Bacteriological profile and antibiogram of urinary tract infections at a tertiary care hospital

V.P. Sarasu[1], S. Ramalatha Rani[2,*]

[1,2]Senior Assistant Professor, Dept. of Microbiology, KAPVGMC, Trichy

***Corresponding Author:**
Email: drrlrrmr@gmail.com

Abstract

A prospective laboratory based observational study collaborating with medicine, surgery, paediatrics, nephrology, urology, obstetrics & gynaecology and STD departments was carried out over a period of one year with 500 patients suffering from UTI, which included 49% of males and 51% of females. Among the 500 patients, significant bacteriuria was detected in 150 patients that constitute 30% of the sample tested. The highest incidence of urinary tract infection (UTI) 41.3% was recorded in paediatric age group followed by reproductive age group (33.3%). In this study, 129 Gram Negative Bacilli and 21 Gram Positive Cocci were isolated among which, *E. coli* (57%) was the commonest organism followed by *Klebsiella spp* (20 %). In addition, presence of *Pseudomonas aeruginosa* (3%), Proteus (2%), Enterobacter (1.35%) and *Citrobacter* (1.35%) and Acinetobacter (2 %) were also detected from the test samples. Among the Gram Positive cocci, 7% were CONS, 5% were *Staphylococcus aureus* and 1.3% were *Enterococcus faecalis*. All the Gram negative bacilli were mostly found sensitive to Imepenam (100%) whereas, the Gram positive cocci were sensitive to Vancomycin (100%) followed by Amikacin (84%), Levofloxacin (83%), Cefipime (81%), Cefoxitin (76%) and Nitrofurantoin (61%). Ampicillin and Co-trimoxazole were highly resistant showed only 11% and 13% sensitivity. Cephlosporins, Nalidixic acid and Norfloxacin were resistant to all the isolated pathogen by 50% and more.

Keywords: Urinary Tract Infection, Significant bacteriuria, Uropathogens, *E. coli*, Mid stream urine, Cephalosporins, Aminoglycosides.

Introduction

Urinary Tract Infection (UTI) is one of the most common infectious diseases in clinical settings.(1) This problem spans all age groups, beginning from the neonates to the geriatric age groups.(2) Apart from being the most common cause of nosocomial infection among hospitalized patients, it is also the second most common cause of hospital visit.(3) UTI was found the cause of 1.0 million visits to the Emergency departments, 7 million visits to outpatient department and about 1,00,000 cases of hospitalizations all over the world annually.(4)

More than 90% of acute UTI in community acquired infection is caused by *Escherichia coli* and 10–20% by CONS especially *Staphylococcus saphrophyticus* which is the second most common cause in young sexually active women and 5% or less by other Enterobacteriaceae and Enterococci. In complicated cases of UTI resulting from anatomical obstruction and catheterization, the most common causative agents are *E. coli* followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus* spp.(5,6)

In general, there is a need to start treatment before the final microbiological reports are available, which may lead to frequent misuse of antibiotics. For better decision making the physician must have current knowledge about the uropathogens and should advice a bacteriological examination of urine sample along with their anti-biogram to know the trend of anti-biogram of uropathogens in their region. This study was done because 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 appropriate treatment and to prevent development of multi drug resistant organisms.

Objectives

1. To determine the prevalence of UTI according to the age, sex and department
2. To find out the bacteriological profile of Urinary Tract Infections.
3. To study the antimicrobial sensitivity pattern of isolated Uropathogens

Materials and Method

A prospective Laboratory based observational study was carried out in the Department of Microbiology, Thanjavur Medical College over a period of one year collaborating with Medicine, Surgery, Paediatrics, Nephrology, Urology, Obstetrics, Gynaecology and STD departments with 500 patients suffering from UTI, which included 49% of males and 51% of females. Prior approval obtained from Ethical Committee and Informed consent obtained from each patient. The inclusion criteria were fresh case of UTI, no H/O antibiotic intake, no H/O instrumentation, non pregnant women, no H/O recent delivery and no H/O liver or renal dysfunction. The exclusion criteria were those with Diabetes mellitus and associated co-morbid conditions, promiscuous individual, repeated catheterization, instrumentation and on antimicrobial therapy. The antimicrobial sensitivity pattern for all the isolates were done in Muller Hinton Agar by modified

Kirby – Bauer disc diffusion method as per CLSI guidelines using antibiotic discs.

