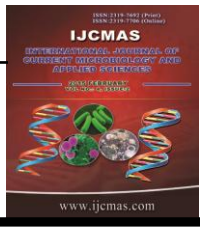
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 1010-1016

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 1010-1016**
http://www.ijcmas.com



Original Research Article

Characterization and Antimicrobial susceptibility testing of Uropathogens from Urinary Tract Infections

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A B S T R A C T

Urinary Tract Infection (UTI) is an infection of one or more structures in the urinary system. The urinary tract includes urethra, bladder, ureters, prostate and kidneys. Clinical presentation of UTI varies from asymptomatic infection to full blown pyelonephritis. Some symptoms may be non-specific and frequently symptoms overlap in patients with lower UTIs and upper UTIs. Present study was undertaken to isolate and characterize pathogens causing UTI in patients. A total of 1000 midstream urine (MSU) samples were taken from both male and female patients who had clinically suspected UTI. They were asked to collect a fresh sample of MSU in a sterile container after cleaning the genitals with soap and water. The sample was transported to the microbiology laboratory and processed by wet film microscophy, Gram's stain and semiquantitative culture in MacConkey's agar and CLED agar. All the isolates were indentified by standard procedures and antimicrobial susceptibility test was done for the bacterial isolates. Bacteria were grown in culture in 25.9 % samples. Mixed infection was observed in 0.08% of the samples. Sex wise, 25.16% infection was seen in females and 28.21% in males. E.coli was the major isolate (61.0%) followed by Klebsiella spp. (8.9%). Coagulase negative staphylococci (6.6%), Citrobacter spp. (6.2 %),Candida spp. (4.2%) and Pseudomonas aeruginosa (3.5%) were the other major uropathogens isolated in that order in our study. Overall resistance pattern among uropathogens in the present study was within the range of that was observed in many Indian studies. Since most of the organisms are showing resistance to routinely used antimicrobials in UTI, especially fluroquinolones, no guidelines for empirical treatment of UTI can be given. The antimicrobial sensitivity testing is needed for selection of antibiotics for treatment.

Keywords

Urinary tract infection, *E.coli*, *Pseudomonas aeruginosa* Antimicrobial susceptibility

Introduction

Urinary tract infections (UTI) are one of the most common infectious diseases and nearly 10% of people will experience a UTI during their lifetime (1,2). UTI are the most common infections after upper respiratory tract infections (3).The urinary tract includes

urethra, bladder, ureters, prostate and kidneys. The infections may be symptomatic or asymptomatic, and either type of infections can result in serious sequelae if left untreated (4). It often results in serious complications like secondary bacteremia and sepsis.

UTI spans all age groups from neonates to elderly. It is much more common in boys during first 3 months, often in association with urologic abnormalities. During pre-school years it is common is girls than boys. Presence of bacteriuria in childhood defines a population at higher risk for development of bacteriuria in adulthood (5).Once adulthood is reached, prevalence of asymptomatic bacteriuria increases in the female population. Up to 40%-50% of female population will experience asymptomatic UTI at some time during their life(6). The prevalence of bacteriuria in adult men is low, until later years, when it rises. In young men lack of circumcision increases the risk for UTI caused by uropathogenic strains of Escherichia coli including the development of symptomatic urethritis (7)

Although several different microorganisms can cause UTI, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases (8). Most infections are caused by retrograde ascent of bacteria from the faecal flora via the urethra to the bladder and kidney especially in the females who have a shorter and wider urethra and are more readily transferred by microorganisms Escherichia coli is the most prevalent causative organism of UTI and is solely responsible for more than 80% of these infections. Other Gram-negative colonic bacteria like Proteus and *Klebsiella* species and occasionally *Enterobacter* species account for a smaller proportion of uncomplicated infections. These organisms along with *Serratia* species and *Pseudomonas* species play a major role in nosocomial catheter associated infections. Proteus species through production of urease and *Klebsiella* species, along with urease, production of extracellular slime and polysaccharides predisposes to urinary stone formation. Gram-positive cocci play a lesser role in UTI. *Staphylococcus saprophyticus* accounts for 10-15% of acute symptomatic UTI in young female patients. Enterococci and *Staphylococcus aureus* cause infections in patients with renal stones or with previous instrumentation and surgery. *Staphylococcus epidermidisis* a common cause of catheter associated UTI (7).

In India antimicrobial susceptibility pattern of uropathogens vary widely by region. High resistance rates to oral antibiotics have been observed, probably due to uncontrolled consumption of these antibiotics. Resistance to amikacin, piperacillin-tazobactum and meropenem are low, likely reflecting lower usage of these drugs. The Worldwide trend of empirically treating community acquired UTI may not apply for specific geographical regions such as India, where decreased susceptibility rates are documented for common urinary pathogens. In the Indian setting, routine urine cultures may be necessary, since treatment failure with empirical therapy is likely to occur(10).

Methodology

Outpatients as well as inpatients attending various departments such as Medicine, Surgery, Obstetrics & Gynaecology, Orthopaedics and Paediatrics of Navodaya Medical College Hospital, Raichur were included in the study group. A total of 1000 MSU samples were analyzed.

