Section: Pharmacology

Original Research Article

SCREENING OF BACTERIAL UROPATHOGENS CAUSING URINARY TRACT INFECTION AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN IN A TERTIARY CARE TEACHING HOSPITAL

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Abstract

Background: Urinary tract infection (UTI) is a common health problem in both community and nosocomial settings. UTI is commonly caused by bacteria and very less extent by fungi. High recurrence rates and increasing trends of antimicrobial resistance among uropathogens threaten economic burden. This study was performed to determine the prevalence of uropathogens causing UTI and to determine their pattern of antimicrobial susceptibility. **Materials and Methods:** This prospective observational study was conducted between January and August 2023. Urine samples were collected from suspected UTI patients and positive urine culture samples were considered for further analysis. All the selected urine samples were processed by standard microbiological methods and susceptibility testing was carried out by Kirby–Bauer disk diffusion method. **Results:** A total of 450 urine samples showed culture positive. Escherichia coli (n=197, 43.8%) was the commonest uropathogen identified followed by Klebsiella spp. (n=96, 21.3%), Enterococcus spp. (n=68, 15.1%), Pseudomonas aeruginosa (n=25, 5.6%) and so on respectively. E. coli was resistant to amoxicillin + clavulanic acid, cephalosporins and ciprofloxacin while it was sensitive to meropenem, piperacillin + tazobactam and amikacin. **Conclusion:** Suspected patients with UTI are at high risk of antibiotic resistance. Time to time identification of uropathogens and their susceptibility pattern is very crucial for treatment options and in patients with UTI.

INTRODUCTION

Urinary tract infection (UTI) is defined as any kind of infection which affects the upper parts of urinary system (kidneys and ureters) termed as upper UTI and lower parts (urinary bladder and urethra) termed as lower UTI.[1] However, lower UTI are the commonest as compared to upper UTI.[2] Hospital-acquired infections accounts nearly 35% of total UTI which leads to frequent hospital visits, longer hospital stays which contributes significant morbidity.[3] In addition to the hospitalized patients, individuals who are suffering from diabetes mellitus, urinary tract and neurological anatomical abnormalities also prone to get UTI with impaired urinary flow. There are several risk factors, in which the major factor includes female gender, older age, prolonged hospital stays, frequent urinary catheterizations, recurrent renal caliculi and chronic renal diseases. Serious complications related to UTI are sepsis and progressive renal damage.[4]

Commonest uropathogens causing the UTI are bacterial infections followed by fungi (Candida). However, the commonest Gram-negative organisms are Escherichia coli, Klebsiella spp., Enterobacter spp., and Proteus spp. Whereas commonest Gram-positive bacteria are Enterococcus spp. and Staphylococcus aureus.[5] Frequent prescribing or repeated administration of antibiotics and irrational way of approaching to treat UTI may lead to development of drug resistance is one of the key problems in the treatment failure.[6] If we screen out the organisms causing UTI and their sensitivity pattern towards the antibiotics, it will helpful to combat the emergence development of drug resistance among uropathogens.

Hence, the present study was aimed to investigate the commonest uropathogens, their antimicrobial sensitivity pattern and outcomes in patients with UTI in a tertiary care teaching hospital.

MATERIALS AND METHODS

This was an observational, prospective study conducted in the Department of Microbiology in collaboration with Department of Pharmacology at Government Siddhartha Medical College, Vijayawada, Andhra Pradesh. After taking the approval by the Institutional ethics committee (IEC) and the study was carried out between January 2023 and August 2023.

Suspected study participants who required urine culture and sensitivity were screening for eligibility at both outpatient and inpatient departments. Based on the culture and sensitivity report treatment had been initiated and individualised, clinical outcome was assessed on the level of clinical symptoms of UTI. All the study participants were carefully followed up during the study period.

Participants aged more than 18 years of either gender with significant microscopic growth of one or more microorganisms after 24 hours of urine culture were included in the study. Contaminated urine samples, samples with no growth after 24 hours and samples showing insignificant bacteriuria were excluded from the study.

From all eligible study participants, midstream fresh clean-catch urine samples and urinary catheter samples were collected under aseptic conditions in a sterile urinary container. 0.01 mL of collected samples were streaked on MacConkey agar and 5% sheep blood agar plates using calibrated loop then incubated at 37°C overnight for growth. Kass count more than l05 CFU/ml were considered as significant growth were subjected to antimicrobial sensitivity testing with exception of Staphylococcus aureus whereas <10 colonies (l0 CFU/ml) were considered for sensitivity testing. Kirby–Bauer disc diffusion methods on Mueller–Hinton agar was used for antimicrobial sensitivity testing on identified isolates and National Committee for Clinical Laboratory Standards guidelines (NCCLS) were followed to interpret results.[7,8]

RESULTS

A total of 1000 samples were assessed for eligibility at different clinical areas. Figure 1 shows the STROBE flow diagram. 550 out of 1000 samples were excluded from the study due to culture negative samples, insignificant growth of bacteria and lower age criteria. At the end of the study, a total of 450 urine samples had a significant growth over time and these were processed and analysed for antibiotic susceptibility testing.

