Urinary tract infections: a retrospective, descriptive study of causative organisms and antimicrobial pattern of samples received for culture, from a tertiary care setting

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Abstract

Introduction **Urinary tract infections (UTI) are common infections encountered by physicians either on an outpatient or inpatient basis. These infections have taken center stage due to increasing resistance being reported for commonly used antibiotics. Understanding the distribution and antibiotic susceptibility patterns of uropathogens would facilitate appropriate therapy.**

Methods **A retrospective analysis of the culture isolates obtained from urine samples received at the Department of Microbiology, St. John's Medical College Hospital, Bengaluru India, was performed between January 2012 and May 2012.**

Results **Of the 5592 urine specimens received, 28.2% showed significant growth. A total of 1673 identified pathogens were used in the analysis. *Escherichia coli* (54.6%) was the most common Gram-negative bacillus, followed by *Klebsiella* species (9.7%) and *Pseudomonas* species (7.5%). The most common Gram-positive coccus was *Enterococcus* (8.8%). Most of the Gram-negative isolates were resistant to ampicillin (79.3%) and cephalosporins (60%). Resistance to cephalosporins and fluoroquinolones was higher in isolates from inpatients. Other than *Klebsiella* spp., all other Enterobacteriaceae were susceptible to carbapenems (93%) and aminoglycosides (85%), whilst fluoroquinolones were effective for all Gram-positive bacteria.**

Conclusion **Due to a high level of antimicrobial resistance amongst the pathogens causing UTI in India, it is cautious to advise or modify therapy, as far as possible, after culture and sensitivity testing have been performed. Regional surveillance programs are warranted for the development of national UTI guidelines.**

Keywords **Antibiotic susceptibility, uropathogens, *Escherichia coli,* India**

Introduction

Urinary tract infections (UTI) are common bacterial infections, with an estimated 150 million UTI per annum worldwide.[1,2] UTI are a significant cause of morbidity in elderly men, and in females of all ages.[3] The manifestations of UTI may vary from mild asymptomatic cystitis to pyelonephritis and septicemia.[4] Untreated UTI can result in serious complications such as recurrent infection, pyelonephritis with sepsis, pre-term birth in pregnant females, and renal damage in young children. Additionally, complexities brought on by inappropriate antimicrobial use could result in high rate of antimicrobial resistance and *Clostridium difficile* colitis.[3]

UTI can be caused by both Gram-negative and Gram-positive bacteria, in addition to certain fungi. The major etiological agent is *Escherichia coli* followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, Group B *Streptococcus*, *Proteus mirabilis*,

Received: 17 May 2016; revised: 28 August 2016, 08 September 2016; accepted: 18 September 2016

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Published December 2016

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Candida* spp.[5,6]

Patients suffering from symptomatic UTI are commonly treated with antibiotics and these are usually given empirically before the laboratory results of urine culture are available.[7,8] Recently, several studies have revealed increasing trends of antibiotic resistance.[9] The antibiotic susceptibility pattern of uropathogens may vary according to the type of healthcare provided (primary or tertiary care at hospitals or other healthcare settings), different environments and geographical location; periodic evaluation of such pattern is necessary to update this information.[10,11] In this context, in the present study, we report the microbiological and antimicrobial profile of uropathogens documented from hospitalized patients with UTI, and those visiting the outpatient department with UTI in a tertiary care hospital in Bengaluru, India.

Methods

We retrospectively studied 1673 proven isolates obtained from 1574 urine samples, collected from January 2012 to May 2012 from both the inpatient (IP) and the outpatient (OP) sections of our tertiary care setting, St. John's Medical College Hospital, Bengaluru, India. Most of the samples were midstream collections, especially for the outpatient group and for many inpatients, as mentioned on the request. The pathogen(s) grown from the first sample of urine were considered in the analysis. Repeated samples (from patients who were already included), samples that grew more than two types of organism, or had evidence of perineal contamination were not included for analysis.

All samples were processed for determining colony count, semi-quantitatively on 5% sheep blood and cystine lactose electrolyte-deficient (CLED) agar medium using calibrated loops, as per standard protocol.[12] Samples showing significant growth, bacteria growing $>10^5$ colony-forming units (CFU/mL) with single morphotype or up to 2 types, were considered significant and processed further for identification and susceptibility testing. Gram-positive organisms were processed, if isolated as pure growth even when the colony counts were $<10^4$ CFU/mL. Susceptibility testing was done by Kirby-Bauer disk diffusion method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2012.[13] Except for vancomycin and meropenem, which were obtained from Oxoid (Basingstoke, UK), all other antibiotic discs were procured from HIMEDIA (Mumbai, India). Quality control of media and discs were performed using ATCC control strains.