Both male and female patients were included in the study. Patients who had clinically suspected UTI were asked to

collect a fresh sample of midstream specimen of urine in a sterile container after cleaning the genitals with soap and water. The sample was transported to laboratory and processed.

Inclusion criteria

- Both male and female patients having clinically suspected UTI and asymptomatic
- bacteriuria were included in the Study.
- Clinical diagnostic criteria: dysuria, frequency, urgency and fever

Exclusion criteria

- Patients on long term antibiotic therapy prior to or during the investigation
- Patient on catheterization

Specimen collection

Midstream urine sample from male and female patients were collected in a wide mouthed universal container with a secure lid. A proper instruction was given to the patient regarding the method of collection of midstream urine sample. Male patients were asked to retract the prepuce, cleanse the glans penis with soap and water and then collect the sample from middle of the urine flow.

Female patients were instructed to thoroughly clean the ano-genital area from front to back, pass urine with labia separated and collect sample from middle portion of the stream.

Since urine is an excellent culture medium supporting the rapid growth of many bacteria, it was transported to the laboratory within an hour and processed and Investigation tests were carried out.

Result and Discussion

A total of 1000 midstream urine samples were processed from patients having clinically suspected UTI attending Navodaya Medical College & Hospital, Raichur. Among them 759 were females and 241 were male patients.

Among 1000 samples 259 (25.9 %) samples yielded growth on culture. Eight of the 259 (0.08%) samples grew 2 organisms (mixed infections) in culture. 109 (10.9 %) showed insignificant bacteriuria. Out of the 759 samples collected from females, 191 (25.16 %) showed growth. Of the 241 male urine samples, 68 (28.21 %) grew uropathogens in culture. Among study subjects, 1-10 years age group had 9.8 % infection. In the age group from 11-30 years the occurrence of UTI ranged between 13.0% to 35.3%. In the age group of 31-40 yrs 19.5 % had UTI. Above 40 years, the occurrence of UTI was 22.4 %. Five Male children of 1-10 years age group had 7.35 % UTI and age group from 11-40 years, the occurrence of UTI was between 7.35% to 8.82%. 25 % of UTI was seen in the age group of 41 – 50 yrs which was high compare to the other age groups .In females, age group from 21-30yrs showed maximum rate of UTI at 38.21% followed by age group from 31 -40yrs at 25.13%.

Among the isolated organisms, 84.2 % of them were Gram negative and 15.8 % were Gram positive organisms. Major bacterial isolate from UTI was *E. coli* (61.0%). The second common isolate was *Klebsiella* species (8.9%). The frequency of other uropathogens in descending order were CONS (6.6 %), Citrobacter species (6.2%), Candida species (4.2%), *Pseudomonas aeruginosa* (3.5%), *Staphylococcus aureus* (2.3%), Proteus species (1.9 %), *Enterobacter* and Enterococcus species

(1.5% each), Acinetobacter species and Streptococcus species (1.1% each). The major isolates namely *E. coli*, *Klebsiella* species, Citrobacter spp. and *Pseudomonas aeruginosa* uniformly exhibited approximately more than 50% resistance to fluoroquinolones.

The antibiotic sensitivity pattern of the major isolate, E. coli (n = l58) is provided in Table 2.

There was varying sensitivity and resistance pattern shown by each organism forthe same antimicrobial. *E.coli* showed high percentage of resistance to Ampicillin (95.6%), Nalidixic acid (90.5%), trimethoprim – sulfamethoxazole (80.0 %), second generation cephalosporins (86.7), third generation cephalosporins (58.9), fluoroquinolones (65.2-67.7%), Nitrofurantoin and Aminoglycosides and Imipenem were better effective with susceptibility rate ranging between 74.1% to 99.4%. *Klebsiella* spp also displayed high percentage of resistance to Trimethoprim – sulfamethoxazole, Ampicillin Nitrofurantoin, fluoroquinolones, third generation cephalosporins. Imipenem and aminoglycosides were found to be having better coverage with relatively low reported resistance.

Citrobacter spp showed maximum sensitivity to aminoglycosides followed by Nitrofurantoin with high percentage of resistance to other drugs. For *Pseudomonas aeruginosa*, Imepenem, Amikacin, ceftazidime, Piperacillin tazobactum were found to be having better coverage with relatively reported low resistance. Amongst the Gram positive isolates, Coagulase negative staphylococci were the most commonly isolated organisms. They were highly resistant to Ampicillin, Nalidixic acid, Trimethoprim – sulfamethoxazole and second generation cephalosporins. Aminoglycosides, Ciprofloxacin and Nitrofurantoin were relatively effective drugs.

