

## Anti-biogram pattern of uro-pathogens isolated from patients in a Tertiary Care Hospital in Karnataka, India

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### Abstract

**Introduction:** Urinary tract infection (UTI) is one of the important causes of morbidity in the community. Development of antibiotic resistance among uro-pathogens pose problem in the treatment of UTI. Hence it is essential to examine the prevalence of such uro-pathogens and study their anti-biogram.

**Materials and Methods:** Bacteria isolated from midstream urine samples collected aseptically from 266 patients over a period of 6 months from November 2016 to April 2017 were identified by standard microbiological procedures. Anti-biogram was done by Kirby-Bauer disc diffusion method and interpreted as per the CLSI guidelines.

**Result:** Out of 266 urine samples, 136 (51.12%) were found to be positive for microbial isolates, of which 96 (71.3%) samples were from females and 40 (29.4%) samples were from males. *Escherichia coli* was the predominant organism (47.05%) followed by *Pseudomonas* species (13.2%), *Enterococcus* species (11.7%), *Klebsiella* species (7.3%), *Citrobacter koseri* (4.4%) and *Proteus* species (2.9%). Enterobacteriaceae shows highest sensitivity towards nitrofurantoin (86%), amikacin (68%) and gentamicin (59%) and resistance towards norfloxacin (74%). Sensitivity of *Pseudomonas* spp was 72.2% for piperacillin/tazobactam, 44.4% for amikacin and 66% resistance to ceftazidime. Sensitivity pattern of Enterococci spp to nitrofurantoin 96.6%, aminoglycosides 78%, and fluoroquinolones 50%.

An attempt was made to study ESBL production among Enterobacteriaceae members. The isolates were 54.4% multidrug resistant (MDR) and 38.8% were ESBL producers.

**Conclusion:** In our study *E. coli* was the most common uropathogen isolated. Patterns of antibiotic resistance in a wide variety of pathogenic organism were noted. To prevent the development of resistance, periodic evaluation of antibiotic susceptibility patterns is necessary.

**Keywords:** Enterobacteriaceae, Extended spectrum beta-lactamase, Uro-pathogens.

### Introduction

Urinary tract infections (UTI) are a common, yet a major health problem in all ages accounting for high morbidity and financial cost.<sup>1</sup> About 150 million people develop UTI each year globally. It accounts for nearly 7 million office visits and 1 million emergency department visits, resulting in about 1 million hospitalizations.<sup>2</sup>

Urinary tract infection actually refers to any type of urothelial inflammatory response resulting from bacterial invasion of the urinary tract. It is commonly following procedures like catheterization and cystoscopy, making it the most common nosocomial infection, accounting for upto 40% of all nosocomial infections.<sup>3</sup> It is also commonly seen in patients with diabetes and other diseases which suppress the immune system and also in those with structural and neurological abnormalities which interfere with urinary out flow. Although UTI is seen in both genders, studies suggest a higher prevalence in women than in men.<sup>4</sup> Nearly 1 in 3 women have atleast 1 episode of UTI requiring antimicrobial therapy by the age of 24 years. Almost half of all women experience 1 UTI during life time.<sup>5</sup> In elderly men, an enlarged prostate can hinder the flow of urine, thus increasing the risk of infection. Renal calculi can also be a risk factor in both the

gender. The manifestations may vary from mild asymptomatic cystitis to pyelonephritis and septicemia.<sup>6</sup>

Even though, the microbiology of UTI has been studied for many years, there has been a subsequent change in the characteristics of the micro-organisms particularly due to antimicrobial resistance.<sup>6</sup> This increasing antimicrobial resistance complicates uncomplicated UTI treatment by increasing patient morbidity, cost of reassessment, retreatment and use of broader spectrum of antibiotics. In most cases of UTI, an empirical treatment is initially given before results of urine culture and sensitivity are available. But the increasing problem of antibiotic resistance necessitates a periodic evaluation of antimicrobial activity to update the information.<sup>7</sup>

Thus, this study was carried out to determine the prevalent uropathogens in our area and antibiotic resistance patterns among them.