Statistical analyses were performed using SPSS version 16 (SPSS, Inc., Chicago, IL, USA). The difference between age groups (≤14; 15–29; 30–59; ≥60 years) and the frequency of each uropathogen was analyzed by the Chi-square (χ^2) test. P-values of less than 0.05 were regarded as statistically significant.

Results

A total number of 5592 urine specimens were received for culture from January 2012 to May 2012, of which 1574 (28.2%) samples showed significant growth. Of the 1574 patients with suspected UTI, 1085 (69%) were IP (619 males and 466 females) and 489 (31%) (214 males and 275 females) were from the OP department. Median age was 49 years (IQR 28, 63), and 61% were males.

Importantly, a total of 1673 isolates were obtained from 1574 urine samples (1475 samples grew a single pathogen; 99 samples grew two pathogens including 86 Gram-positive and Gram-negative bacteria, 9 Gram-negative bacteria with yeast, and 4 Gram-positive bacteria with yeast). Gram-negative bacteria represented 70.4% of the isolates and *E. coli* (54.8%) was the leading pathogen followed by *Klebsiella* spp. (9.7%) and *Pseudomonas* spp. (7.5%). The frequency of isolation of uropathogens was significantly higher in the 30-59 years age group ($p<0.001$, $\chi(3)=76.850$) (Table 1). *E. coli* (75.7%) and *Klebsiella* spp. (70.1%) were significantly more frequent in patients with ages over 30 years ($p<0.001$, $\chi(3)=27.620$ and $p<0.001$, $\chi(3)=16.880$, respectively). *Enterococcus* spp. (8.5%) was the most frequent Gram-positive pathogen; it was identified more commonly in

Table 1. Frequency distribution of uropathogens among different gender and age groups. Data are reported as number of isolates and percentages (within brackets) of total patients in each age group.

Isolates		Age groups (in years)								[a]Chi-square (degrees of freedom)	p-value
		≤14		15–29		30–59		≥60			
	Total 1574	Male 104	Female 43	Male 100	Female 183	Male 321	Female 325	Male 308	Female 190		
Citrobacter spp.	21	1 (4.8)	0 (0)	0 (0)	5 (23.8)	3 (14.3)	6 (28.6)	4 (19.0)	2 (9.5)	6.865(3)	0.076
Escherichia coli	916	54 (5.9)	23 (2.5)	55 (6.0)	91 (9.9)	185 (20.2)	200 (21.8)	176 (19.2)	132 (14.4)	27.62(3)	**<0.001**
Enterobacter spp.	26	3 (11.5)	1 (3.8)	1 (3.8)	4 (15.4)	2 (7.7)	7 (26.9)	4 (15.4)	4 (15.4)	4.429(3)	0.219
Klebsiella spp.	162	11 (6.8)	4 (2.5)	6 (3.7)	27 (16.7)	33 (20.4)	37 (22.8)	25 (15.4)	19 (11.7)	16.88(3)	**<0.001**
NFGNB	46	6 (13.0)	2 (4.3)	4 (8.7)	5 (10.9)	11 (23.9)	12 (26.1)	6 (13.0)	0 (0)	6.974(3)	0.073
Proteus spp.	28	2 (7.1)	0 (0)	2 (7.1)	5 (17.9)	9 (32.1)	4 (14.3)	5 (17.9)	1 (3.6)	6.087(3)	0.107
Pseudomonas aeruginosa	125	14 (11.2)	7 (5.6)	14 (11.2)	14 (11.2)	20 (16.0)	15 (12.0)	33 (26.4)	8 (6.4)	8.044(3)	**0.045**
Enterococcus spp.	148	12 (8.1)	3 (2.0)	11 (7.4)	18 (12.2)	35 (23.6)	24 (16.2)	29 (19.6)	16 (10.8)	8.594(3)	**0.035**
CoNS	44	1 (2.3)	0 (0)	4 (9.0)	11 (25.0)	8 (18.2)	8 (18.2)	9 (20.5)	3 (6.8)	7.267(3)	0.064
Staphylococcus aureus[*]	12	0 (0)	1 (8.3)	0 (0)	2 (16.7)	4 (33.3)	1 (8.3)	3 (25.0)	1 (8.3)	5.623(3)	0.131
Candida spp.	109	3 (2.8)	4 (3.7)	5 (4.6)	7 (6.4)	21 (19.3)	24 (22.0)	27 (24.8)	18 (16.5)	2.395(3)	0.494
Other isolates[#]	36	2 (5.6)	0 (0)	4 (11.1)	5 (13.9)	6 (16.7)	6 (16.7)	11 (30.6)	2 (5.6)	6.029(3)	0.110
Total	1673	109	45	106	194	337	344	332	206	76.85(3)	**<0.001**

