

Prevalence and Antibiotics Susceptibility of Uropathogens in Patients from a Rural Environment, Tamilnadu

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Abstract: Problem statement: Urinary Tract Infections (UTIs) in human, rural environment cause significant morbidity due to insanitary condition, lack of knowledge of personal hygiene, lack of patient's compliance and economic burden. In our study, the bacterial etiologies and the resistance patterns found in human with serious UTIs and selecting optimal antimicrobial therapy. **Approach:** A total of 105 patients first morning mid stream urine samples, culture was done by the calibrated loop technique delivering 0.001 mL of urine plated on Cystine-Lactose-Electrolyte Deficient (CLED) agar, MacConkey agar and Blood agar medium (Hi Media, India) for isolation of uropathogens. All pathogens were analyzed for drug susceptibility by disk diffusion method. **Results:** More than 50% of them were confirmed to have UTI bacteriological positive. Women and man especially in the age group of 20-29 had higher incidence of bacterial infection. A total of 58 isolates seven different uropathogens were identified among the *Escherichia coli* (31%) was dominant pathogens followed by *Citrobacter* spp., (20%) and *Pseudomonas aeruginosa* (17.24%). In all uropathogens antibiotics susceptibility the more active agents were norfloxacin (87.93%), ciprofloxacin (70.68%), gentamicin (70.68%), nalidixic acid (68.96%), nitrofurantoin (60.34%) and tetracycline (60.34%) and less active in ampicillin (8.62%), rifampicin (37.93%) and carbenicillin (41.37%). **Conclusion:** Among commonly used antimicrobial agents for the treatment of UTI, there is a trend towards increasing resistance to ampicillin and a persistently low resistance rate to norfloxacin, ciprofloxacin and gentamicin should be consider for treatment.

Key words: Uropathogens, drug susceptibility, UTI patients

INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common infectious diseases and nearly 10% of people will experience a UTI during their life time. (Delanghe *et al.*, 2000; Hoberman and Wald, 1997) it is serious ailment in human due to the frequency, recurrence and difficulty in eradication UTI poses stiff challenge to the medical professionals. UTI is much more common in women than in men, due to anatomic and physiological reasons (Fihn, 2003). By virtue of its position urinogenital tract is more vulnerable to bacterial infections caused by both internal and external flora. It is not always possible to trace the mode of entry of bacteria into the urinary tract. Many authors have suggested four possibilities which are ascending infection, haematogenous spread and lymphogenous spread and direct extension from another organ. UTI

with increased risk include infants, pregnant women and the elderly, as well as those with indwelling catheters, diabetes and underlying urologic abnormalities (Foxman and Brown, 2003). Incidence of uro-genital tract infection in hospital environment is on the rise due to cross infection and lowered immune status of the patients. Furthermore indiscriminate use of antibiotics has resulted in the emergence of drug resistant pathogens. Even though several different microorganisms can cause UTIs, including protozoan parasites, fungi and viruses, bacteria are the major causative organisms and are accountable for more than 95% of UTI cases (Bonadio *et al.*, 2001).

Common pathogens that have been implicated in UTIs are primarily gram-negative organisms with *Escherichia coli* having a more prevalence than other gram-negative pathogens include *Klebsiella pneumoniae*, *Enterobacter* spp., *Proteus mirabilis*, *Pseudomonas*

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aeruginosa and *Citrobacter* spp., (McLaughlin and Carson, 2004; Llenerrozos, 2004; Mittal and Wing, 2005; Blair, 2007). Some enteric organisms such as *Pseudomonas* also adhere to the urinary catheter and form a biofilm on the surface, which then acts as a reservoir for growth (Shigemura *et al.*, 2006). An accurate and prompt diagnosis of UTI is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract and renal failure. This problem of persistent uro-tract infection is more pronounced in rural environment due to insanitary condition, lack of knowledge of personal hygiene, non availability of clinical diagnostic facilities and lack of patient's compliance. In this view we were planned to study the incidence of uro-tract infection with symptomatic evaluation of patients for UTI, assess the incidence of UTI among the population in terms of sex and age groups, to determine the causative agents of UTIs and their susceptibility patterns to commonly used antibiotics in patients.

