Original Article

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Bacteriological Profile and Antibiogram of Urinary Tract Infections at a Tertiary Care Hospital

Microbiology Section

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ABSTRACT

Introduction: Urinary tract infections (UTI) are amongst the most common cause of bacterial infections in humans. It encompasses a wide array of infections, accounting for a vast number of community as well as hospital acquired infections in developing countries. Thus, it is of utmost importance to distinguish between complicated and uncomplicated UTI because in complicated UTI the infecting pathogens are more likely to be resistant to antimicrobial agents and lead to higher mortality.

Aim: To study the bacteriological profiles of UTI in patients attending the tertiary care hospital and to study the antimicrobial sensitivity pattern of uropathogens.

Materials and Methods: A total of 500 "mid-stream" urine samples were obtained from suspected UTI patients in Microbiology Department, Smt. Kashibai Navale Medical College, Pune, India over a period of six months from September 2015 to February 2016. They were tested microbiologically and antimicrobial susceptibility tests were performed for the isolated pathogens using Kirby- Bauer disk diffusion method. Detection of Extended Spectrum Beta-Lactamase (ESBL) production in Gram negative organisms and Methicillin resistance in *Staphylococcus* was carried out according to Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: Significant bacteriuria accounted for 46% of the samples. UTI was more common in female (66%) compared to males (34%). The overall infection rate was highest in the age group of 26-44 years (38%). *Escherichia coli* (41.3%) was the most common uropathogen isolated followed by *Klebsiella* spp (18.5%) and *Enterococcus* spp (12%). Isolated pathogens were sensitive to Nitrofurantoin (85.30%), Gentamicin (78.20%), Amikacin (72.40%) and showed resistance to Ampicillin, Norfloxacin and Co-trimoxazole. ESBL production was 32.7% in *Escherichia coli* and *Klebsiella* spp. Among Enterococci 100% sensitivity was seen to Vancomycin and Teicoplanin. Methicillin resistance was seen in 1 out of 5 *S. aureus* isolates.

Conclusion: The commonly isolated uropathogens have a changing resistance pattern due to indiscriminate use of antibiotics resulting in reduced efficacy and safety of the treatment. Antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications.

Keywords: Drug resistance, *Escherichia coli*, Uropathogens

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious diseases in a clinical setting [1]. This problem spans all age groups, beginning from the neonates to the geriatric age group [2]. Apart from being the most common cause of nosocomial infection among hospitalised patients, it is also the second most common cause of hospital visits [3]. It was estimated that in a year UTI was the cause of 1 million visits to the Emergency Departments, 7 million visits to Outpatient department and about 100,000 cases of hospitalizations all over the world [4].

Prevalence of UTI is 3% in girls and 1% in boys [5]. The incidence is greater in women as compared to men due to anatomical predisposition or large bacterial load in urothelial mucosa or other host factors including obstruction in the urinary tract, sexual activity, and pregnancy [6]. Paediatric UTIs are associated with high morbidity and long term complications like renal scarring, hypertension and chronic renal failure [7,8]. Diagnosis is difficult in the neonatal period because the signs and symptoms are non-specific in this age group [9]. With advancing age, the incidence of UTI increases in men due to prostate enlargement and neurogenic bladder [10].

Common pathogenic organisms include gram negative enteric bacilli especially *Escherichia coli* and *Klebsiella* species and gram positive organisms like *Staphylococcus saprophyticus* and *Enterococcus* species [3].

In almost all cases, there is a need to start treatment before the final microbiological results are available which may lead to antibiotic resistance due to frequent misuse

of antibiotics. To aid better decision making the physician must have current knowledge of the organisms and should advice a bacteriological examination of urine sample along with their antibiogram to know the trend of antibiogram of uropathogens in the regions. Prompt diagnosis and timely antimicrobial treatment help to minimize renal scarring and progressive kidney damage [6].

It is universally accepted that UTI can only be ascertained on the basis of microscopy and microbial culture [11,12]. The dipstick/dip-slide method used in many centres serves only as a screening method but culture is needed for the final diagnosis [10]. Thus, this study was done because the causative organisms and their susceptibility pattern vary in regions and change through times. Knowledge of current local trend in our hospital is important to update recommendations for appropriate treatment and to prevent development of multi-drug resistant organisms. Ours is a laboratory based observational study. As a routine protocol, clinicians send urine samples for culture and sensitivity. Hence, no informed consent and ethical committee approval are taken.

MATERIALS AND METHODS

This observational study was carried out in the Department of Microbiology over a period of six months from September 2015 to February 2016. A total of 500 "mid-stream" urine samples were collected from suspected UTI patients of all age-groups who presented to the Outpatient and Inpatient departments of Smt. Kashibai Navale Medical College and General Hospital, Pune, India with complaints of dysuria, increased frequency and urgency of voiding, suprapubic discomfort or loin pain and fever. Out of 500 samples 378 (75.6%) samples were from females and the rest 122 (24.4%) samples were from males. Screening of the urine sample was done by gram staining. Urine culture was done by a semi-quantitative technique [13,14]. With the help of a calibrated bacteriological loop, urine (0.001ml) was cultured on blood agar and Mac Conkey's agar.