The patients of all age groups belonging to both the sex with fever, dysuria, frequency, urgency, lower abdominal pain / flank pain and supra pubic tenderness that are suggestive of upper and lower Urinary tract infections were considered and included in the study.

Urine samples were collected in 50ml wide mouth sterile container as per CLSI guidelines for urine Group16-A2.[8,9] The clean catch mid-stream urine collection[8] method were followed in the study. The female patients were asked to clean the area around the urethral opening with soap and water, and instructed to rinse well and collect the urine with the labia held apart, male patients were asked to wash periurethral region and by retracting the foreskin, and they were asked to void few ml of urine initially then collect about 20ml during midstream. Immediately after collection, the samples were labelled and transported to the laboratory and processed within two hours.

Urine specimen were examined macroscopically for the presence of colour and turbidity [8,9] followed by Gram staining and culture. Gram staining was done and examined under oil immersion. Presence of ≥1 to 5 bacteria / OIF was taken as significant bacteriuria accounts for $>10^5$ CFU/ml, and the presence of pus cells >1 / OIF taken as definite indication of UTI.[8]

The culture was processed by the following method.[8,10] Uncentrifuged urine was mixed well by gently rotating the container by keeping it over the table. Using a calibrated loop (0.001), each sample was inoculated in the nutrient agar, Mac Conkey agar, blood agar. Loop was flamed, after cooling, it was inserted vertically into the urine to allow urine to adhere to the loop. And the culture plate was inoculated by keeping this loop in the centre of the plate and the inoculum was spread in a line on either side. Then without flaming, loop was drawn across the entire plate, crossing the first inoculums, streak numerous times to produce isolated colonies.

Plates were incubated for 24 hrs at 35-37ºC. Colonies were counted on each plate and the number of colonies were multiplied by 1000 to determine the number of microorganism per ml in the original specimen. A single type of colony was counted and more than 1,00,000 CFU /ml was interpreted as, *significant bacteriuria*. The isolated colony was identified by adopting the procedures of Gram staining, motility and routine biochemical reactions.[10,11]

The antimicrobial sensitivity pattern for all the isolates were done in Muller Hinton Agar by modified Kirby – Bauer disc diffusion method as per CLSI guidelines using antibiotic discs (Himedia, Mumbai).

Results

Collection of samples based on age and gender: Among the samples collected from 500 patients both inpatient and outpatients, 49% were male, 51% were female, mean age was 39 years with the range from new born to 78 years. The observations showed 53.8% (27.2% of male and female 26.6%) of the patients were less than 14 years of age. 29% (11.6% + 17.4%) of patients were in reproductive age group, 12.8% (7.4% + 5.4%) of patients from middle age group, 4.4% (2.8% + 1.6%) of patients were in older age groups more than 60 years.

Age and gender wise prevalence of UTI: Among the 500 samples tested only 30% showed significant bacteriuria. In 150 bacterial isolates 54% were from female patients and 46% from male patients with a male to female ratio of 1:1.2 (Table 1). Large numbers of isolates were found in paediatric age group 41% (17.3% + 24%) followed by 33.4% (12.7% + 20.7%) were from reproductive age group and 15.3% (10% + 5.3%) from middle age and the rest 10% (6% + 4%) were from old age group.

Department wise prevalence of UTI: Among the 150 patients showing UTI, 130 patients i.e., 86.4% were in-patients and the remaining 20 patients (13.6%) were out-patients. The department wise distribution of patients revealed that, the prevalence of UTI was maximum in the patients admitted in the department of Paediatrics which registered the highest number of 57 UTI cases (38%) followed by departments of medicine (26.6%) and surgery (15.3%). Of the 15 UTI cases in the department of obstetrics and gynaecology, 13 were from in-patients and only two were from out-patients. Among all the departments, the departments of nephrology and STD recorded the least cases of UTI (1.4% each) Table 2.

Prevalence of urinary pathogens: Among the 150 pathogens isolated from the samples, the Gram Negative Bacilli (GNB) with 129 isolates (86.0%) was the major cause for UTI while only 21 isolates were Gram positive cocci (GPC). Among the 129 GNB, the *Escherichia coli* and *Klebsiella spp* alone constituted 77% of total isolates with 85 isolates of *E. coli* and 30 isolates of *Klebsiella* spp. The remaining GNB isolates includes, 4 isolates of *Pseudomonas aeruginosa*, 3 isolates from *Proteus spp* and Acinetobacter. *Citrobacter* and Enterobacter accounted 2 each. Among the 21 isolates of GPC, 11 were coagulase negative *Staphylococcus*, 8 were *Staphylococcus aureus* and 2 isolates were *Enterococcus faecalis.* Out of 11 CONS, 6 were *Staphylococcus epidermidis* and 5 isolates were *Staphylococcus saprophyticus.* (Fig. 1).