MATERIALS AND METHODS

Sampling and patients: A total of 105 patients 40 samples from female and 65 from male with suspected urinary tract infection were screened in this study. With the help of trained nursing staff the first morning mid stream urine sample was collected and transported to the laboratory in an icepack and analyzed within 6 h. simultaneously baseline data was collected as regards to patient identification, age, sex, provisional diagnosis with help physician. This study was conducted in a period of six months at Post graduate department of Microbiology, Sri Paramakalyani College, Tamilnadu and South India.

Isolation and identification of organisms: All the samples were midstream urine specimens and culture was done by the calibrated loop technique delivering 0.001 mL of urine and plated on Cystine-Lactose-Electrolyte Deficient (CLED) agar, MacConkey agar and Blood agar medium(Hi Media, India). The inoculated plates were incubated at 37°C for 24 h and for 48 h in negative cases. A specimen was considered

positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ cfu mL⁻¹, or when a single organism was cultured at a concentration of 10⁴ cfu mL⁻¹ and ≥ 5 pus cell per high-power field were observed on microscopic examination of the urine (Collee *et al.*, 1996). Bacterial identification was based on standard culture and biochemical characteristics of isolates. Gram-negative bacteria were identified by standard biochemical tests (Foxman and Brown, 2003; Foxman *et al.*, 2000). Gram-positive microorganisms were identified with the corresponding laboratory tests: catalase, coagulase, Manitol test for *Staphylococcus aureus* (Andreu *et al.*, 2005).

Susceptibility testing: Antimicrobial susceptibility of isolates was tested by the Kirby Bauer disk diffusion method (Bauer *et al.*, 1966). Antimicrobial agents tested were nitrofurantoin (300 µg), tetracycline (30 µg), chloroamphenicol (30 µg) representative antibiotics of quinolones such as norfloxacin (10 µg), ciprofloxacin (10 µg), nalidixic acid (30 µg); Aminoglycosides such as gentamycin (10 µg), rifampicin (5 µg); Beta lactam drug such as ampicillin (10 µg), penicillin G (1 unit) and carbenicillin (10 µg) (Hi Media, India).

RESULTS

Over a 6 month period, 105 midstream urine samples from outpatients were analyzed, of which 58 (55.23%) had significant bacteriuria. Of 105 suspected patients results presented as a sizable number patients with symptoms is found to have bacterial culture was negative (Table 1). The detail analysis of the relationship of bacterial infection with the sex and age of the patients (Table 2).The incidence was found to be more in female especially in the age groups of 20-29 than the male counter parts. A total of 65 male suspected UTI samples 29 samples were revealed positive culture of pathogens. The percentage of incidence was 44.61%. Where as in female 29 out of 40 samples were positive culture the percentage of incident was 72.50%.

Table 1: Symptomological correlation of bacterial infection in UTI

Symptoms	Patients with positive growth and symptoms		Patients with negative growth and positive symptoms	
	N = 56	Percentage	N = 49	Percentage
Burning micturation	49	87.50	40	81.16
Increased frequency of urination	36	64.20	28	57.14
Fever (Acute prelates)	25	44.60	23	46.93
Pain in abdomen	18	32.10	12	24.48
Difficulty in micturation	17	30.40	0	0.00
Haematuria	10	17.85	5	10.20
Pyuria	29	51.78	0	0.00

Table 2: Patient of different sex and age groups with percentage of UTI

Age groups	Male			Female			Total no. of samples (N = 105)	Infection	
	Total number (N = 65)	Infection number	Percentage	Total number (N = 40)	Infection number	Percentage		Male and Female (N = 58)	UTI percentage
1-10	04	02	50.00	03	2	66.66	07	04	06.89
20-29	20	12	60.00	11	9	81.81	31	21	36.20
30-39	13	06	46.15	14	9	64.25	27	15	25.86
40-49	19	06	31.57	09	7	77.77	28	13	22.41
50-60	09	03	33.33	03	2	66.66	11	05	08.62

Table 3: Prevalence and percentage of uropathogens isolated from different age groups