Culture results were interpreted as significant and insignificant, according to the standard Kass criteria [13,15]. A growth of $\geq 10^5$ colony forming units/ml indicated an active urinary infection and considered as significant bacteriuria [13,15-17]. Patients with significant bacteriuria were considered as having UTI. Cultures with more than three types of colonies were discarded as contaminants.

Bacterial pathogens were identified by gram reactions, motility and biochemical characteristics as per standard Microbiological techniques [18]. The antibiotic susceptibility pattern of the isolates was determined by the Kirby-Bauer disk diffusion method [19]. Gram negative bacilli (GNB) were tested against Amikacin (30 µg), Ampicillin (10 µg), Co-trimoxazole (1.25/23.75 µg), Gentamicin (10 µg), Doxycycline (30 µg), Norfloxacin (10 µg), Nitrofurantoin (300 µg). For gram positive organisms Penicillin (1u), Amikacin (30 µg), Doxycycline (30 µg), Gentamicin (10 µg), Norfloxacin (10 µg) and Nitrofurantoin (300 µg), Vancomycin (30 µg), Teicoplanin (30 µg) were used. Second line antibiotics were tested only for organisms resistant to all 1st line antimicrobials. These included Imipenem (10 µg), Cefepime (30 µg), Ofloxacin (5 µg), and Piperacillin-Tazobactam (100/10 µg).

The detection of Extended Spectrum Beta-Lactamase (ESBL) production in GNB and Methicillin resistance in *Staphylococcus* was carried out according to Clinical Laboratory Standards Institute (CLSI) guidelines [20]. Test for ESBL production was performed on Muller-Hinton agar via the disc diffusion method using Cephalosporin, Ceftazidime (30 µg) and Cefotaxime (30µg) discs alone and in combination with clavulanate. The organism was considered ESBL producing if the zone diameter was ≥ 5 mm for either antimicrobial tested in combination with clavulanate versus its zone diameter when tested alone. Statistics was not employed.

RESULTS

Out of 500 urine samples that were analysed, 230 samples (46%) were found to have significant bacteriuria and remaining 270 samples were either non-significant bacteriuria or very low bacterial count or sterile urine.

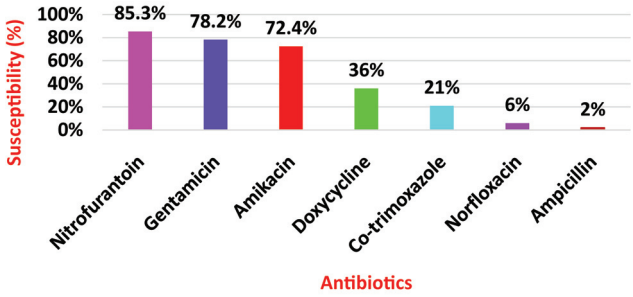
Out of the 230 samples, prevalence was higher in females (66%) compared to males (34%). The overall infection rate was highest in the age group of 26-44 years (38%).

Gram negative and gram positive organisms contributed to 82.6% and 17.4% of infections respectively [Table/Fig-1]. *Escherichia coli* (41.3%) was the predominant uropathogen isolated followed by *Klebsiella* spp (18.5%) and *Enterococcus* spp (12%).

Antibiotic susceptibility pattern among gram negative bacilli has shown maximum sensitivity to Nitrofurantoin, Amikacin and Gentamicin. Maximum resistance was seen against Ampicillin and Norfloxacin as depicted in [Table/Fig-2]. ESBL production was 32.7% in *Escherichia coli*

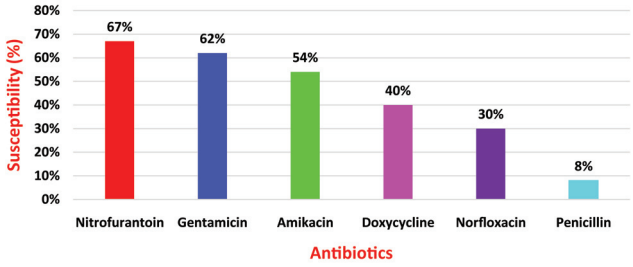
Gram negative organisms (N=190)	
E.coli	41.3%
Klebsiella	18.5%
Pseudomonas	9.8%
Acinetobacter	7.6%
Proteus	5.5%
Total	82.6%
Gram positive organisms (N=40)	
Enterococcus	12%
S. aureus	5.4%
Total	17.4%

[Table/Fig-1]: Pathogens isolated from urine samples.