### Material and Methods

**Study site and duration:** The present study was retrospective, hospital based and carried out in the Department of Microbiology KVG Medical College and Hospital, Sullia from November 2016 to April 2017.

**Inclusion criteria:** All suspected cases of UTI

**Exclusion criteria:** Patients on antibiotics in prior week.

**Collection and processing of samples:** Mid-stream clean catch urine samples were collected from all patients suspected of UTI and transported immediately to the laboratory without any delay. Microscopic examination (wet mount) of urine was done. The wet preparation technique is a quick and simple method ideal for the identification of crystals, casts and pus cells.

Urine culture was done on blood agar, MacConkey's agar and cysteine lactose electrolyte deficient (CLED) agar by semi-quantitative method. Significant growth was considered in case of colony count amounting to  $\geq 10^5$  colony forming unit/mL (CFU/mL) as per standard. Symptomatic cases with a lower count were also considered for the study. Repeated isolation of a single pathogen in freshly collected MSU or in symptomatic cases were considered significant even with a low bacterial count.

Identification of bacteria was done by using standard microbiological procedures.<sup>8,9</sup> Candida species were identified by Gram's staining, germ tube formation<sup>10</sup> and by using Chrome agar.

**Antimicrobial susceptibility testing:** This was done by the modified Kirby Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines.<sup>11</sup> All Enterobacteriaceae members were tested against nitrofurantoin (300 $\mu$ g), amikacin (30 $\mu$ g), gentamicin (10 $\mu$ g), cefotaxime (30 $\mu$ g), norfloxacin (10 $\mu$ g), nalidixic acid (30 $\mu$ g), ceftriaxone (30 $\mu$ g), ceftazidime (30 $\mu$ g). *Pseudomonas aeruginosa* were tested against ceftazidime (30 $\mu$ g), sparfloxacin (30 $\mu$ g), ciprofloxacin (5 $\mu$ g), cotrimoxazole (1.25/23.75 $\mu$ g), ceftriaxone (30 $\mu$ g), amikacin (30 $\mu$ g), gentamicin (10 $\mu$ g), piperacillin/tazobactam (100/10 $\mu$ g), imipenem (10 $\mu$ g), meropenem (10 $\mu$ g), and ceftriaxone/tazobactam (30/10 $\mu$ g). Enterococcus spp. were tested against nitrofurantoin (300 $\mu$ g), gatifloxacin

(5 $\mu$ g), norfloxacin (10 $\mu$ g), nalidixic acid (30 $\mu$ g), Amikacin (30 $\mu$ g) high level gentamicin (120 $\mu$ g). E.coli ATCC 25922, and P.aeruginosa ATCC 27853 were used as controls.

### ESBL detection

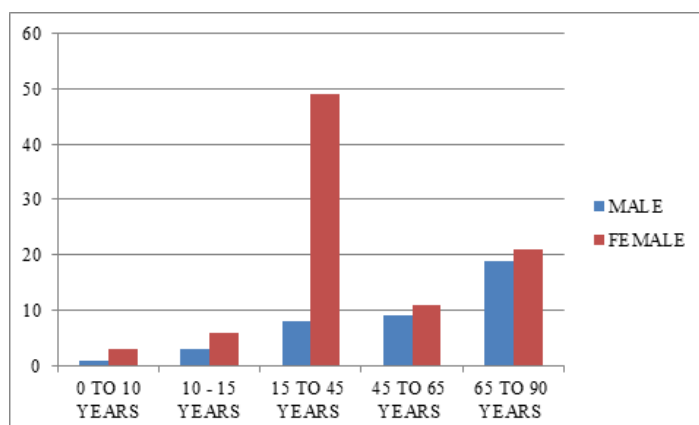
All the isolates showing resistance to third generation cephalosporins, namely ceftazidime, ceftriaxone and cefotaxime, were further tested for confirmation of extended spectrum  $\beta$ -lactamase production by phenotypic methods.

