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Original Research Article

Recent pattern of drug sensitivity of most commonly isolated uropathogens from Central India

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ABSTRACT

Background: The objective of the study is to assess the latest antibiotic sensitivity pattern of most commonly isolated organisms in urinary tract infection (UTI). Widespread and irrational use of antibiotics has led to development of highly resistant microorganisms. As the antibiotic sensitivity patterns of the microorganisms are frequently changing, this study was performed to assess the recent antibiotic sensitivity pattern in urinary tract infection among the human population.
Methods: The study conducted from July 2016 to April 2017, in clinical microbiology laboratory, Sara Diagnostics (fully automated) laboratories, Nagpur, Maharashtra, India. 3000 urine samples were studied. The processing of the samples were done by standard microbiological methods. The antibiotic susceptibility was measured by disk diffusion test. CLSI guidelines were used for the antibiotic susceptibility evaluation.
Results: Out of these, 1729 (57.63%) samples were culture positive showing significant bacteriuria. The most common bacteria isolated was *E. coli* (n=1040, 60.15%), followed by *Enterococcus spp.* (n=275, 15.90%), *Klebsiella pneumoniae* (n=210, 12.14%), *Staphylococcus aureus* (n=105, 6.07%) and *Pseudomonas spp.* (n=50, 2.89%) in this study. The study showed marked resistance to doxycycline, quinolones, cephalosporins and greater sensitivity to nitrofurantoin and fosfomycin, showing them better treatment options.
Conclusions: The study confirmed, *E.coli* is still the most common bacteria to cause UTI, irrespective of geographical area. Nitrofurantoin, fosfomycin showed very high susceptibility to urinary tract infection pathogens, which include most commonly isolated gram-negative bacteria and gram-positive cocci, which were resistant to other various antibiotic groups. Thus, nitrofurantoin and fosfomycin seemed to be promising drugs.

Keywords: *E. coli*, *Enterococcus spp.*, Fosfomycin, Nitrofurantoin, UTI

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infection in the human population affecting all the age groups.[1] It remains a major public health problem in terms of morbidity and financial cost. Worldwide about 150 million people are being diagnosed with urinary tract infection every year.[2] Studies suggest that the overall prevalence of UTI is higher in women, an estimated 50% of women experience at least one episode of UTI at some point of their lifetime and between 20% and 40% of women can have recurrent episodes.[3,4] UTI is less common in males as compared to females.[4]

Urinary tract infection is due to an inflammatory response of urothelium to the invading pathogenic organisms.[2] *E-coli* is the most common bacteria associated with the etiology of UTI. Other organisms

such as *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Enterococcus sp. and P. aeruginosa* are on the rise.[3,4] *E. coli* causes about 80% of acute infections [both cystitis and pyelonephritis] in patients without catheters, urologic abnormalities or calculi. To treat UTIs, updated knowledge of the organisms causing UTI and their antibiotic susceptibility is required.[5] Antibiotic susceptibility of various pathogenic organisms may vary even over short periods and depend on site of isolation and on different environments. Periodic evaluation of antibacterial activity is needed to update the information.[5-7]

High prevalence of infection, irrational uses of antibiotics, over-the counter availability of antibiotics and poor infection prevention practices leads to emergence of multi drug resistance.[8] In the context of this, present study was undertaken with the aims to study the various uropathogens and their sensitivity pattern, so that this knowledge can be utilized for betterment of the community as well as for controlling the drug resistance.

METHODS

A prospective study conducted in clinical microbiology laboratory, Sara diagnostics (fully automated) laboratories, Nagpur, Maharashtra. The study duration was from July 2016 to April 2017.

Inclusion criteria

Positive urine cultures showing pure growth of organism and their sensitivity pattern with the following criteria were included in the study

- Age above 18 years
- From all departments
- Both sex
- Both inpatients and out patients
- Only bacterial isolate.

Exclusion criteria

- Age below 18 years
- Urine cultures showing mixed organisms
- Urine cultures showing other than bacteria.