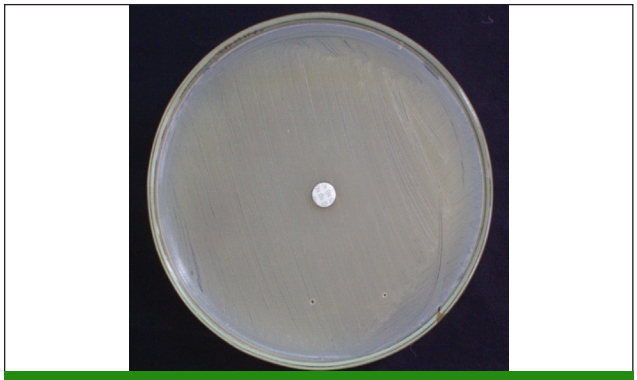


[Table/Fig-2]: Antibiotic susceptibility pattern - gram negative organisms.

and *Klebsiella* spp. Among the gram positive organisms higher sensitivity was seen with Nitrofurantoin (67%)[Table/Fig-3] and 100% sensitivity was seen to Vancomycin and Teicoplanin. Methicillin resistance was seen in 1 out of 5 *S. aureus* isolates [Table/Fig-4]. No Vancomycin resistance was observed in enterococcal isolates. 5% of *E.coli* and 2% of *Klebsiella* isolates have shown resistance to all antibiotics used. Most of the ESBL isolates and Methicillin resistant *S. aureus* isolates were multidrug resistant.



[Table/Fig-3]: Antibiotic susceptibility pattern - gram positive organisms.



[Table/Fig-4]: Testing for methicillin resistance in isolated *S.aureus* strains using Cefoxitin disk.

DISCUSSION

In a hospital setting the cause and antimicrobial susceptibility pattern of uropathogens has been changing over years. Enterobacteriaceae have shown an increase in resistance to drugs like Ampicillin, Tetracycline, Co-trimoxazole and Gentamicin as compared to a decade ago [21,22]. Proper treatment of the patients with UTI depends on correct identification of the causative organism and selection of susceptible antibiotics.

In this study, significant bacteriuria accounted for 46%. It was lower to the isolation rate reported by some studies [3,10,23] but higher than that reported by Tambekar DH et al.,[24]. Whereas, insignificant bacteriuria and sterile cultures were observed in 11.6% and 42.4% respectively. Prior antibiotic therapy before submitting the urine sample and clinical conditions like non-gonococcal urethritis or other conditions that mimic UTI could be responsible for such results [10].

A female preponderance has been reported in patients with UTI [5,9,24]. In this study also prevalence of UTI was seen more in females (66%) as compared to males (34%). Females are more prone to UTI either because the urethra is shorter and closer to the anus [3] or urothelial mucosa adherence to the mucopolysaccharide lining or other host factors [24]. Higher proportion of patients were in the age group of 26-44 years (38%). This was comparable with a study [3] in which 49% were in the age group between 21-40 years.

Several factors such as adhesion, pili, fimbriae and P1-blood group phenotype receptor are responsible for the attachment of the gram negative *Enterobacteriaceae* to the uroepithelium making them the most commonly isolated organisms [15]. In this study *E.coli* (41.3%) followed by *Klebsiella* species (18.5%) and *Enterococcus* species (12%) were the most commonly isolated organisms. *E.coli* is a normal inhabitant of the gastro-intestinal tract and thus maybe a potential source for the development of UTI [24]. Similar results were observed in other studies also [3,6,24].

In the present study, gram negative bacilli showed resistance to Ampicillin (98%), Norfloxacin (94%), Co-trimoxazole (79%) as well as to 2nd line drugs like Cefepime (91%) and Ofloxacin (82%). This may be due to the indiscriminate use of antibiotics [24]. They were sensitive to Nitrofurantoin, Gentamicin, Amikacin and Imipenem. Similar resistance patterns were seen among uropathogens in other studies [3,5,24]. Nitrofurantoin is still a sensitive antibiotic as compared to the other commonly used drugs which show significant resistance because it acts on multiple sites unlike Ampicillin which has a single target and Co-trimoxazole which has two targets. Thus, resulting in few side effects and being a safe drug even in pregnancy [25]. First generation Cephalosporins and Fluoroquinolones are commonly prescribed antibiotics in a tertiary care hospital resulting in frequent antibiotic resistance [25]. ESBL production was detected in 45 out of 138 isolates (32.7%) of *E.coli* and *Klebsiella*. ESBL is plasmid mediated and thus easily transmitted among members of *Enterobacteriaceae* resulting in resistance not only to β-lactams but also to other commonly used antibiotics

like Quinolones and Aminoglycosides [26]. Several studies have shown that ESBL producing *E.coli* isolates are also susceptible to Nitrofurantoin ranging from 70-95% [25]. The gram positive organisms were resistant to Penicillin, Norfloxacin and Gentamicin and showed 100% sensitivity to Vancomycin and Teicoplanin. No case of Vancomycin resistant enterococci was detected. Amongst the *S. aureus* 1 out of 5 isolate showed resistance to Cefoxitin. Similar results were seen in other studies [27,28].

LIMITATION

Sample size is too small to generalise the findings.

CONCLUSION

This study concludes that *E.coli* is the principal pathogen of UTIs. It also indicates a high resistance to the most commonly used antibiotics due to indiscriminate use of antibiotics. Thus in order to prevent development of resistance, antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications.

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