[*]Including one methicillin-resistant *S. aureus*

[#]Other isolates were: *Providencia rettgeri* (10), *Morganella* spp. (6), *Staphylococcus saprophyticus* (1), alpha-hemolytic streptococci (14 including one *S. viridans*), beta-hemolytic streptococci (3) and *Trichosporon* spp.(2)

[a]Frequency of each uropathogen among different age groups was analyzed by the Chi-square test.

CoNS – coagulase negative staphylococci; **NFGNB** – non-fermenting gram negative bacilli.

patients belonging to middle age group (30-59 years, 40% of cases) and was associated with male gender (p=0.035, $\chi(3)=8.594$). The incidence of *Candida* spp. was significant in the age group >50 years (61.5%) (p=0.016, $\chi(1)=29.1$). The rate of isolation of coagulase negative staphylococci (CoNS) was higher (75%) in patients aged ≥30 years but the gender difference was not significant (p=0.060, $\chi(1)=3.536$). Non-fermenting Gram-negative bacilli (NFGNB, presumptively identified as *Acinetobacter* spp.) were more commonly noted (91%) among IP (p=0.001, $\chi(1)=13.29$ for NFGNB vs. other isolates compared in OP and IP).

The percentage of resistance to commonly used antibiotics among these groups (IP and OP) is shown in Table 2. Most of the isolates were resistant to ampicillin (80%) and cephalosporins (60%); resistance to cephalosporins and fluoroquinolones (ciprofloxacin) was found to be higher among IP. UTI associated with *Klebsiella* spp. and NFGNB did not demonstrate susceptibility to the commonly used antimicrobial agents. *Enterobacteriaceae* other

Table 2. Percentage of antibiotic resistance of individual bacterial pathogens isolated from inpatients and outpatients.

Isolates	Ward	N	Amp[a]	Cz	Ctx	Cpz	Caz	PT	Gn[b]	Net	AK	Cot	Nit	Nx	Cip	Lef	Mrp	Cpm
Citrobacter spp.	OP	10	70.0	40.0	20.0	20.0	20.0	0.0	10.0	0.0	0.0	30.0	20.0	20.0	20.0	20.0	0.0	20.0
	IP	11	72.7	63.6	45.5	45.5	45.5	27.3	18.2	9.1	9.1	45.5	27.3	72.7	72.7	54.5	9.1	45.5
CoNS	OP	12	75.0	-	-	-	-	-	0.0	33.3	41.7	58.3	-	-	0.0	-	-	-
	IP	32	78.1	-	-	-	-	-	9.4	40.6	56.3	56.3	-	-	3.1	-	-	-
E. coli	OP	316	82.3	69.9	65.5	65.2	64.9	19.0	40.2	6.6	5.4	61.4	19.9	76.6	75.3	59.2	1.3	57.9
	IP	600	89.8	81.5	77.2	76.8	76.7	28.7	48.8	15.5	11.7	67.0	25.8	84.5	83.7	68.7	7.3	72.7
Enterobacter spp.	OP	12	100	83.3	58.3	50.0	50.0	25.0	33.3	16.7	16.7	25.0	50.0	41.7	41.7	33.3	8.3	50.0
	IP	14	100	92.9	64.3	64.3	64.3	28.6	57.1	28.6	28.6	64.3	50.0	57.1	57.1	42.9	14.3	64.3
Enterococcus spp.	OP	27	40.7	-	-	-	-	-	29.6	-	-	-	3.7	7.4	7.4	-	-	-
	IP	121	53.7	-	-	-	-	-	37.2	-	-	-	0.8	1.7	1.7	-	-	-
Klebsiella spp.	OP	53	100	45.3	41.5	41.5	37.7	22.6	32.1	20.8	20.8	34.0	73.6	45.3	37.7	26.4	11.3	39.6
	IP	109	100	80.7	72.5	72.5	72.5	55.0	61.5	44.0	36.7	62.4	73.4	74.3	71.6	61.5	32.1	66.1
NFGNB	OP	4	-	-	-	25.0	25.0	0.0	0.0	0.0	0.0	-	-	-	0.0	-	0.0	-
	IP	42	-	-	-	83.3	81.0	59.5	69.0	54.8	59.5	-	-	-	69.0	-	59.5	-
Proteus spp.	OP	13	61.5	58.8	46.2	46.2	46.2	15.4	30.8	23.1	15.4	53.8	61.5	61.5	53.8	30.8	7.7	46.2
	IP	15	73.3	73.3	46.7	53.3	53.3	13.3	33.3	33.3	26.7	66.7	86.7	66.7	46.7	26.7	6.7	33.3
P. aeruginosa	OP	39	-	-	-	20.5	17.9	7.7	33.3	20.5	23.1	-	-	-	28.2	-	15.4	-
	IP	86	-	-	-	48.8	46.5	27.9	55.8	44.2	45.3	-	-	-	51.2	-	24.4	-
S. aureus	OP	6	83.3	-	-	-	-	0.0	33.3	33.3	33.3	16.7	-	-	0.0	-	-	-
	IP	6	100	-	-	-	-	16.7	50.0	83.3	83.3	33.3	-	-	0.0	-	-	-
Mean	OP	492	76.6	59.5	46.3	38.3	32.7	11.2	24.3	17.1	17.4	39.9	38.1	42.1	26.4	33.9	6.3	42.7
	IP	1036	83.5	78.4	52.5	63.5	62.8	32.1	44.0	39.3	39.7	56.5	44.0	59.5	45.7	50.9	21.9	56.4