Study population and sample processing

Total 3000 patients were included in the study, of which 1279 were females and 450 were males with age above 18 years. Majority of the samples were midstream clean catch urine and others included catheterized urine samples. Samples were collected in a sterile screw-capped wide-mouth container after informing patients about proper urine collection method. Informed consent of the patients taken. The containers were labelled with a unique sample number, date and time of collection. The urine samples were processed within an hour after collection in the clinical microbiology laboratory.

Urine microscopy, gram staining, culture and antimicrobial susceptibility testing followed by MIC by Vitek 2 Compact were done during processing of the specimens. The presence of more than 1 or 5 bacteria per oil immersion field in un-centrifuged well mixed urine corresponds to a colony count of 10^5 cfu/ml (significant bacteriuria).[9] Microscopic findings in well mixed un-centrifuged urine as > 1 white blood cells (pus cells) per 7 high power fields considered as significant pyuria.[10] Semi quantitative urine culture was done using a calibrated loop. A loopful (0.001 mL) of well mixed un-centrifuged urine was inoculated onto the surface of cysteine lactose electrolyte deficient medium.[9] The culture plates were incubated aerobically at 37°C for 18-24 hours and count were expressed as colony forming units (cfu) per milliliter (ml). Single bacterial species from the urine sample with concentration of $>10^5$ cfu/ml were included for microbiological analysis. The culture isolates were identified by standard microbiological methods.[11] All culture media were procured from Hi Media laboratories, Mumbai, India.

Isolates were then tested for antimicrobial susceptibility testing on Mueller-Hinton agar plates by the standard Kirby-Bauer disc diffusion method and reported as per clinical and laboratory standards institute guidelines (CLSI).[12] Plates were incubated at 37ºC for 18-24 hours, after that inhibition zones were measured. The following antibiotic discs were used penicillin G (10 units/disc), ampicillin (10 mcg/disc), amoxiclav i.e. amoxicillin + clavulanic acid (20/10 mcg/disc), cefaclor (30 mcg), cefuroxime (30 mcg/disc), cefoxitin (30mcg/disc), ceftriaxone (30 mcg/disc), piperacillin+tazobactam (100+10mcg/disc), imipenem (10mcg/disc), co-trimoxazole (23.75/1.25 mcg/disc), novobiocin (5mcg/disc), nitrofurantoin (300 mcg/disc), ciprofloxacin (5 mcg/disc), ofloxacin (5 mcg/disc), levofloxacin (5 mcg/disc), doxycycline (30 mcg/disc), gentamicin (10 mcg/disc), amikacin (30 mcg/disc), colistin (10mcg/disc), polymyxin B (300 units/disc), tigecycline (15mcg/disc), vancomycin (30mcg/disc), linezolid (30mcg/disc), fosfomycin (200mcg/disc). Antibiotic discs were obtained from HiMedia laboratories, Mumbai, India. The results were interpreted according to clinical and laboratory standards institute guidelines.[12] The quality control strains used were *E. coli* American type culture collection (ATCC) 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus fecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 for antimicrobial discs.

RESULTS

A total of 3000 urine samples from clinically suspected patients were analyzed for UTI. Of these, 1729 (57.63%) samples were found to be culture positive showing significant bacteriuria and the remaining 1271 (42.36%) samples were either non-significant bacteriuria, polymicrobial flora or had sterile urine. Out of 1729 (culture positive cases), 1279 (73.97%) were females and

450 (26.02%) were males. Female cases outnumbered male cases. Female gender found to be significant risk factor for acquiring UTI (with statistically significant P value of <0.0000).

The urinary tract infections were found highest within 18-40 years of age group (50.82%), followed by 40-60 years (21.50%), among the female patients. Whereas majority of the isolates of male patients were obtained from age group of 61-80 years (Table 1).

Table 1: Age and gender wise distribution of the UTI cases.