### Phenotypic confirmatory test for extended-spectrum beta-lactamase production

As per the guidelines of CLSI, the test was done with confluent growth of the test isolates (0.5 McFarland standard) on Mueller-Hinton agar plates, with a distance of 20 mm of disc containing cefotaxime disc (30  $\mu$ g/disc) and ceftazidime disc (30 $\mu$ g/disc) with the combination of cefotaxime with clavulanate (30  $\mu$ g + 10 $\mu$ g/disc) and ceftazidime with clavulanate (30  $\mu$ g + 10 $\mu$ g/disc). Plates were incubated overnight at 37°C, and after incubation a zone of inhibition that showed  $\geq 5$  mm increased in the cephalosporin discs and their respective cephalosporin/clavulanate discs was taken to be a phenotypic confirmation of ESBL production.<sup>12</sup>

### Results

Out of 266 urine samples, 136 (51.12%) were found to be positive for microbial isolates. Overall prevalence of UTI was higher in female 96 (71.3%) and prevalence among males was 40 (29.4%). Significant bacteriuria was seen in 136 (51.1%) patients and 13 (4.8%) patients had an insignificant colony count. No growth was seen in 100 (37.5%) specimen and mixed growth was seen in 17 (6.3%) samples. Department wise distribution showed that maximum numbers of samples were from obstetrics & gynecology (40%) followed by surgery (22%), medicine (15%), pediatrics (9%), Orthopedics (8%) and ENT & Dermatology (3%).



**Fig. 1: Age/Sex wise distribution of patients with UTI**

Most of the cases were recorded in 15-49 (41.9%) years of age group followed by 65-90(29.4%) years. In both age groups females showed higher risk of developing UTI than male. Age/sex wise distribution is given in (Fig. 1).

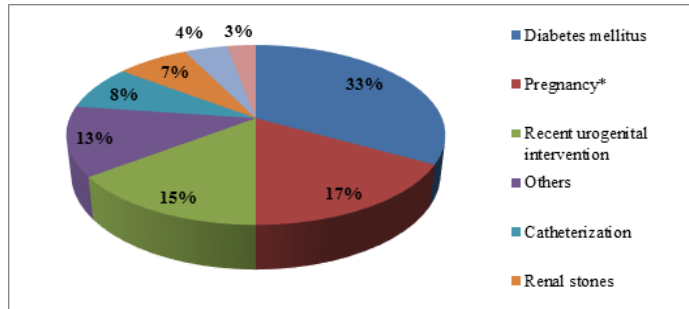


Fig. 2: Risk factors in subjects with UTI

\*23.9% females with UTI were pregnant.

Diabetes (33.08%) was the most common factor associated with UTI in our study. Pregnancy (16.9%) was the second most common risk factor associated with UTI. While the recent history of uro-genital instrumentation other than catheterization was present in 14.7 % of the study subjects, catheterization alone posed a significant risk factor seen in 8.08% of the patients. Others (12.5%) include patients in reproductive age group without any other complication. (Fig. 2)

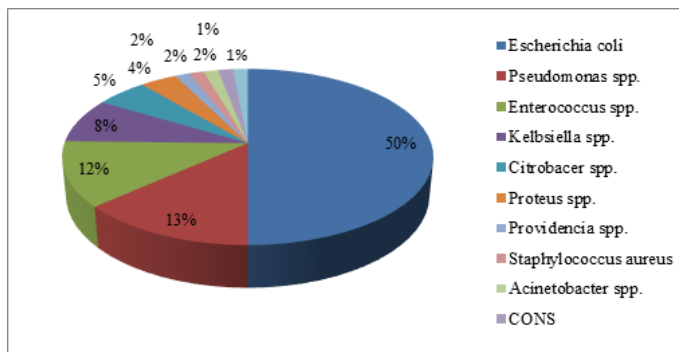


Fig. 3: Frequency of various pathogenic organisms in the study (n=136)