Organisms	Age groups					Total number of organisms	Percentage of organisms
	1-10	14-29	30-34	40-49	50-60		
<i>Escherichia coli</i>	1	8	4	2	3	18	31.03
<i>Citrobacter</i> spp.	2	3	4	2	1	12	20.68
<i>Pseudomonas aeruginosa</i>	0	5	1	4	0-	10	17.24
<i>Proteus vulgaris</i>	0	4	3	1	0	8	13.79
<i>Klebsiella aerogens</i>	0	0	3	3	1	7	12.06
<i>Staphylococcus aureus</i>	0	0	0	1	0	1	1.72
<i>Salmonella</i> spp.	1	1	0	0	0	2	3.44
Total	4	21	15	13	6	58	----

Table 4: Antimicrobial susceptibility rates of the tested uropathogens isolates against 11 antimicrobial agents

Antimicrobial agents	Susceptibility, n/N (%)						
	<i>Escherichia coli</i> (n = 18)	<i>Citrobacter</i> spp. (n = 12)	<i>Pseudomonas aeruginosa</i> (n = 10)	<i>Proteus vulgaris</i> (n = 8)	<i>Klebsiella aerogens</i> (n = 7)	<i>Staphylococcus aureus</i> (n = 1)	<i>Salmonella</i> spp. (n = 2)
Norfloxacin (10 µg)	17 (94.44%)	10 (83.33%)	9 (90.00%)	7 (87.50%)	5 (71.42%)	1 (100%)	2 (100%)
Ciprofloxacin (10 µg)	14 (77.70%)	8 (66.60%)	7 (70.00%)	4 (50.00%)	6 (85.71%)	1 (100%)	1 (50.00%)
Gentamycin (10 µg)	15 (83.33%)	6 (66.60%)	8 (80.00%)	5 (62.50%)	4 (57.00%)	1 (100%)	2 (100%)
Ampicillin (10 µg)	2 (11.11%)	0	1 (10.00%)	0	2 (28.57%)	0	0
Rifampicin (5 µg)	4 (22.22%)	2 (16.60%)	7 (70.00%)	2 (25.00%)	5 (71.42%)	1 (100%)	1 (50.00%)
Penicillin G (1 unit)	0	0	0	0	0	0	0
Nalidixic acid (30 µg)	13 (72.22%)	4 (33.30%)	8 (80.00%)	4 (50.00%)	5 (71.42%)	1 (100%)	1 (50.00%)
Nitrofurantoin (30 µg)	7 (38.88%)	9 (75.00%)	13 (50.00%)	4 (50.00%)	0	0	2 (100%)
Tetracycline (30 µg)	12 (66.66%)	8 (66.66%)	7 (70.00%)	3 (37.50%)	4 (57.00%)	0	1 (50.00%)
Chloroamphenicol (30 µg)	11 (61.11%)	0	5 (50.00%)	0	5 (71.42%)	1 (100%)	2 (100%)
Carbenicillin (10 µg)	8 (44.44%)	3 (25.00%)	2 (20.00%)	0	4 (42.85%)	1 (100%)	2 (100%)

Spectrums of seven different uropathogens were isolated. It was varied importance and their distribution in patients of different sex and age groups (Table 3). Overall more than 95% of isolates were gram negative organisms. *E. coli* was the most frequently isolated uropathogens (31.03%), followed by *Citrobacter* spp., (20.68%) and least isolated *Salmonella* spp., (3.44%) and *S. aureus* (1.72%) (Table 3). A total of 11 antibiotics tested against all uropathogens among which norfloxacin, ciprofloxacin and gentamycin more effective than other antibiotics such as nitrofurantoin and ampicillin. The rates of resistance of isolates to a panel of antibiotics are routinely used to treat UTI infections, are shown in (Table 4) *E. coli* as the predominant cause of UTI, showed the highest percentage of resistance to penicillin in 100% and ampicillin in 93.1% and the lowest resistance to norfloxacin in 5.56%. *Citrobacter* spp., as the second

most prevalent pathogen of UTI displayed a similar resistance pattern and were resistant to ampicillin in 100% of cases and susceptible to norfloxacin in 83.33% of cases. Followed by *P. aeruginosa* and *P. vulgaris* showed the highest antibiotic resistance rate and was significantly resistant to most of the antibiotics (Table 4). In this study, *S. aureus* was responsible for about 1.72% of UTI cases and were resistant to penicillin, ampicillin, nitrofurantoin and tetracycline in 100% respectively.