Age groups in years	No. of males (UTI)	No. of females (UTI)
18-40	65 (14.4%)	650 (50.82%)
41-60	70 (15.5%)	275 (21.50%)
61-80	200 (44.4%)	204 (15.94%)
>_81	115 (25.5%)	150 (11.72%)
Total	450	1279

The most common bacteria isolated was *E. coli* (n=1040, 60.15%), followed by *Enterococcus spp*. (n=275, 15.90%), *Klebsiella pneumoniae* (n=210, 12.14%), Staphylococcus aureus (n=105, 6.07%), *Pseudomonas spp*. (n=50, 2.89%) and *Staphylococcus saprophyticus* (n=20, 1.15%) in this study. The other less frequently isolated Gram-negative bacteria were *Proteus spp*. (n=24, 1.38%), *Morganella morganii* (n=03, 0.17%), *Serratia spp*. (n= 02, 0.11) (Table 2).

Table 2: Distribution of urinary bacterial isolates.

Bacterial isolates	No. of isolates (%)
Escherichia coli	1040 (60.15)
Enterococcus spp.	275 (15.90)
Klebsiella pneumoniae	210 (12.14)
Staphylococcus aureus	105 (6.07)
Pseudomonas spp.	50 (2.89)
Proteus spp.	24 (1.38)
Staphylococcus saprophyticus	20(1.15)
Morganella morganii	03 (0.17)
Serratia spp.	02 (0.11)
Total	1729 (100)

Table 3 shows sensitivity pattern of *E. coli* (60.15%) and *Klebsiella pneumoniae* (12.15%), which are most common gram-negative bacilli isolated from UTI cases. Over all *E. coli* showed high resistance to cephalosporins, levofloxacin (52.01%) and doxycycline (65.28%). Among the 1040 *E. coli*, 541(52%) isolates were ESBL producer.[12,13] In this study, we found ESBL isolates also show co-resistance with levofloxacin and doxycycline. About 84% isolates of both *E. coli* were sensitive to nitrofurantoin and 100% were sensitive to fosfomycin. These drugs were highly active against ESBL producing *E. coli*. Isolates of *E. coli* showing resistant to various groups of antibiotics (quinolones, tetracycline group, cephalosporins) are sensitive to nitrofurantoin and fosfomycin. Gentamicin (82%), amikacin (95%) showed good sensitivity pattern in *E. coli* (Table 3).

Table 3: Antibiotic susceptibility pattern of gram-negative urinary isolates.

Drugs	*Escherichia coli* (n=1040)			*Klebsiella pneumoniae* (n=210)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin	364 (35)	_	676 (65)	_	_	210 (100)
Cefuroxime	416 (40)	_	624 (60)	77 (36.6)	-	133 (63.33)
Ceftriaxone	499 (48)	_	541 (52)	95 (45.23)	-	115 (54.76)
Ceftazidime	499 (48)	_	541 (52)	95 (45.23)	-	115 (54.76)
Piperacillin-Tazobactam	988 (95)	_	52 (5)	175 (83.33)	-	35 (16.66)
Cefoperazone-Sulbactam	1019 (98)	_	21 (2)	175 (83.33)	-	35 (16.66)
Gentamicin	853 (82)	41 (3.94)	146 (14.03)	170 (80.95)	-	40 (19.04)
Amikacin	988 (95)	20 (1.92)	32 (3.08)	174 (82.85)	-	36 (17.14)
Cotrimoxazole	572 (55)	_	468 (45)	132 (62.85)	-	78 (37.14)
Levofloxacin	468 (45)	31 (2.98)	541 (52.01)	52 (24.76)	-	158 (75.23)
Doxycycline	343 (33)	18 (1.73)	679 (65.28)	36 (17.14)	-	174 (82.85)
Tigecycline	1034 (99.42)	-	6 (0.57)	178 (84.76)	-	32 (15.23)
Imipenem	1033 (99.32)	2 (0.19)	5 (0.48)	172 (81.90)	-	38 (18.09)
Colistin	1034 (99.42)	-	6 (0.57)	182 (86.66)	-	28 (13.33)
Nitrofurantoin	874 (84)	84 (8.02)	82 (7.88)	178 (84.76)	-	32 (15.23)
Fosfomycin	1040 (100)	_	_	210 (100)	-	-

Note: *Klebsiella pneumoniae* is intrinsically resistant to ampicillin (CLSI).