Escherichia coli were the predominant organism (47.05%) and second most common organism isolated were Pseudomonas species (13.2%) and third most common organism was Enterococci species (11.7%) followed by Klebsiella species(7.3%), Citrobacter koseri (4.4%), Proteus species (2.9%). Incidence of Providencia spp, Staphylococcus aureus, Acinetobacter spp, Coagulase negative Staphylococcus (CONS) and Candida species were (1.4%). (Fig. 3)

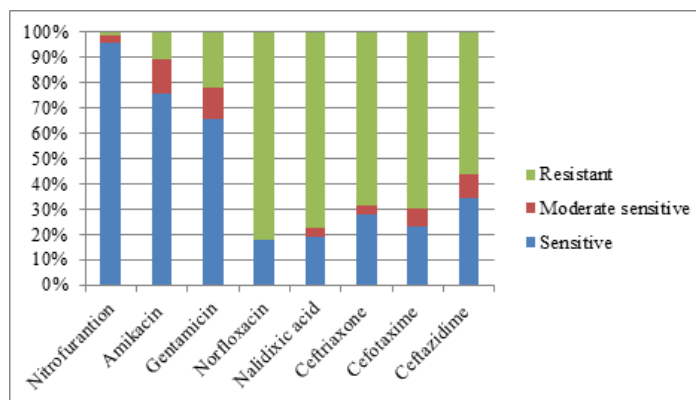


Fig. 4: Antibiotic sensitivity pattern for members of Enterobacteriaceae (n=92)

A total of 92 Enterobacteriaceae members were isolated from the urine samples. They showed maximum sensitivity towards nitrofurantion (95.5%), amikacin (68%) and gentamicin (59%) and maximum resistance towards norfloxacin (82.6%), nalidixic acid (70%) and third generation cephalosporin (56-63%). Antibiotic sensitivity pattern is given in Fig. 4.

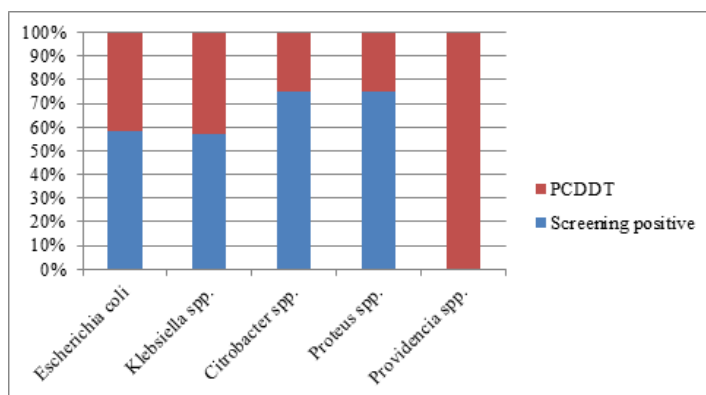


Fig. 5: Frequency of ESBL production among Enterobacteriaceae (n=92)

Out of 92 Enterobacteriaceae 53 (57.6%) were showing multidrug resistance (MDR).<sup>13</sup> These were subjected for ESBL detection by phenotypic disc diffusion test. Among these 36(38.8%) were confirmed ESBL producers. Highest prevalence (75.0%) was shown by strains of *E. Coli* followed by *Klebsiella spp.* (16.6%), *Citrobacter spp.*, *Proteus spp.* and *Providencia spp.* (2.7%) as shown in Fig. 5

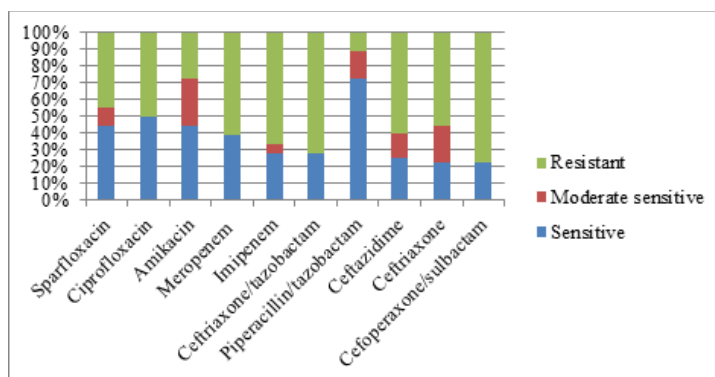


Fig. 6: Antibiotic sensitivity pattern for *Pseudomonas spp* (n=18)