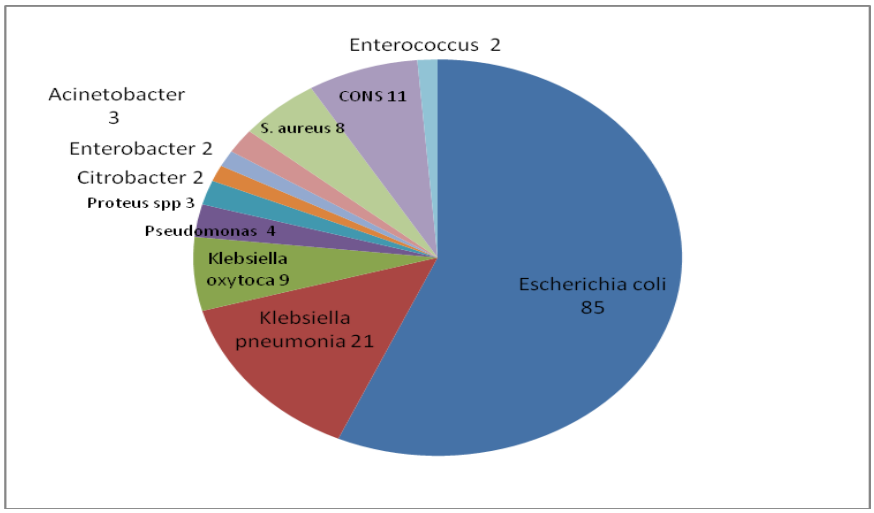

Fig. 1: Distribution of pathogens isolated in UTI

Antimicrobial susceptibility pattern: The antibiogram revealed that, all the isolated bacteria such as *E.coli, Klebsiella spp, Pseudomonas aurogenosa*, Citrobacter, Enterobacter, Acinetobacter, had a maximum sensitivity pattern to imipenem (100%) and *Staphylococcus aureus,* CONS and *Enterococcus faecalis* had a maximum sensitivity to vancomycin(100%) followed by amikacin (84%), levofloxacin (83%), cefepime (81%), cefoxitin (76%), nitrofurantoin (61%) and ciprofloxacin (48%). Lower sensitivity pattern observed in ampicillin (11%), co-trimoxazole (13%), nalidixic acid (21%), cephalexin (15%), cefuroxime (25%), norfloxacin (25%) and gentamicin (35%). Fig. 2.

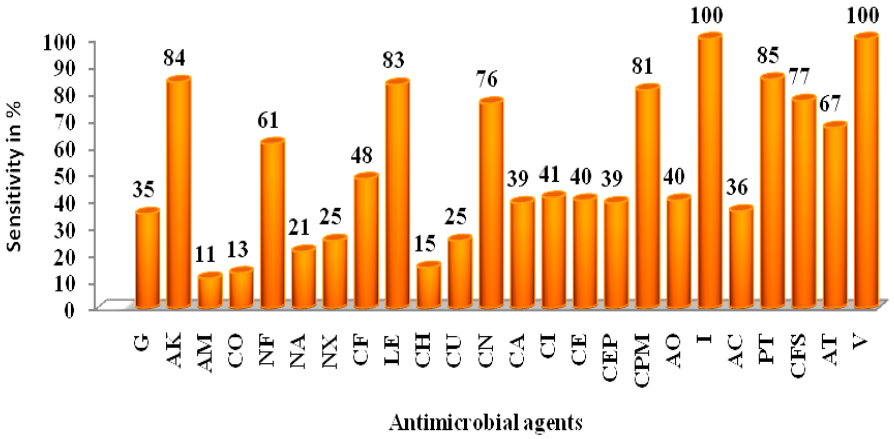

Fig. 2: Antimicrobial susceptibility pattern of isolated pathogens

Table 1: Age and gender wise prevalence of UTI

Age Group	Male	%	Female	%	Total	%
0 - 13 years	26	17.3	36	24.0	62	41.3
15-44 years	19	12.7	31	20.7	50	33.4
45-60 years	15	10.0	8	5.3	23	15.3
Old age (>60 years)	9	6.0	6	4.0	15	10.0
Total	69	46.0	81	54.0	150	100