In case of *Klebsiella pneumoniae*, 54.76% (115) isolates showed ESBL production which is higher than *E. coli*.[12,13] Though the number of isolates of *Klebsiella pneumoniae* is less, but showed greater amount (percentage) of resistance among all the antibiotic groups, as compared to *E. coli*. 84% isolates of *Klebsiella pneumoniae* were sensitive to nitrofurantoin and 100% were sensitive to fosfomycin (Table 3).

Majority of the *E. coli* and *Klebsiella pneumoniae* were sensitive to drugs like piperacillin-tazobactam, Cefoperazone-sulbactam, tigecycline, imipenem, colistin. Thus, these drugs seemed to be highly active against these organisms (Table 3).

Enterococcus spp. found to be second most common spp. to be isolated after *E. coli*, in current study. Most of the isolates were resistant to doxycycline (76%) and levofloxacin (69.09%). Majority of *Enterococcus* were sensitive against elevated level of gentamicin (85.81%), vancomycin (96%), linezolid (97.81%), nitrofurantoin (94.9%). 100% sensitivity shown by tigecycline and fosfomycin. 11(4%) isolates were found resistant to vancomycin. Vancomycin resistant enterococci (VRE) showed simultaneous resistance to the majority of antibiotic group. These isolates (VRE) were found sensitive to linezolid, fosfomycin, tigecycline (Table 4).

Table 4. Antibiotic susceptibility pattern of *Enterococcus sp*.

Drugs	*Enterococcus sp.* (n=275)		
	S (%)	I (%)	R (%)
Penicillin G	198 (72)	-	77 (28)
Amoxicillin	198 (72)	-	77 (28)
Piperacillin-Tazobactam	247 (89.8)		28 (10.2)
High-level Gentamicin	236 (85.81)	-	39 (14.2)
Levofloxacin	85 (30.90)	-	190 (69.09)
Doxycycline	66 (24)	-	209 (76)
Tigecycline	275 (100)	-	-
Vancomycin	264 (96)	-	11 (4)
Linezolid	269 (97.81)		6 (2.18)
Nitrofurantoin	261 (94.9)	-	14 (5.09)
Fosfomycin	275 (100)	-	-

Note: *Enterococci* found intrinsically resistant to all cephalosporins, cotrimoxazole (CLSI guidelines).

Present study found, *S. saprophyticus* found sensitive to all antibiotics. Also, *Staphylococcus aureus* showed 100% sensitivity to tigecycline, vancomycin, linezolid and fosfomycin. Fosfomycin showed 100% sensitivity against proteas isolates which are intrinsically resistant to nitrofurantoin. So, though the nitrofurantoin is not available, fosfomycin can be used to treat UTI by Proteus spp. Majority of proteus isolates shown resistance to ampicillin, cephalosporins and cotrimoxazole. Though these isolates are less frequently isolated in the study, but their antibiotic susceptibility outcome impacts the treatment options.

DISCUSSION

The study finding showed that UTI is more common in females (73.97%) as compared to males (26.02%), which is similar with the earlier studies.[3,15,16] Short female urethra, proximity of the female urethral meatus to the vagina and rectal mucosa with their abundant microbial flora and sexual intercourse have been reported as influencing factors for the higher occurrence of UTI in women.[15,16] The study shows that UTI is high among females of age groups 18-40 years (50.82%). This group includes sexually active females which contributes as risk factor for UTI.[2,3,14] Similar findings shown by Dash M et al and Nalini R et al.[2,3]

Study showed, UTI is most common in the age group of 61-80 years among males (44.4%). Elderly males had a higher incidence of UTI when compared with the elderly females (19.54%). This finding is like study conducted by Shah et al and Sood et al.[16,17] This is probably because with advancing age, the incidence of UTI increases among males due to prostate enlargement, neurogenic bladder and requirement of catheterization in the form of intervention.[16,17]