*Pseudomonas spp.* was the second most common isolated organism, it showed maximum sensitivity 72.2% to piperacillin/ tazobactam and maximum resistance 77.7% towards combination of cefoperzone/ sulbactam. Antibiotic sensitivity pattern of *Pseudomonas spp.* is given Fig. 6

The third most common isolated organism was *Enterococci spp.* showed maximum sensitivity towards nitrofurantoin (96.6%), amikacin (81.2%), gentamicin (75.0%) and maximum resistance to fluoroquinolones (50%).

## Discussion

UTI ranks among the most common infection in developing countries (47%-55%)<sup>4,16-18</sup> and in the present study it was 51.12%.

Our study showed a high prevalence of UTI in females (73.57%) than in males (35.14%) which correlates with other findings which also revealed that

the frequency of UTI is greater in females as compared to males.<sup>4,5,14,15</sup> The reason behind this high prevalence of UTI in females may be due to close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse favouring the entry of bacteria into urethra and other common reasons are incontinence, and pregnancy.<sup>4,5,14</sup> Females of the age group 15-49 years were found to be more susceptible (90.69%) to UTI followed by 65-90 years (82.93%). These findings are similar to other reports which showed that females are more prone to UTIs than males during adolescence and old age.<sup>15-17</sup> While increasing incidence of UTI in young age females are associated with high sexual activity, recent use of a diaphragm with spermicide, and a history of recurrent UTIs,<sup>16,17</sup> in elderly female it is due to less vaginal acidity and waxing defense mechanism.

In the present study, diabetes mellitus (33.08%) was the most frequently associated risk factor for UTI

which corroborates with the incidence of UTI as reported by Rawal *et al* (42.9%) in Ahmedabad.<sup>17</sup> Diabetes mellitus is associated with many complications and in the long run it has some major effects on the urinary system which makes diabetic patients more vulnerable to UTI.<sup>18</sup> Hyperglycemic urine promotes rapid bacterial growth and colonization. Autonomic neuropathy in diabetes mellitus impairs bladder emptying and subsequent urological manipulation pre-dispose to UTI.<sup>18</sup> Pregnancy also showed high association with UTI (23.9%) among females. Manjula *et al* in Bangalore reported 49.9% incidence of UTI in their study.<sup>19</sup> The hormonal changes in pregnancy leads to decreased bladder tone, diminished peristalsis and dilatation of renal pelvis and ureter. Pregnancy produces physical obstruction in the female urinary tract, leading to retention of urine which is one of the important risk factor for the development of the infection.<sup>20,21</sup> Catheterization was also a common risk factor in the present study leading to catheter-associated UTI in any age group. For either short- or long-term catheters, the infection rate is 8.08%. Infection spreads by biofilm formation on both internal (intraluminal route) and external (periurethral route) catheter surface.<sup>19,22,23</sup>

The uropathogens isolated were similar to many other studies from different regions of India. Our study corroborates with the studies done by Bhargavi *et al*<sup>24</sup> in southeast India, Murugan *et al*<sup>25</sup> in Tamil Nadu and Prakash *et al*<sup>26</sup> in U.P.

Gram-negative organisms are the most common organisms causing UTIs, and they collectively account for more than 75% of cases. Higher incidence of Gram negative bacteria belonging to Enterobacteriaceae, in causing UTI is associated with many factors which are responsible for their attachment to the uroepithelium. In addition, they are able to colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor.<sup>19</sup>

In our study *Escherichia coli* was the most common uropathogen accounting for 47.05% of cases. The findings are similar to studies done by Lakshmi *et al*<sup>28</sup> (66.45%) and Raval *et al*<sup>17</sup> (35%). The incidence of *E. coli* as a causative pathogen of UTI in India varies from 48% to 65% as reported by various studies done earlier.<sup>18-22</sup>

