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RESEARCH ARTICLE OPEN ACCESS

Prevalence of Community Acquired Uropathogens and their Antimicrobial Susceptibility in Patients from the Urology Unit of A Tertiary Care Medical Center

Manzoor Ahmad[1], Pushkar Kumar[2], Asfia Sultan[2], Anees Akhtar[2], Bhanu Chaudhary[2] and Fatima Khan[2]*

[1]Department of Surgery, Jawaharlal Nehru Medical College, Aligarh, Aligarh - 202 002 U.P. India.
[2]Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh, Aligarh - 202 002 U.P. India.

Abstract

Urinary tract infections (UTI) are one of the most common community acquired infections and can also present with similar lower urinary tract symptoms (LUTS). Moreover, UTI can be a complication of these urological diseases. Thus, this study was conducted in patients with LUTS to find out the prevalence of uro-pathogens and their antibiotic susceptibility pattern so that appropriate antibiotics can be started on clinical suspicion of UTI. The study was conducted over a period of 12 months. Culture and susceptibility of urine specimen was done as per standard microbiological guidelines. Apart from the growth of common bacteria with their antibiogram, Methicillin-resistant *Staphylococcus aureus* (MRSA), high level aminoglycoside resistance (HLAR) was investigated among *Enterococci*, and metallo beta-lactamases(MBL) production was production was investigated among gram-negative pathogens. Out of 407 urine samples included in the study, 80 (19.6%) samples showed bacterial growth. The commonest isolate was *E. coli* 44 (55 %). Majority of the isolates were multidrug resistant with two *E.coli* strains showing pan-resistance to the first line drugs tested. Carbapenem resistance was seen in 67.2% of all gram negative isolates tested. Metallo beta-lactamases production was found to be highest among the *Klebsiella* isolates. Among the Enterobacterales, highest susceptibility was noted to Fosfomycin (87.7%) followed by Nitrofurantoin (62.7%). Similarly in gram positive group, highest susceptibility was again to Fosfomycin followed by Nitrofurantoin. Overall the prevalence of MDR is increasing however Fosfomycin or Nitrofurantoin, both oral antibiotics, can be the considered for starting empirical antibiotic therapy.

Keywords: UTI, antimicrobial resistance, urology, LUTS, BOO

***Correspondence:** fatimasalmanshah@gmail.com; +91 7060317021

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INTRODUCTION

Urinary tract infections are considered as one of the most common infections developing in a community setting. Estimates show that more than 150 million episodes of UTIs occur annually world over[1]. They are a major cause of outpatient attendance as well as indoor admissions. It is also responsible for a major share of antibiotic consumption worldwide with negative socio-economic repercussions[2,3]. One of the major concerns for last many years is the fear of developing antibiotic resistance due to non-judicious use by health care professionals[4]. As the urine culture sensitivity report takes more than 2 days, clinicians often face difficulties in deciding the right antibiotic to be prescribed in an outpatient setting.

In Urology Outpatient clinics, we see lot of patients with lower urinary tract symptoms (LUTS) which refers to a group of symptoms like frequency, urgency, hesitancy, dribbling, poor flow of urine and straining at urination[5]. These symptoms start developing in 5th decade of life with higher prevalence in males than females[6,7]. One of the commonest causes of LUTS in males is Benign prostatic hypertrophy (BPH) which produces these symptoms secondary to bladder outlet obstruction[8]. It is estimated that about half of the male population has histopathological evidence of BPH with the prevalence increasing to around 90% at the start of ninth decade of life[9] .Urinary tract infection is also one of the complications of bladder outlet obstruction due to various causes some of which requires surgical intervention at some point of their clinical course[10]. Both UTI and bladder outlet obstruction can present with similar LUTS. Thus UTI needs to be ruled out in patients with LUTS before commencing cause specific medications or advising operation. Thus this retrospective study was designed to know the prevalence of uropathogens and their susceptibility pattern in patients presenting with LUTS in our Urology OPD. The ultimate aim is to make a local protocol for starting empirical treatment whenever a patient presents in outpatient clinic with clinical features suggestive of UTI.

