   |                                 |
| Piperacillin + tazobactam | 262 (51.77) | 89 (35.17) | 111 (54.14) | 27 (38.02) | 17 (68) |                               |                                 |
| Polymyxin B      |                                 | 189 (92.19)                   | 70 (98.59)                      | 3 (12)                          |                         | 31 (53.45)                   |                                 |
| Tetracycline     | 183 (36.16)                   | 112 (44.27)                   |                                 | 3 (12)                          |                         |                               |                                 |
| Ticarcillin + clavulanic acid | 223 (44.07) | 103 (40.71) | 121 (59.02) | 28 (39.43) | 18 (72) |                               |                                 |
| Vancomycin       |                                 |                               |                                 | 51 (87.93)                      | 11 (100.00)               |                                 |                                 |

Susceptibility rate %: n/N; where n: Susceptible isolates; N: Total isolates; Blank rows: Test not done.

Table 5: Susceptibility rates of isolated Candida spp. to various tested antifungals

| Antifungal drugs | Candida spp. (N=260), n (%) |
|------------------|----------------------------|
| Amphotericin B   | 253 (97.30)                |
| Clotrimazole     | 131 (50.38)                |
| Fluconazole      | 169 (65.00)                |
| Itraconazole     | 201 (77.30)                |
| Ketoconazole     | 198 (76.15)                |
| Micafungin       | 178 (68.46)                |
| Nystatin         | 233 (89.61)                |

Susceptibility rate %: n/N; where n: Susceptible isolates; N: Total number of isolates.

The susceptibility rate of 39.5% to imipenem.[19] Susceptibility of *E. coli* to nitrofurantoin was found high at 72.33%, in trend with other studies across India.[14,15,17] Susceptibility of *E. coli* to cotrimoxazole was 32.02% in this study, while in other studies across India it varied from 15.15% to 52.3%.[14,15] Susceptibility of *E. coli* to ciprofloxacin was found to be 18.97% which lower compared to susceptibility rates seen in other studies across India and Pakistan.[14,16,19] High susceptibility rates of *E. coli* to ciprofloxacin were reported in studies done in Iran (68.1%), Poland (65.8%) and Ethiopia (54.8%) showing geographical variations in antibiotic susceptibility trends.[20–22] [Table 6].

*Klebsiella* spp. was the third most commonly isolated uropathogen at rate of 18.06%, similar to isolation rate of 18.71% reported in a study from Meerut, India.[18] *Klebsiella* spp. however, was the second most commonly isolated organism in various studies across India.[14,15] Susceptibility to nitrofurantoin, cotrimoxazole and ciprofloxacin was found to be 51.77%, 27.27% and 22.13% respectively. Susceptibility of *Klebsiella* isolates to nitrofurantoin varied from 38% to 67% in studies across India.[15,17] Susceptibility of *Pseudomonas* isolates to anti-pseudomonas cephalosporin cefazidime was found to be 40.48%.

*Candida* spp. was the second most common isolated organism in this study at rate of 18.56% which was higher compared to other studies across India, with isolation rate as low as 8% reported in a study in Kerala, India.[16] The isolation rate of *Candida* spp. in the current study could be influenced by various patient factors such as urinary catheterisation and stenting, diabetes, immunocompromised status, hospitalization and use of broad spectrum antibiotics. *Candida* isolates were most susceptible to amphotericin B (97.30%) which was close to susceptibility rate of 91% observed in a study done in Mangalore, India.[23]

Resistance to antibiotics is higher in India compared to nations like UK, USA, Australia and South Africa.[24] Decreased susceptibility of uropathogens to empiric antibiotics for UTI like cotrimoxazole and ciprofloxacin and even to broad spectrum antibiotics in India, as evident by this and other studies, could be because of rampant use of antibiotics predisposed by many factors. Tendency to self-medicate, noncompliance to treatment, financial constraints and lack of education on part of patient, sale of antibiotic drugs without proper prescription and failure to educate patient on part of pharmacists, negligible surveillance of susceptibility patterns, poor regulatory controls over antibiotics and lack of will to make change on part of health care system, and administering antibiotics before obtaining sample for culture, failure to educate patient and
Table 6: Comparison of susceptibility rates of *Escherichia coli* to various antibiotics, found in various studies across India and abroad

| Studies                  | Country     | Nitrofurantoin (%) | Ciprofloxacin (%) | Gentamicin (%) | Amikacin (%) | Imipenem (%) | Cotrimoxazole (%) |
|--------------------------|-------------|--------------------|-------------------|---------------|--------------|--------------|-------------------|
| George et al.[16]        | India       | 84.15              | 34.1              | 63.6          | 90.9         | 97.7         | 52.3              |
| Prakash and Saxena[17]   | India       | 74.24              | 30.3              | 30.3          | 90.91        | 98.48        | 15.5              |
| Somashekar et al.[18]    | India       | -                  | 28                | -             | 84           | 92           | 31.2              |
| Singhal et al.[19]       | India       | 88.66              | 16.22             | -             | 65           | -            | -                 |
| Kulkarni et al.[20]      | India       | 92.41              | 34.18             | 59.24         | 90.89        | 96.71        | -                 |
| Sabir et al.[21]         | Pakistan    | -                  | 29.2              | 26.4          | 71.7         | 39.5         | -                 |
| Kashef et al.[22]        | Iran        | 71.3               | 68.1              | 49.3          | -            | -            | 38.2              |
| Stefanik et al.[23]      | Poland      | 64                 | 65.8              | 92.7          | 98.9         | 100          | 65.1              |
| Mamuye[24]               | Ethiopia    | 20.8               | 54.8              | 22.6          | -            | -            | 22.6              |
| Current study            | India       | 72.73              | 18.97             | 53.16         | 61.46        | 91.69        | 32.02             |

poor prescribing practices on part of physicians are among many factors that lead to injudicious and inappropriate use of antibiotics in India, hence causing rapid development of resistance.[25-28]

Based on this study, it can be recommended that nitrofurantoin be preferred instead of cotrimoxazole and ciprofloxacin for use as empiric antibiotic for uncomplicated cystitis.[29,30] For uncomplicated and complicated pyelonephritis, aminoglycosides and carbapenems should be preferred over fluoroquinolones and cephalosporins.[29,30] In all cases, urine sample for culture and susceptibility testing should be collected before administration of antibiotics and then therapy should be modified to narrow spectrum agent as per urine culture and susceptibility report.[30] This study emphasizes the need for hospital or regional antibiograms in order to combat the problem of antibiotic resistance. Antibiograms help monitor antimicrobial resistance trends and help clinicians select appropriate antibiotic therapy.[31]

The major limitation of this study is that since direct laboratory data was used, it does not take into account risk factors that can cause drug resistant and complicated UTIs like diabetes, compromised immunity, cancer chemotherapy, HIV, prolonged urinary catheterisation, recent antibiotic use, incomplete treatment of prior UTIs, urinary tract malformations and old age.[32,33] This study is also limited by the fact that those patients who were treated on outpatient basis might have had uncomplicated UTIs and physicians treating them might not have requested urine culture and susceptibility reports.

**Conclusion**

This study provides important data to monitor and compare with other studies, the trend of antimicrobial susceptibility of uropathogens and helps us towards deciding empirical treatment of UTIs at this referral healthcare center. Similar studies should be done on a larger scale periodically in different regions, so that empiric antibiotic therapy guidelines can be framed according to local antimicrobial susceptibility trends improving patient outcomes and minimizing antibiotic misuse.

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**Conflicts of interest**

There are no conflicts of interest.

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