*Pseudomonas spp.* was the second most common uropathogen accounting for 13.2% of cases which was similar to study done by Prakash *et al* (12.90%).<sup>26</sup> In our study only 11.7% of the isolates were *Enterococci spp.* According to study of Sood *et al.* from Jaipur *Enterococci spp.* accounted for 9.24% of urinary tract isolates.<sup>15</sup>

The antibiotic sensitivity pattern was similar to other studies done in India.<sup>12,24,28,29</sup> The members of Enterobacteriaceae were showing maximum sensitivity towards nitrofurantoin (95.5%), amikacin(75.5%), gentamicin (65.5%) and maximum resistance towards

norfloxacin (82.6%), nalidixic acid(76.0%) which correlates with the study results of Aruna *et al*<sup>33</sup> and Eshwarappa *et al*<sup>32</sup> and resistance towards third generation cephalosporin like ceftazidime (37.7%), ceftriaxone (27.7%) and cefotaxime (23.3%) were lower as compared to studies done by Gupta *et al.*<sup>29</sup> The sensitivity pattern for third generation cephalosporin reported by Gupta *et al*<sup>29</sup> was ceftazidime 48.3%, ceftriaxone 33.7% and cefotaxime 39.4%. Resistance towards cefotaxime (70%) ceftriaxone (68.8%) and ceftazidime (62.2%) was analogous to studies by Bhargavi *et al*<sup>24</sup> (cefotaxime 69%, ceftazidime 65%, ceftriaxone 60%) and Manjunath *et al*<sup>30</sup> (cefotaxime 68%, ceftriaxone 65%, ceftazidime 63%).

*Pseudomonas spp* was sensitive to piperacillin/tazobactam is 72.2%, ciprofloxacin 50%, for amikacin and sparfloxacin 44.4%, meropenem 38%, for both imipenem and ceftazidime 27.7%, for both ceftriaxone and cefoperazone/sulbactam 22.2%. It was resistant to cefoperazone/sulbactam 77.7%, ceftriaxone/tazobactam 72.2%, resistance for both imipenem and ceftazidime was 66%. Sensitivity and resistance pattern were similar to studies done by Sood *et al*<sup>15</sup> in Jaipur and Kalal *et al*<sup>36</sup> in Bangalore.

The antibiotic sensitivity pattern for third highest recorded organism *Enterococcus spp.* was as follows: (96.6%) sensitive for nitrofurantoin, (81.2%) for amikacin, (75%) for gentamicin, (62.5%) for nalidixic acid, (50%) for both norfloxacin and gatifloxacin. Sensitivity pattern was similar to studies by Sood *et al*<sup>15</sup> in Jaipur and Llyods *et al*<sup>27</sup> in South Michigan.

In our study most of Enterobacteriaceae were showing multi-drug resistance and which were subjected for ESBL detection. Out of 53(57.6%) isolates were tested for ESBL production and 36(67.9%) were confirmed for ESBL production and findings corroborates with previous reports from Bangalore<sup>32</sup> and Mumbai.<sup>33</sup>

Extended spectrum  $\beta$ - Lactamase producers do not respond to the usually prescribed empirical therapy and there is an increased risk of associated morbidity and mortality. Factors which might have led to the high prevalence of the ESBL producers could be indwelling catheters, invasive procedures, and severity of the illness and excessive use of cephalosporins. Presently, alternative antimicrobial therapy to treat ESBL-positive UTI on outpatient basis is limited. Carbapenems are the most effective drugs in this situation<sup>34,35</sup> but need to be administered intravenously or intramuscularly. All this and the high rate of ESBL positivity in the present study warrant a change in the empirical therapy of UTI to prevent the complications.

## Conclusion

*Escherichia. coli* was the most common uropathogen isolated in our hospital, and is showing highest sensitivity towards nitrofurantoin and resistant towards norfloxacin and third generation

cephalosporins. Extended spectrum beta- lactamase producers were also reported. Diabetic patients were at a higher risk of developing UTI. Higher prevalence of UTI was seen in females.

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