MATERIALS AND METHODS

Patients and study design

The study was conducted over a period of 12 months (March 2019 to February 2020) in the Urology unit of Department of Surgery and Department of Microbiology, JNMCH, AMU, Aligarh. This study was approved by Institutional ethics committee and informed consent of all patients was obtained.

Specimen Collection and processing

Urine samples were collected from all new patients of age 15 years and above presenting with lower urinary tract symptoms like pain and burning during urination, frequency, urgency, supra pubic pain etc. Freshly collected mid-stream clean catch urine samples were collected from the non-catheterized, alert, conscious patients with above mentioned indications. The urine samples were processed as soon as possible after being received in the laboratory. The urine samples were plated by semi-quantitative method with standard loop technique on CLED Agar incubated

Table 1. Distribution of microbiological flora in two age groups in urine specimen

Organism	Age 15-60 years		Age > 60 years		Total = 80
	Male (%)	Female (%)	Male (%)	Female (%)	
E.coli	19 (43.18)	15 (34.10)	9 (20.45)	1 (2.27)	44 (55)
Klebsiella sp.	11 (73.33)	2 (13.33)	2 (13.33)	-	15 (18.75)
Citrobactor sp.	2 (40)	2 (40)	1 (20)	-	07 (8.8)
Enterococcus sp.	4 (57.14)	2 (28.57)	-	1 (14.28)	05 (6.2)
CONS	3 (75)	1 (25)	-	-	04 (5)
Pseudo sp.	2(100)	-	-	-	02 (2.5)
S. aureus	2 (100)	-	-	-	02 (2.5)
Proteus vulgaris	1 (100)	-	-	-	01 (1.25)
Total					80 (100)

at 37°C overnight. The growth of organisms (single or double) based on colony count (≥10^3) were taken[11]. Isolates significant clinically, as described in the standard guidelines of ICMR with some modifications, were included in the study[12].

Exclusion criteria

Patients with catheters or ureteral stents, recent history of antibiotic consumption, inadequate urine samples (<10 ml urine), samples collected from urine bag, specimens collected more than 2 hours before submission, specimens submitted in leaking, or dirty unsterile containers and specimens revealing growth of more than two types of bacteria on culture.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing of all isolates was performed as per clinical laboratory standards institute guidelines[13] on Mueller Hinton agar by Kirby-Bauer disk diffusion method.. The antimicrobial discs were procured from Hi-Media Laboratories, Mumbai, India.

Enterobacterales isolates

Cotrimoxazole (1.25/23.75µg), Amikacin (30µg), Ceftriaxone (30 µg), Ceftriaxone-Sulbactam (75µg, 1:1), Cefixime (5µg), Amoxicillin-Clavulanic acid (20µg/10µg), Fosfomycin (200µg), Nitrofurantoin (300µg), Norfloxacin (10µg) and Meropenem (10 µg) as the first line drugs. Pathogens resistant to the first line drugs were taken as multi-drug-resistant and were tested against second line drugs, Piperacillin-Tazobactam (100:10 µg), Tobramycin (10 µg), Imipenem (10 µg), Polymyxin B (300 µg) and Colistin (10 µg).

***Pseudomonas* spp.**

Piperacillin-Tazobactam (100:10µg), Meropenem (10µg) and Colistin (10µg), Amikacin (30µg), Aztreonam (30µg), Cefepime (30µg), Nitrofurantoin (300µg), Gentamycin (10µg), Levofloxacin (5µg) and Ceftazidime (30µg).

***Staphylococcus aureus* and CONS isolates**

Amikacin (30µg), Amoxicillin (20µg), Azithromycin (15µg), Vancomycin (30µg), Cefoxitin (30µg), Cotrimoxazole (1.25/23.75µg), Fosfomycin (200µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg).

***Enterococcus* species**

Ampicillin (10 µg), Azithromycin (15 µg), Doxycycline(30 µg), Fosfomycin (200µg), High Content Gentamycin (120µg), High Content Streptomycin (300µg), Vancomycin (30µg),Nitrofurantoin (300µg) and Norfloxacin (10µg).