[a]Penicillin was used for *Staphylococcus aureus*
[b]High level gentamicin for *Enterococcus* spp.
–Not tested
Underlined numbers indicate variable sensitivity among the species according to the Clinical Laboratory Standards Institute (CLSI) 2014 guidelines
IP – inpatient; **N** – no of isolates; **OP** – outpatient; **Amp** – ampicillin; **Cz** – cefazolin; **Ctx** – cefotaxime; **Cpz** – cefoperazone; **Caz** – ceftazidime; **PT** – piperacillin+tazobactam; **Gn** – gentamicin; **Net** – netilmicin; **AK** – amikacin; **Cot** – co-trimoxazole (trimethoprim-sulfamethoxazole); **Nit** – nitrofurantoin; **Nx** – norfloxacin; **Cip** – ciprofloxacin; **Lef** – levofloxacin; **Mrp** – meropenem; **Cpm** – cefepime; **CoNS** – coagulase negative staphylococci; **NFGNB** – non-fermenting gram negative bacilli.

than *Klebsiella* spp. demonstrated higher susceptibility to carbapenems (93%), aminoglycosides (amikacin 85.8%) and nitrofurantoin (83%). Fluoroquinolones were effective (>93%) for all Gram-positive bacteria isolated.

Discussion

Correct diagnosis and treatment of UTI have important ramification into patients' health, selective pressure for antibiotic resistance, and healthcare costs.[3,14] The prevalence and antimicrobial susceptibility of uropathogens may vary with time and geographical area, and therefore monitoring the local etiology of UTI is beneficial to guiding empiric treatment.[15,16]

The present retrospective study highlights the age- and gender-wise distribution of UTI and antibiotic resistance patterns of uropathogens in the population seeking healthcare services from a tertiary care setting in South India. The proportion of affected males was high among young children and those with ages over 60 years old. We identified a higher number of males in the age groups ≤14 and ≥60 years. Similarly, there was a greater predominance of young females (15-29 years), whereas in the middle age group (30-59 years), equal proportions of males and females had UTI, which was in concordance with the findings of similar studies.[6,17] Females are more prone to develop UTI, probably due to the characteristic anatomy of the urethra and the effect of normal physiological changes that affect the urinary tract – short urethra, its proximity to the anus, urethral trauma during intercourse, dilation of the urethra and stasis of urine during pregnancy.[18,19]