Present study showed majority of isolates were gram negative organisms. *E. coli* (60.15%) is the most common bacteria isolated among them followed by *Enterococcus spp.* (15.90%) and *Klebsiella pneumoniae* (12.14%). Similar findings shown by Bhadan et al, Dash M et al of having *E. coli* is the predominant isolate followed by *Enterococcus spp.*[3,18] *E. coli* is still the most common urinary isolate irrespective of geographical area and study confirms it. The data collected from other places around the world, also showed that *E. coli* and *Klebsiella spp*. are still the commonest uropathogens isolated in UTI patients.[19,20] Gram-negative bacteria including *Enterobacteriaceae* have several factors responsible for their attachment to uroepithelium. They colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor.[21]

Overall, gram-negative isolates showed higher resistance in the present study. The study revealed that among gram-negative bacteria, the most common isolate *E. coli* showed high resistance to commonly used empirical antibiotics like cephalosporins (60%), levofloxacin (52.01%), doxycycline (65%). Also *K. pneumoniae* showed high resistance to cephalosporin (63.33%), levofloxacin (75.23%), doxycycline (82.85%). These findings are similar to previous studies in India.[3,17,22] This high antibiotic resistance is mostly due to widespread use of antimicrobials as well as irrational prescription of antimicrobials which are available over-the-counter.[3] Study findings thus suggest that empirical treatments with these drugs are no longer be appropriate and effective. This study showed that for hospitalized patients

gentamicin, amikacin, piperacillin-tazobactam, imipenem, colistin can be better treatment options available, as they showed good amount of sensitivity. Thus, these drugs can be used as empirical treatment in hospitalized patients for urinary tract infections.

About 84% isolates of both *E. coli* and *Klebsiella pneumoniae* were sensitive to nitrofurantoin, which stand out as effective treatment option. Also, all gram-negative isolates showed 100% sensitivity to fosfomycin, found to be most active drug against them. So, for out patients, nitrofurantoin and fosfomycin serve as a perfect treatment options. Multi drug resistant isolates and isolates producing extended spectrum beta-lactamases were also found sensitive to nitrofurantoin and fosfomycin making them as one of the treatment of choice for these isolates. Re-emergence of sensitivity to nitrofurantoin is probably due to non-usage of the drug, for an extended period of time.[2] Similar findings of high susceptibility to nitrofurantoin and fosfomycin shown by Gupta V et al, Fajfr M et al, which is supportive to this study.[22,23]

In current study 15.90% *Enterococci* were isolated, found to be second most common organism causing UTI after *E. coli*. Among these enterococci, 69.09% and 76% of them were found resistant to levofloxacin and doxycycline respectively which are commonly and frequently prescribed drugs for UTI. 14.2% isolates were resistant to gentamicin. A similar rising trend of enterococcal resistance to various antibiotics was noted in another Indian study by Shinde et al in a tertiary care hospital in Mumbai.[24] Similar increasing resistance to quinolones and tetracycline group of drugs shown by Ali S et al.[25] Study has revealed very encouraging results for nitrofurantoin and linezolid, as 94.9% and 97.81% of enterococcal isolates were susceptible to them respectively. Another antimicrobial which has given increasingly encouraging results is that all our isolates remained susceptible to fosfomycin (100%) and tigecycline (100%). Ali S et al showed linezolid 100%, vancomycin 97% and nitrofurantoin 81% sensitive to enterococcal isolates.[25] One study at Rawalpindi found 144 enterococcal isolates, 99% of them were susceptible to vancomycin, 88% to nitrofurantoin which is somewhat similar to this study.[26] In one study by Allerberger F et al, fosfomycin showed an excellent bactericidal activity against gram-positive cocci, such as *Enterococcus* species, even in vancomycin-resistant strains which supports study findings, as our VRE isolates remained susceptible to fosfomycin.[27] So, even in gram positive cocci such as *Enterococcus* species, nitrofurantoin and fosfomycin stand out as treatment of choice, like that of gram negative bacilli.

CONCLUSION

In this era of ever increasing antimicrobial resistance it is mandatory that antimicrobials like nitrofurantoin, fosfomycin should be given due importance and encouragement. These are orally available and found active against most of the multi-drug resistant isolates, hence use of these drugs should be preferred and promoted. One should have vigilance on their usages so that future must have such choices available. Thus, there is need for the making and following of policy for antibiotic usages that will guide the prescription and use of antibiotics through the regular surveillance of resistant organisms in the environments.

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