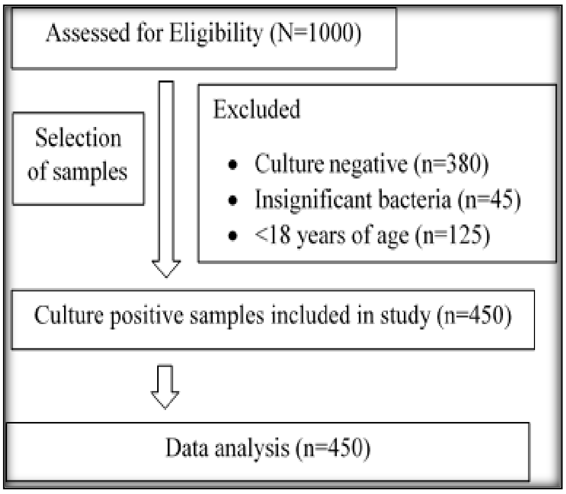

Figure 1: STROBE flow diagram

Demographic details and sample distribution was showed in Table 1. Two-thirds (68%) of study population were aged between 18-60 years and female gender was two times (62.4%) greater than males (37.6%) and mean age was 51.64 years. Samples collected from ICUs has following the reasons for hospitalization which includes acute kidney injury (n=16), chronic kidney disease with suspected UTI (n=15), ischemic heart diseases (n=7) and bronco-pulmonary diseases (n=4). Most of the inpatient sample population were admitted in the hospital due to fever with chills and suspected UTI (n=158). Urine culture for the outpatients was done in cases of suspected UTI (n = 226). Out of the 450 total culture positive samples, 390 samples had growth of only one organism, 26 samples showed multi-drug resistant strains, 11 samples showed fungal candida species and 22 samples showed mixed organisms. Klebsiella pneumoniae (n=16, 3.6%), E. coli (n=4, 0.9%), Klebsiella oxytoca (n=2, 0.4%) and each one (0.2%) of Pseudomonas aeruginosa, Acinetobacter spp., and Enterococcus spp., were showed multi-drug resistance uropathogens. More than one-fourth (84.0%) of samples were collected from fresh midstream urine and urinary catheter samples (16.0%) were obtained from ICUs and post-operative surgical wards.

Escherichia coli (n=197, 43.8%) was the commonest uropathogen identified followed by Klebsiella spp. (n=96, 21.3%), Enterococcus spp. (n=68, 15.1%), Pseudomonas aeruginosa (n=25, 5.6%) and so on respectively. Microbiological profile of culture positive uropathogens was showed in. [Table 2]

Resistance and sensitivity pattern of isolated culture positive uropathogens towards antimicrobial agents were showed in Table 3. Antibiotic drug class (broad category) was mentioned in table and drugs which are resistant to that particular broad category was mentioned in the footnote. E. coli was resistant to amoxicillin + clavulanic acid, cephalosporins and ciprofloxacin while it was sensitive to meropenem, piperacillin + tazobactam and amikacin. Klebsiella species were found to be resistant to amoxicillin + clavulanic acid and cephalosporins while being

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sensitive to aminoglycosides and meropenem. Staphylococcus aureus was sensitive to gentamicin, cefepime and ciprofloxacin and on the other hand, resistant to amoxicillin + clavulanic acid, co-trimoxazole and erythromycin. [Table 3]

Table 4 shows the WHO priority pathogens.[9] None of these organisms were encountered in the present study. [Table 4]

Table 1: Demographic details and Sample distribution

Parameter	n (%)
Total samples (n=)	450
Age	
Mean±SD, years	51.64±18.12
Range, years	19-84
Adults (18-60 years)	306 (68.0)
Elderly (>60 years)	144 (32.0)
Gender	
Female	281 (62.4)
Male	169 (37.6)
Distribution of the samples	
Total culture positive	450 (100)
Culture positive with single organism	390 (86.7)
MDR (>1 AMA)	26 (5.8)
Candida species	11 (2.4)
Mixed organisms (>1)	22 (4.9)
Source of the cases	
Outpatient	226 (50.2)
Inpatients	158 (35.1)
ICUs	42 (9.3)
Post-operative surgical wards	24 (5.3)
Source of the urine specimen	
Fresh midstream urine	378 (84.0)
Urinary catheter	72 (16.0)