Detection of Metallo beta-lactamases

Imipenem resistant isolates were tested for Metallo beta-lactamases (MBL) production by modified Hodge test and Double Disc synergy test using EDTA[14].

Screening for methicillin resistance in *Staphylococcus* species, high-level aminoglycoside resistance in *Enterococci* and Vancomycin resistance in *Enterococci*

Test was performed on Muller Hilton agar with 4% NaCl using Cefoxitin (30 µg) disc. Isolates showing a zone size <22 mm for *Staphylococcus aureus* and <25 mm for Coagulase negative staphylococcus species were considered resistant[13].

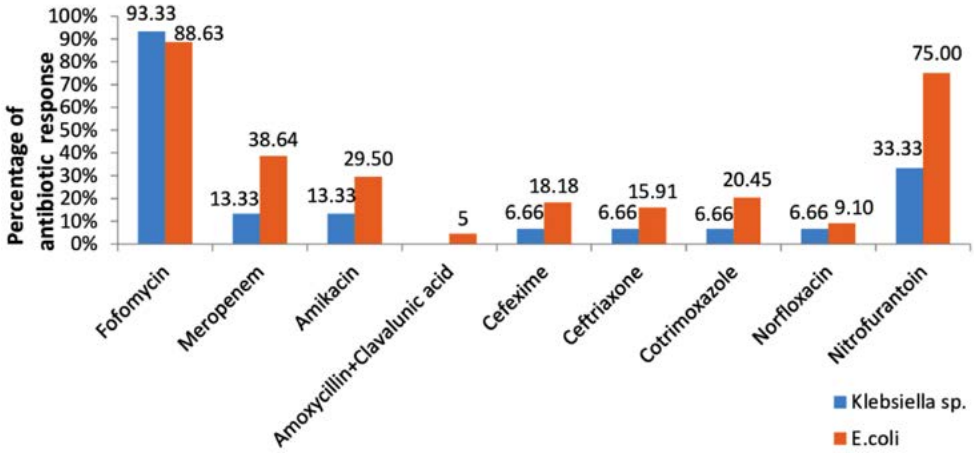

Fig. 1. Antibiotic sensitivity pattern of *Klibsiella* sp. and *E.coli.*

For *Enterococcal isolates*, high-level aminoglycoside resistance (HLAR) was tested using high content gentamycin (120 µg) and high level streptomycin (300 µg). Zone size ≥10 mm is considered as sensitive and zone size <6mm (no zone) is considered to be resistant (HLAR)[14] and for the detection of VRE, 30 µg disc of Vancomycin disc is used along with the routine susceptibility testing and zone of inhibition ≤ 14 are considered as VRE. Those with intermediate zones are confirmed by MIC methods[13].

RESULTS

Out of 407 urine samples included in the study, 316 (77.6%) were from males and 91 (22.4%) were from female patients. Of these 80 (19.6%) samples showed bacterial growth & 327 (80.4%) samples were sterile. Culture positivity was higher in females 26.4% (24 out of 91) as compared to males 17.7% (56 out of 316).

Of all positive urine cultures, 56 (70%) patients were males while 24 (30%) were females. Male to female ratio was 2.3:1. Categorization was also done on the basis of age group of patients; Group 1 consisted of the patients with the age of 15-60 years and group 2 with patient's age above 60 years. Majority of the culture positive patients were in group 1; 66 (82.5%) consisting of 44 (66.7%) males and 22 (33.3%) females. Out of total 14 elderly patients in Group 2 with UTI, 12 (85.71%) were males and 2 (14.28%) were females. Out of the eighty culture positive specimen majority 67 (83.8%) were gram negative isolates while gram positive organisms were cultured in 13 (16.2%) specimen. The frequency of isolation of these organisms is shown in Table 1. Almost all the specimens included the study showed mono-microbial growth. The most common isolate was *E coli* 44 (55 %) followed by *Klebsiella* species 15 (18.8%). Among Gram positive isolates, *Enterococci* was the commonest 07 (8.8%). Other organisms, which were identified included *Citrobactor* species 07 (6.2%), Coagulase negative *Staphylococcus* species 04 (5 %), *Pseudomonas* species 02 (2.5 %), *Staphylococcus aureus* 02 (2.5 %), and *Proteus species* 01 (1.25 %).