E. coli was the most frequently encountered uropathogen in our study followed by *Klebsiella* spp., *P. aeruginosa*, and *Enterococcus* spp. The isolation rate of urinary pathogens is consistent with reports of other recently published studies.[6,18,20-24] However, studies from some other parts of the country have shown different isolation rates, probably due to variation in sample size, geographical location or population.[5] NFGNB isolation was more common in IP as seen in a study by Malini et al.[25] Non-fermenters are ubiquitous in the environment, able to survive in the hospital environment and can spread among hospitalized patients. They are emerging nosocomial pathogens especially in seriously ill patients and are responsible for causing a variety of infections.[25]

Antibiotic resistance has become a major clinical problem worldwide and has increased over the years.[24,26] Antimicrobial susceptibility patterns of the pathogens vary widely by region, patient population and type of healthcare facility.[20] Most of the isolates were resistant to multiple antibiotics at our setting. Resistance to ampicillin and the cephalosporins (first, second, third and fourth generation) was seen most commonly among Gram-negative bacilli. Fluoroquinolones, which are the mainstay for treatment of urinary tract infections, were not found to be useful even among Gram-negative bacilli (76.6%). This is similar (>74%) to previous studies in India.[20] *P. aeruginosa*, *Acinetobacter* spp. and *Klebsiella* spp. demonstrated higher resistance to relevant antibiotics, and were resistant to most antibiotics. *Klebsiella* spp. have the ability to acquire resistance genes by mutations and more commonly by transmissible plasmids.[27] Progressive spread and increasing incidence of carbapenem resistance among *Klebsiella* spp. has become a severe public health issue.[28] Since carbapenems are often the last line of defense against resistant Gram-negative infections, resistance to these antibiotics could result in greater morbidity, mortality, costs, and prolonged hospital stay.[28]

Carbapenems showed the highest efficacy on uropathogens among all tested antibiotics, with a susceptibility rate of 95% and 81% in OP and IP cases respectively. Inappropriate use of antibiotics may lead to the emergence and spread of resistance genes among bacteria.[29] UTI due to these multi-drug resistant bacteria associate increased morbidity and mortality, higher treatment costs, and prolonged hospital stay, thereby adding to the economical burden.[29] The Infectious Diseases Society of America (IDSA) guidelines consider nitrofurantoin and co-trimoxazole as current standard therapy for uncomplicated UTI in women.[7] However, the guidelines specify that local antimicrobial susceptibility patterns should be taken into account. As shown by our study and some previous studies,[20,22,30] aminopenicillins, ciprofloxacin and co-trimoxazole may not be appropriate choices for empirical treatment of UTI in our setting. Clinical correlation and culture results of catheter samples should be given additional emphasis prior to starting antibiotic therapy especially when the culture demonstrates resistant bacteria. Recently, fosfomycin has been introduced for the treatment of infections with multidrug-resistant uropathogens for which there are limited treatment options. It has a unique mechanism of action, which may provide a synergistic effect with other antibiotics including β-lactams, aminoglycosides and fluoroquinolones.[31] Good clinical practice should guide the use of the limited antibiotics left. Additionally, regional surveillance programs would be necessary to update the treatment guidelines of UTI in India.

Overall, this study provides important data on antimicrobial resistance among uropathogens in India. The limitations of the study were that it was laboratory based and limited to the cases for which cultures were requested from the clinic. Information on antibiotics administered prior to culture or data on subsequent treatment and its outcome in this study population would have added meaningful data to allow a better understanding of the prevalent practice in diagnosis and treatment of UTI in South India. Details on the method of collection were not available for all patients, limiting the analysis of the pathogens from these samples.

The rate of resistance to carbapenem among Gram-negative bacilli seen in our institution could possibly be due to the fact that this is a reference center, and that many of the patients had prior contact with other healthcare institutions and history of antibiotic use. Therefore, some data may be skewed and not entirely representative for similar patient populations at other types of healthcare institutions.

Conclusion

The uropathogens showed high levels of resistance to multiple urinary antimicrobial agents. Therefore, therapy should only be advocated, as far as possible, after culture and sensitivity has been performed and if required. Antibiotic resistance is becoming a huge public health problem that can lead to limited options for treatment, increasing hospitalization and associated costs.

Authors' contributions statement: SN designed and supervised the study. BSK collected and analyzed the data, and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

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Please cite this article as:
Kalal BS, Nagaraj S. Urinary tract infections: a retrospective, descriptive study of causative organisms and antimicrobial pattern of samples received for culture, from a tertiary care setting. GERMS 2016;6(4):132-138 doi: 10.11599/germs.2016.1100