SD=Standard deviation; MDR= Multi-drug resistant; AMA=Antimicrobial agent; ICU=Intensive care unit

Table 2: Microbiological profile of culture positive uropathogens

Culture positive organisms (n=450)	n (%)
Escherichia coli	197 (43.8)
Klebsiella spp.	96 (21.3)
Klebsiella pneumoniae	62 (13.8)
Klebsiella oxytoca	34 (7.5)
Enterococcus spp.	68 (15.1)
Enterococcus faecalis	42 (9.3)
Enterococcus faecium	26 (5.8)
Pseudomonas aeruginosa	25 (5.6)
Acinetobacter spp.#	18 (4.0)
Enterobacter spp. #	16 (3.6)
CoNS	11 (2.4)
Staphylococcus spp.	8 (1.8)
Staphylococcus aureus	4 (0.9)
Staphylococcus saprophyticus	4 (0.9)
Proteus spp.	7 (1.6)
Proteus mirabilis	5 (1.1)
Proteus vulgaris	2 (0.4)
Citrobacter spp. #	2 (0.4)
NFGNB	2 (0.4)

CONS= Coagulase-negative Staphylococcus aureus; NFGNB=Non-fermenting Gram-negative bacilli; #Speciation was not performed

Table 3: Resistance and sensitivity pattern of isolated culture positive uropathogens towards antimicrobial agents

Organisms	Resistance shown to AMAs	Sensitivity shown to AMAs
Escherichia coli (n=197)	β-lactam antibiotics* (n=135) Fluoroquinolones# (n=42)	Piperacillin + tazobactam (n=130) Meropenem (n=39) Amikacin (n=18) Netilmicin (n=10)
Klebsiella spp. (n=96)	β-lactam antibiotics* (n=69) Fluoroquinolones# (n=27)	Meropenem (n=58) Amikacin (n=38)
Enterococcus spp. (n=68)	β-lactam antibiotics* (n=41) Aminoglycoside‡ (n=22) Lincosamide† (n=5)	Amoxicillin + clavulanic acid (n=39) Levofloxacin (n=15) Vancomycin (n=14)
Pseudomonas aeruginosa (n=25)	β-lactam antibiotics* (n=19) Aminoglycoside‡ (n=6)	Tobramycin (n=16) Meropenem (n=9)

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Acinetobacter spp. (n=18)	β-lactam antibiotics* (n=11) Urinary antiseptics§ (n=7)	Tobramycin (n=5) Meropenem (n=5)
Enterobacter spp. (n=16)	β-lactam antibiotics* (n=9) Urinary antiseptics§ (n=6	Meropenem (n=11) Tobramycin (n=5)
CONS (n=11)	β-lactam antibiotics* (n=5) Fluoroquinolones# (n=4) Macrolides¶ (n=2)	Gentamicin (n=6) Vancomycin (n=3) Levofloxacin (n=2)
Staphylococcus spp. (n=8)	β-lactam antibiotics* (n=4) Fluoroquinolones# (n=2) Macrolides¶ (n=2)	Gentamicin (n=3) Levofloxacin (n=3) Vancomycin (n=2)
Proteus spp. (n=7)	β-lactam antibiotics* (n=4) Urinary antiseptics§ (n=3)	Piperacillin + tazobactam (n=3) Meropenem (n=2) Amikacin (n=1) Ciprofloxacin (n=1)
Citrobacter spp. (n=2)	β-lactam antibiotics* (n=1) Urinary antiseptics§ (n=1)	Meropenem (n=1) Netilmicin (n=1)
NFGNB (n=2)	β-lactam antibiotics* (n=2)	Piperacillin + tazobactam (n=1) Tobramycin (n=1)

*Amoxicillin, Ampicillin, Cefoperazone, Cefepime, Cefoxitin, Cefuroxime, Ceftriaxone, Cefexime, Ceftazidime and Piperacillin+Tazobactam, #Ciprofloxacin and Levofloxacin, †Clindamycin, ‡Gentamicin and Amikacin, §Nitrofurantoin and Nalidixic acid, ¶Erythromycin; AMAs=Antimicrobial agents, CONS= Coagulase-negative Staphylococcus aureus; NFGNB=Non-fermenting Gram-negative bacilli.