E.coli was the most common bacteria isolated in all age and gender groups. Among the Enterobacterales, highest susceptibility was noted to Fosfomycin (87.7%) followed by Nitrofurantoin (62.7%). Almost all the gram negative strains (94.4%) were resistant to Amoxicillin-Clavulanic acid.

Majority of the isolates were multidrug resistant with two *E.coli* strains showing resistance to all the first line drugs tested. Of these two isolates tested for 2nd line drugs, one was susceptible only to Colistin whereas the other one

Table 2. Antimicrobial Sensitivity Pattern of Gram Negative Bacterial Isolates in percentages

	Fo	Mrp	Ak	Amc	Cfm	Ctr	Cot	Nx	Nit
E.coli (n=44)	88.6	38.6	29.50	4.5	18.18	15.91	20.45	9.10	75
Klebsiella sp. (n=15)	93.33	13.33	33.33	0	6.66	6.66	6.66	6.66	33.33
Citrobactor sp. (n=5)	60	40	20	20	20	40	20	40	60

Fo = Fosfomycin, Mrp = Meropenem, Ak = Amikacin, Amc = Amoxycillin + Clavalunic acid, Cfm =Cefixime, Ctr = Ceftriaxone, Cot = Co–trimoxazole, Nx = Norfloxcin, Nit = Nitrofurantoin

Table 3. Antimicrobial Sensitivity Pattern of Gram Positive Bacterial Isolates in percentages

Uropathogens	Fo	Ak	Cot	Nx	Nit	HG	HS	Va	Amp	Azm	Amx	Cx	Do
Enterococcus sp. (n=7)	100	0	0	14.28	71.42	85.72	57.14	100	57.14	14.29	0	0	28.57
CONS (n=4)	100	100	50	25	100	_	_	100	_	75	75	50	_
S. aureus (n=2)	100	100	50	50	100	_	_	100	_	50	50	50	_

HG = High sensitive gentamycin, HS = High sensitive streptomycin, Va = Vancomycin, Amp = Ampicillin, Azm= Azithromycin, Cx = Ceftriaxone, Do = Doxycycline, Fo =Fosfomycin, Ak = Amikacin, Cot = Co–trimoxazole, Nx = Norfloxcin, Nit = Nitrofurantoin, Cx=Cefoxitine, Amx= Amoxicillin.

was sensitive to all the 2nd line drugs tested i.e., Colistin, Tigecycline, Polymixin B and Gentamycin. Out of 15 Klebsiella isolates, one tested for 2nd line antimicrobials which showed sensitivity to Tigecycline only and was resistant to Polymixin B, Colistin, Gentamycin and Ceftazidime. The antibiotic resistance profile of *E. coli* and *Klebsiella* sp. is depicted in Fig. 1.

The only *Proteus vulgaris* isolate was found to be sensitive to Fosfomycin and Nitrofurantoin and was resistant to Meropenem, Amikacin, Cefixime, Co- Trimoxazole and Norfloxacin.

Carbapenems resistance was seen in 67.2% of all gram negative isolates. Metallo-beta-lactamases production was found to be highest among the *Klebsiella* isolates followed by *E.coli* and *Citrobactor* species. The antibiogram of the different bacterial organisms isolated from the urine specimen is reported for gram negative and gram positive isolates in Table 2 and Table 3 respectively.

Both of the *Pseudomonas* growth was sensitive to Colistin and one of them was also sensitive to Meropenem, Piperacillin-Tazobactum and Aztreonam. However both of them were resistant to Cefepime, Gentamycin, Levofloxacin, Ceftazidime, and Nitrofurantoin.