UTI is considered to be the most common bacterial infection. Approximately10% of humans will have a UTI at some time during their lives (1). Women are significantly more likely to experience UTI than men. Almost half of all women will experience at least one UTI during their lifetime. 20-30% of adult women will have recurrence within 3 - 4 months after an initial UTI. Specific subpopulations at increased risk of UTI include infants, pregnant women, and the elderly. Patients with spinal cord injuries and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome/human immunodeficiency virus and patients with underlying urologic catheter-associated urinary tract infections remain the most common nosocomial infection accounting for as many as *35%* of nosocomial infections. Although usually benign, catheter associated UTI cause bacterimia in 2 - 4% of patients and has been associated with a case fatality rate three times as high as nonbacteriuric patients. Although majority of UTI are acute and short lived, they contribute to significant morbidity in thepopulation (11). UTI are important complications of diabetes, renal disease, renal transplantation and structural and neurologic abnormalities that interfere with urineflow. Therefore the incidence of UTI varies between populations and geographical areas depending upon the life style and other factors.

In the present study, midstream urine samples (MSU) from 1000 patients were collected belonging to both sexes and all ages ranging from 4 months to 85years, in whom UTI was suspected on clinical grounds.

Table.1 Frequency of Uropathogens in UTI

Isolate	Total	Percentage
Escherichia coli	158	61.00
Klebsiella species	23	8.89
Coagulase negative staphylococci	17	6.57
(CONS)		
Citrobacter species	16	6.18
Candida species	11	4.24
Psedomonas aeruginosa	9	3.47
Staphylococcus aureus	6	2.32
Proteus species	5	1.93
Enterobacter species	4	1.54
Enterococcus species	4	1.54
Acinetobacter species	3	1.15
Streptococcus species	2	0.78
Diphtheroids	1	0.39
Total	**259**	**100**

Table.2 Antibiotic resistance pattern of bacteria isolated from UTI

Antibiotic	***E.coli***	***Klebsiella* spp**	**CONS**	***Citrobacter* spp**	***P. aeruginosa***
Nitrofurantoin	25.9	47.8	11.8	43.8	88.9
Nalidixic acid	90.5	91.3	82.4	93.8	100
Norfloxacin	67.7	56.5	47.1	62.5	66.7
Ciprofloxacin	65.2	65.2	33.4	68.8	77.8
Cefuroxime	86.7	73.9	52.9	75.0	100
Cefotaxime	58.9	69.6	47.1	62.5	77.8
Ceftazidime	58.8	61	-	48.2	33.3
Cefepime	52.7	52.4	46.2	40.3	-
Cotrimoxazole	80.0	82.6	66.7	87.5	100
Gentamicin	33.5	39.1	29.4	12.5	33.3
Amikacin	10.8	8.7	5.9	6.2	22.2
Ampicillin	95.5	91.1	58.8	81.3	100
Imipenem	0	0	-	0	11.1
Amoxyclav	91.8	87	52.9	75.0	88.9
Piperacillin	90.4	89.6	-	-	22.2
Tazobactum					

Table.3 Comparison of percentage of uropathogens isolated in the present study with previous Indian reports

Isolate	Kothari et al 10 2005	Taneja et al 12 2006	Sonavan et al13 2008	Arjunan et al14 2010	S.Banerjee 15 2011	Murugan et al 16 2012	Niladri et al 17 2013	Present study
E.coli	68	47.1	41.3	31.03	72.3	66.02	54	61
Klebsiella spp.	16.9	15.9	15.8	12.06	13.33	5.83	10	8.9
CONS	2.8	-	-	-	17.24	-	8	6.6
Citrobacter spp.	-	-	-	-	1.53	-	-	6.2
Pseudomonas aeruginosa	-	5.9	11.4	17.24	5.64	--	1	3.5
S. aureus		1.7	7.8	1.72	2	-	10	2.3

Table.4 Comparison of antibiotic resistance of E. coli with other Indian reports

Antibiotic	Mahesh et al 18 2005 ESBL		Sonavan et al13 2008	Arjunan et al14 2010	Bhargavi et al 19 2010	Murugan et al 16 2012	Mandal et al 20 2012	Niladri et al17 2013	Present Study
	+	-							
Nitrofurantoin	23.4	12	45.8	61.12	-	1.5	26.9	17.5	25.9
Nalidixic acid	-	-	90.9	27.78	92.6	98.5	-	-	90.5
Norfloxacin	99	52	88.5	5.56	5.56	75.4	-	-	67.7
Ciprofloxacin	99	52	-	22.3	91.9	70.4	73.04	64	65.2
Cotrimoxazole	24.9	43	89.1	-	78.1	-	-	-	80.0
Ampicillin	-	-			-	96.2			95.5
Amoxyclav	-	-	94.7	-	-	-	80.6	79.4	91.8
Amikacin	27.9	5	36.1	-	20.8	10.5	23.2	29.9	10.8
Gentamicin	72.6	5	-	16.67	61.5	30.4	59.6	53.6	33.5
Cefuroxime	-	-	-	-	-	72.6	-	-	86.7
Cefotaxime	-	-	-	-	-	74.8	60.5	62.9	58.9

Major uropathogens showed increased resistance to Nalidixic acid, Cotrimoxazole and fluoroquinolones than other antibiotics. The possible explanation behind the resistance showed to these antibiotics , may be because these antibiotics have been in use for a long period and must have been used and as a result the organisms must have developed mechanisms of circumventing their mode of action

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