Table 4: World health organization priority pathogens

Priority 1: Critical Acinetobacter baumannii - carbapenam-resistant Pseudomonas aeruginosa - carbapenam-resistant Enterobacteriaceae - carbapenam-resistant, ESBL-producing
Priority 2: High Enterococcus faecium - vancomycin-resistant Staphylococcus aureus - methicillin-resistant, vancomycin-intermediate and resistant Helicobacter pylori - clarithromycin-resistant Campylobacter spp. - fluoroquinolone resistant Salmonellae - fluoroquinolone resistant Neisseria gonorrhoeae - cephalosporin-resistant, fluoroquinolone resistant
Priority 3: Medium Streptococcus pneumonia – penicillin non-susceptible Haemophilus influenza - ampicillin-resistant Shigella spp. - fluoroquinolone-resistant

WHO lists the above organisms as priority pathogens

DISCUSSION

UTI contributes to the most common infection, in developing countries. Urine culture is crucial for making a confirmed diagnosis, however, urine being the most common sample tested in microbiology laboratory. Antimicrobial susceptibility pattern changes from region to region and time to time.[10] In the present study, the key focus was to isolate uropathogens and identify their antimicrobial susceptibility pattern. The gender distribution in our study shows >60% positive cultures among females, similar to those of other reported studies.[11,12] This incidence of UTI occurring more in females can be correlated to their anatomy of genitourinary tract and indigenous microflora.[13]

E. coli (43.8%) causing UTI is the highest occurring pathogen followed by Klebsiella species (21.3%), Enterococcus species (15.1%), Pseudomonas aeruginosa (5.6%) in the present study. This data correlates with the study conducted by Ranjbar et al,[14] Amin et al,[15] and Rezia RA et al,[12] E. coli was resistant to amoxicillin + clavulanic acid, cephalosporins and ciprofloxacin while it was sensitive to meropenem, piperacillin + tazobactam and amikacin. Previously, a study was conducted in Beijing in the year of 1997 also claimed that 60% of E. coli were resistant to ciprofloxacin.[16] However in 2005, the resistance to ciprofloxacin and levofloxacin in E. coli drastically reduced to 21.6% and 20.4%, respectively. The reason behind resistance to antimicrobials is due to higher rate of over-the-counter usage despite lack of prescription.

Further, a study done in King Fahd Hospital, Saudi Arabia reported that meropenem was 95.8% sensitive closely followed by amikacin (93.7%) against extended-spectrum β-lactamase-producing E. coli,[17] Klebsiella was responsible for 12.2% of the organisms isolated in a study done in Kuwait,[18] which is concurrent with another study done in Aligarh, India.[19] However, in the present study, Klebsiella species accounts 21.3% which was almost double to the previous studies and it was the second most common uropathogen found in the present study, preceded by the Enterococcus species (15.1%). Klebsiella species were found to be resistant to amoxicillin + clavulanic acid and cephalosporins while being sensitive to aminoglycosides and meropenem.

Staphylococcus is not found commonly in urinary tract infections amongst general population. In the present study Staphylococcus accounted for 1.8% (S. aureus, n=4, 0.9% and S. saprophyticus, n=4, 0.9%) of UTI, which can be well compared with a similar finding obtained from a multi-center, community-based study conducted in Great Britain, where Staphylococcus aureus contributed to 0.5% of all isolates.[20] A laboratory-based study conducted in France also reported that Staphylococcus aureus accounts for 1.3% of the isolates from urine specimens acquired. The reason behind S. aureus isolated from urine was staphylococcal bacteremia.[21] In the present study, S. aureus was sensitive to gentamicin, cefepime and ciprofloxacin and on the other hand, resistant to amoxicillin + clavulanic acid, co-trimoxazole and erythromycin. Onanuga conducted a study and reported that higher resistance of S. aureus to gentamicin and vancomycin.[22]

A unified antibiotic protocol is essential in restricting the use of antimicrobials injudiciously and preventing resistance- therefore reducing the complications of UTI which aggravates from using resistant drugs. The changing susceptibility patterns of the uropathogens can be tracked by a periodical study which will be helpful in emulating an empirical therapy before the actual culture and sensitivity report comes.

CONCLUSION

The present study highlights the varying etiology of UTI and the emergence of drug resistance in pathogens. The spread of drug resistant bacteria can be prevented by the rational use of antibiotics and improving sanitation. This study focused on resistance to commonly prescribed empirical antimicrobials such as amoxicillin, ampicillin, nitrofurantoin, nalidixic acid, cefepime, cefuroxime and cefoperazone, owing to their widespread and prolonged usage in empirical therapy. Therefore, care should be taken to refrain from using these drugs as first-line empirical agents. Hence, this study aims to determine the emerging trends in the resistance of uropathogen with keen emphasis on formulating local antimicrobial policies and finally, to assist clinicians in rational choice of antibiotic therapy so as to prevent misuse or overuse of antimicrobials. This will also guide in infection control measures and other necessary strategies to be applied.

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