Table 2: Department wise prevalence of UTI (n= 150)

Department	IP		OP		Total (n= 150)	
	Number	%	Number	%	Number	%
Paediatrics	52	34.6	5	3.4	57	38
Medicine	35	23.3	5	3.4	40	26.6
Surgery	21	14.0	2	1.4	23	15.3
Obstetrics & gynaecology	13	8.6	2	1.4	15	10

Urology	7	4.6	4	2.6	11	7.3
Nephrology	2	1.3	-	-	2	1.4
STD	-	-	2	1.4	2	1.4
Total	130	86.4	20	13.6	150	100

Table 3: Comparison of Common organisms causing UTI

Organisms	Latin America[49]	India[7]	Tamil Nadu[78]	Present study
E. coli	60.4%	61%	30.2%	57%
Klebsiella	11.1%	22%	22%	20%
Pseudomonas	8.3%	4%	12.35%	3%
Acinetobacter	10%	3%	8.3%	2%
Proteus	4.6%	-	6.7%	2%
Enterobacter	14%	-	35%	1.3%
Citrobacter	7%	2%	2.5%	1.3%
CONS	-	7%	5%	7%
Enterococcus	2.3%	1%	9.5%	1.3%

Table 4: Comparison of *E. coli* isolates among UTI in various part of the world

Sl. No.	Name of the Country/ State	*E. coli* (%)	Reference
A	**International**		*Sana et al. (2005)*[30]
1**.**	Israel	94	
1.	USA	90	
2.	Kuwait	90	
3.	England	75	
4.	Sweden	74	
5.	Italy	69	
6.	Japan	65	
7.	Bangladesh	48.1	Mouse *et al. (2015)*[20]
B	**National**		
1.	Kashmir	90	Chatterjee *et al*. (2009)[37]
2.	Maharashtra	83.4	Chatterjee *et al*. (2009)[37]
3.	Tamil Nadu	49.3	Baby Padmini and Appalaraju (2004)[18]
4.	New Delhi	46	Mohanty *et al.* (2005)[31]
5.	Pune	41.3	Ghadage *et al.* (2016)[21]
5.	Present study	57	

Discussion

Urinary tract infections (UTI) are the most common bacterial infection among the humans. Perhaps one of the most important factors impacting the management of UTI over the past decade is emergence of antimicrobial resistance among uropathogens.[12] During the neonatal period about one per cent of all babies have bacteria in bladder urine then up to three months UTI is more common in male babies after that UTI predominates in females of all age groups. Again after 60 years UTI incidence increases in males.[13]

In the present study 500 samples were collected by MSU. Among the samples 150 (30%) showed significant growth of bacteria. According to Chua *et al*. (1988)[14] in Philippines, the clean-catch midstream urine collection is primarily aimed at avoiding contamination of voided urine by urethral and perineal flora, which might confuse the interpretation of culture results. The normal urethral flora consists primarily of diphtheriods, streptococci and staphylococci.[15] In contrast, Morris *et al*. (1979)[16] concluded that in ambulant adult perineal cleansing before voided urine sample is taken does not influence the bacteriologic finding. In a study, Turner (1961)[17] on pregnant women, demonstrated that vulvar cleansing did not decrease the contamination rate of midstream voided specimen. It is also probable that in some instance, the use of disinfectant and antiseptics in the cleansing procedure might alter and decrease the true bacterial count.

Significant bacteriuria showed in 30% of 500 tested samples. Out of 150 bacterial isolates 46% were males and females were 54% with the male to female ratio of 1:1.2. Similar finding (1:1.3) was shown by

Baby padmini and Appalaraju (2004)[18] in Tamil Nadu. According to Foxman *et al.* (2003),[19] USA the ratio was 1:4.2. In every age group there was a higher incidence of UTI in adult female than male with an annual incidence of 12.6% in women as compared with 3% among men (*Mouse et al. (2015)*[20] *Ghadage et al.* (2016).[21] Incidence of UTI is higher in females because of the shorter urethra, that bacteria have less distance to travel to reach the bladder. In addition, the urethral meatus opens into the moist introitus which is colonized by bacteria having the potential to cause cystitis. Sexual intercourse, pregnancy and postmenopausal state also favours occurrence of UTI in females.[22]