DISCUSSION

Infection of the urinary tract is one of the most common infectious diseases and it would affect all age groups peoples including men, women and children in worldwide (McLaughlin and Carson, 2004; Llenerrzos, 2004; Blair, 2007). In this study, of 105

patients with complaint from who urine samples were taken, only 52.83% had a bacterial urinary tract infection. This is possibly because UTI symptoms are not a reliable indicator of infection. Early diagnosis and timely and appropriate antimicrobial treatment are considered key factors for eliminate the pathogen, to prevent urosepsis and to reduce the risk of renal scarring. Urinary Tract Infection (UTI) can be either symptomatic or asymptomatic (Macejko and Schaeffer, 2007). Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria (Hooton *et al.*, 1996). Asymptomatic bacteriuria is a condition characterized by bacteriuria without classical symptoms attributable to the urinary tract (Nicolle, 1997). In our studies the incidence of infection was highest in the age group of 20-29 followed by 30-39. This finding correlates with the reports of earlier workers which include (Macejko and Schaeffer, 2007). Most UTIs are caused by bacteria and *E. coli* is the most common uropathogen detected in above 30% cases. *Citrobacter* spp., was isolated in 20% of cases and other occasional pathogens include *S. aureus*, *Salmonella* spp., *P. mirabilis* and *K. aerogens* 2-48. Similar results observed Taneja *et al.* (2010) investigated a total of 1974 clean catch mid-stream urine samples from which significant bacteriuria was found in 558 samples (28.3%). Common uropathogens isolated were *Escherichia coli* (47.1%), *Klebsiella* spp. (15.6%), *Enterococcus fecalis* (8.7%), members of tribe *Proteae* (5.9%), *P. aeruginosa* (5.9%) and *Candida* spp., (5.5%). whereas others (Tambekar *et al.*, 2009) investigated a total of 174 urine samples were analyzed from which, 68 found to be significant bacteriuria with *E. coli* (59%), followed by *Pseudomonas aeruginosa* (15%), *K. pneumoniae* (10%), *P. mirabilis* (9%), *S.aureus* (6%) and *C. freundii* (1%). The urinary tract infections were found to most frequently in female (63%) than male (37%). On the other hand Amin *et al.* (2009) reported that the urine culture bacteriological positive were 68% females and 32% males. The most isolated bacterium was *E. coli* with frequency rate of 59%. The other bacteria were *Klebsiella* spp. (11.6%), *Enterobacter* spp. (9.8%), *Pseudomonas* spp. (7.2%), *Proteus* spp. (2.9%), *Acinetobacter* spp. (2.7%), coagulase positive *Staphylococci* (2.2%), coagulase negative *Staphylococci* (2.3%), *Citrobacter* spp. (1.3%) and *Streptococci* α hemolytic (1.1%). Wazait *et al.* (2003) has reported still higher incidence of *Escherichia coli* (47.30%) in urine samples. The presence and distribution of other pathogens namely *P. vulgaris*, *K. aerogens*, *S. aureus* correlate with earlier reports (Ronald, 2002) it is interesting to note that

only few have reported the presence and significance of *Citrobacter* spp., in UTI (Chawla *et al.*, 1998; Kim *et al.*, 2003). Recurrence is an inevitable consequence of UTI. Frequent and abrupt emergence of drug resistant strains is attributed as a major reason for this. Most of the isolates had exhibited resistance to common antibiotics like ampicillin, tetracycline and carbenicilin. Tseng *et al.* (2008) from UTI recovered all bacteria showed the highest degree of resistance to ampicillin, cefalothin. The Gram-negative bacilli isolated from UTI were sensitive to amikacin and, ciprofloxacin (83-100%) and Gram-positive cocci were sensitive to kanamicin and tobramicin (100%). (Nicolle, 2002) has remarked that this increase in resistance may be due to improper treatment and indiscriminate use of antibiotics.

CONCLUSION

In conclusion, the present studies the incidence of urinary tract infection was high in the age group of 20-29. A total of 58 isolates seven different uropathogens were identified among the *E. coli* was dominant pathogens. The drugs norfloxacin and ciprofloxacin, the relatively floor quinolones were found to be the most effective against uropathogenic isolates followed by gentamycin and nalidixic acid.

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