Enterococcus was the most common bacteria in the Gram positive group. All the seven were uniformly (100%) sensitive to Fosfomycin and Vancomycin followed by Nitrofurantoin (84.2%). Amongst the staphylococcal isolates, all were consistently susceptible to Fosfomycin, Amikacin, Nitrofurantoin and Vancomycin. Half of the isolates of *Staphylococcus aureus* and coagulase negative species showed susceptibility to Cefoxitin. The remaining 50% were thus considered to be methicillin resistant. High level aminoglycoside resistance was noted among 6 out of 7 *Enterococcal* isolates.

Multidrug resistance was noted in 91.2% amongst all the isolates tested. MDR frequency was higher among the gram negative isolates i.e. 94 % (63 out of 67) as compared to gram positive isolates 76.9% (10 out of 13). MDR was highest in *Klebsiella sp.* (100%) and *Enterococcus* species (100%) followed by *E.coli* (93.2%) and *Citrobactor sp.* (80%).

DISCUSSION

This study was done to identify the prevalence of UTI and antibiotic sensitivity pattern in patients presenting with LUTS in the Urology clinic of J. N. Medical College, AMU, Aligarh. In our study, males were affected more than females, which is contrary to various previously published studies[1,2,15,16]. The reason for this can be explained by the fact that three fourth of our study subjects were males. Positive culture was found more commonly in the younger age group that is less than 60 yrs. It may be because the condition of bladder outlet obstruction (BOO) which commonly affects the elderly can also present with similar set of lower urinary tract symptoms. This reason can be supported by the fact that majority of our study population were males in whom BOO is commoner than in females.

In this study *E. coli* (55%) was the most common organism isolated followed by *Klebsiella sp.* (18.8%). The commonest gram positive isolate was Enterococcus sp. (8.8%). In the gram negative group, Fosfomycin and Nitrofurantoin has good coverage which confirms the findings from other studies[1,2]. There was poor coverage by Amikacin as compared to previous study from the same center[2]. This may be due to higher empiric use of Amikacin in the region in the indoor patients because of higher sensitivity in previous years leading to the development of resistance.

Amongst the gram positives there was uniform susceptibility to Fosfomycin and Vancomycin. All the *Staphylococcal* isolates were also sensitive to Nitrofurantoin while 25% of *Enterococcus sp*. were resistant to the same. This implies that Fosfomycin and Nitrofurantoin are still showing promising results in terms of efficacy with added advantages that they are comparatively cheaper and are available in oral formulations for prescription in the outpatient setting. The current study demonstrates poor coverage by commonly prescribed Co-trimoxazole and Norfloxacin which is the same finding from previous studies[1,2,15]. Although Norfoxacin has been incorporated in the treatment options according to CLSI, we cannot continue with this antibiotic because of its poor coverage as per our results[17].

Increasing antimicrobial resistance even in uncomplicated UTI as published in various

studies is a matter of concern. From the above study we note that many of the commonly prescribed oral antibiotics are ineffective against even the common pathogens. More than 90% of pathogens in our study are already MDR. This puts pressure on the physicians to prescribe parenteral antibiotics, which can result in poor patient compliance because of cost factors and need for hospital admission. This further spirals the problem of emergence of antimicrobial resistance. Thus we have observed that there are limited options for prescribing oral antibiotics in the OPD setting.

Overall Fosfomycin is the most potent drug against both the Gram positive and Gram negative groups followed by Nitrofurantoin. In one study Fosfomycin had poor coverage in the gram positive group but our study showed that it is 100% effective against Enterococcus and Staphylococcus species[18].

There are few limitations in this study like small study population, lack of information on comorbidities and absence of radiological information to diagnose and correlate any anatomical findings.

CONCLUSION

Similar to the known fact, our study shows that *E. coli* is still the commonest bacteria responsible for lower urinary tract infection. Overall the prevalence of MDR is increasing however Fosfomycin or Nitrofurantoin can be the considered for starting empirical antibiotic therapy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

MA, AS and FK designed the study. PK and BC analyzed the data and PK wrote the manuscript. AK supervised and reviewed the manuscript. All the authors approved the manuscript.

FUNDING

None.

ETHICS STATEMENT

Informed and written consent was taken from each patient before specimen collection and processing and that the results may be included in the study.

DATA AVAILABILITY

All datasets generated or analyzed in this research are included in the manuscript.

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