In the present study, more number of UTI were found in paediatric age group of 41.3%, followed by 33.3% were from reproductive age, 15.3% were in middle age and elderly accounts for 10%. Bacteriuria is common in association with UTI in male new-borns. When infection occurs in pre-school boys, it is frequently associated with serious congenital abnormalities. The prevalence of significant bacteriuria in this age group is 4.5% for girls and 0.5% for boys. About one third of these patients had some referable urinary tract abnormality when bacteriuria was first detected. The presence of bacteriuria in childhood defines a population at higher risk for development of bacteriuria in adult. ESBL producing *E. coli* may be causative agent of UTI in children without any specific risk factor.[29] The similar findings showed in which the prevalence of bacteriuria in adult men is low (0.1% or less). In young men, a lack of circumcision may also increase the risk for UTI caused by uropathogenic strain of *E. coli* including the development of symptomatic urethritis.[24]

In the present study, the results on the department wise prevalence of UTI found to be highest in paediatric and followed by medicine. But, in the study by Ullal *et al.* (2009)[25] Pakistan, patients from gynaecology contributed maximum number of isolates (42.2%) followed by medicine department as in the case of the present study.

In the present study, *E. coli* (57%) was the commonest organism isolated followed by *Klebsiella* (20%), *CONS* (7%) and the least isolated was *Citrobacter, Enterobacter, Enterococcus* each accounting 1.3%. The study conducted in India by Akram *et.al* (2007)[6] and in Latin America by Gales *et.al* (1998)[26] showed that the *E. coli* was the commonest organism isolated followed by *Klebsiella.* In this study 7% of CONS were isolated and the same was found in Akram *et. al* (2007).[6] In the present study, only 1.3% of Enterococcus was isolated, but the study conducted by Ana. C. Gales *et.al* (1998)[26] showed the highest isolation of about 14% and Ramesh *et.al* (2008)[27] Tamil Nadu showed 9.58% of isolation (Table 3).

E. coli was the predominant pathogen isolated from patients with community acquired UTI.[28,29] Among the 500 samples obtained, 150 pathogens were isolated and 129 out of them were Gram negative bacilli were the leading cause of UTI followed by Gram positive cocci with 21 isolates. A higher isolate rate was reported by 61% reported by Akram *et al*., (2007),[6] but lower isolate rate of 43.5% reported by Sana *et al*., (2005)[30] in Kuwait and 46% *E. coli* by Mohanty *et al.* (2005)[31] in New Delhi. While seeing the current status of UTI in world, studies have revealed a preponderance of *E .coli* in urinary isolates 65% in Japan, 69% in Italy, 74% in Sweden, 75% in England up to 90% in USA and as high as 94% in Israel.[30] The study conducted at Bangladesh showed 48.1% by *Mouse et al. (2015)*[20] *and Ghadage et al.* (2016)[21] reported 41.3% in Pune (Table 4).

The most effective antibiotics against all isolates were imipenem (100%) followed by amikacin (84%), levofloxacin (83%), cefepime (81%), cefoxitin (76%), nitrofurantoin (61%) and ciprofloxacin (48%). However, Akram *et. al*. (2007)[6] from India have reported 100% activity of imipenem against *E. Coli* and similar findings were also reported by Ullal *et. al*. (2009) from Pakistan.[25]

Both ampicillin and co-trimoxazole were highly resistant shows only 11% and 13% sensitivity. Studies from USA, Europe and most other countries have shown better susceptibility pattern for pathogens isolated from UTI against co-trimoxazole.[32,33,34,35] But, in this region of the world co-trimoxazole has shown poor activity.[36,6] A reason for this lack of sensitivity may be that in the past, co-trimoxazole has been extensively used in this region. Among the 85 *E. coli*, 70(82.4%) strains were resistant to co-trimoxazole. Hence, co-trimoxazole cannot be recommended as an empiric therapy for the treatment of UTI in India. First, second and third generation cephlosporins, nalidixic acid and norfloxacin were resistant to all the isolated pathogen by 50% and more.

Conclusion

In this study, high prevalence of UTI was found in paediatric age group and in female gender. It concludes that E. *coli* (57%) was the principal pathogen. This study 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. Institutional Antibiotic policy can be tailored to achieve superior therapeutic outcome.

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How to cite this article: V.P. Sarasu, Rani S.R. Bacteriological profile and antibiogram of urinary tract infections at a tertiary care hospital. International Journal of Medical Microbiology and Tropical Diseases 2017;3(3):106-112.