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Prevalence, Microbiologic Profile of Urinary Tract Infection and its Treatment with Trimethoprim in Diabetic Patients

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ABSTRACT

Urinary Tract Infection (UTI) defines a condition in which the urinary tract is infected with a pathogen causing inflammation which is a common, distressing and occasionally life threatening condition. UTI in patients with diabetes mellitus are reported with asymptomatic bacteriuria. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory. This study focused on the frequency of uropathogens and their antibiotic susceptibility in different gender of diabetic patients. Biochemical characterization of uropathogens revealed the prevalence of gram negative organisms and *E. coli* as the predominant isolate. Among the antibiotics tested, trimethoprim was found to be effective for empirical treatment of UTI and has covered the majority of urinary pathogens followed by nalidixic acid, chloramphenicol and kanamycin. Most of the isolates were resistant to oxytetracycline which is more frequently prescribed and indicates that increased consumption of a particular antibiotic can be a pathway to its resistance by the uropathogens. Conclusively, resistance rates among common uropathogens continue to evolve and appear to be increasing too many commonly used antimicrobial agents and a continued surveillance of resistance rates among uropathogens is needed to ensure appropriate recommendations for the treatment of the urinary tract infections.

Key words: Trimethoprim, diabetic, urinary tract infection, uropathogens, *E. coli*

INTRODUCTION

Urinary Tract Infection (UTI) is caused by pathogenic invasion of the urinary tract which leads to an inflammatory response of the urothelium. Proliferation of bacteria in the urinary tract is the cause of urinary tract infection. The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organism(s), the severity of the infection and the patient's ability to mount an immune response to it (Foxman and Brown, 2003). Signs and symptoms may include fever, chills, dysuria, urinary urgency, frequency and cloudy or malodorous urine. Infections are almost always ascending in origin and caused by bacteria in the periurethral flora and the distal urethra. These bacteria inhabit the distal GI tract and colonize the perineal area. *E. coli* usually causes a child's first infection (Brkic *et al*., 2010) but other gram-negative bacilli and *Enterococci* may also cause infection. *Staphylococcal* infections, especially those due to *Staphylococcus saprophyticus* (Assel *et al*., 2009) are common causes of urinary tract infection among female adolescents.

Diabetes mellitus is a metabolic syndrome characterized by an inappropriate elevation of blood glucose as a result of relative or absolute lack of insulin. Diabetes mellitus has a long term effect on genitourinary system and diabetics are more prone to UTIs and particularly to upper urinary tract infections (Patternson and Andriole, 1997). Studies show that diabetes affects many systems that protect against infection in general and against urinary tract infections specifically. Poor circulation of blood in diabetes reduces the ability of infection-fighting white blood cells to get to their target site, even when they do get there, they are less able to ingest the offending bacteria and kill them than normal white blood cells. Many people with diabetes also have dysfunctional bladders that contract poorly; this allows urine to remain in static pools for long periods of time, providing luxurious ponds for bacteria to grow in (Andriole, 2002). The high prevalence of urinary tract infection among diabetic patients and the evidence of rapid parenchymal involvement emphasize the need for knowledge of the prevalence, clinical awareness of the problem and clarification of its consequences in order to define the magnitude of public health resources required to care for the disease (Akbar, 2001).

Urinary Tract Infections (UTIs) are a common burden in patients with diabetes mellitus. Cystitis, ascending infections leading to pyelonephritis, impaired leukocyte function, recurrent vaginitis, emphysematous complications and renal and perinephric abscesses are well recognized in this group of patients especially if glycaemic control is poor. Despite the clinical significance of UTI in diabetes, it is inadequately understood and management regimens are mostly not evidence based. Anticipation of potential complications and earlier interventions are vital to reduce serious adverse outcomes. Asymptomatic bacteriuria has been reported to be commoner in women with diabetes, although data are less convincing for men (Ronald and Ludwig, 2001). Many studies have also shown that bacteriuria in diabetic women involves the upper urinary tract more frequently (Zhanel *et al*., 1995; Aleksandrov *et al*., 2002).

The management of urinary infection in patients with diabetes is essentially the same as patients without diabetes. During the course of a lifetime with diabetes, UTIs would be ranked among the top ten concurrent or complicating illnesses by most experts and patients (Robbins and Tucker, 1994). Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory. Since patterns of antibiotic resistance in a wide variety of pathogenic organisms may vary even over short periods and depend on site of isolation and on different environments, periodic evaluation of antibacterial activity is needed to update this information.

The objective of the study was to investigate the frequency of uropathogens and their antibiotic susceptibility in different gender and age groups of diabetic patients.

MATERIALS AND METHODS

This study focuses on the frequency of uropathogens and their antibiotic susceptibility in different gender and age groups of diabetic patients and was conducted from November 2010- January 2011.

Fourty four diabetic human urine samples were obtained from different locations in Bangalore for this purpose. These samples were processed to monitor urinary tract infection and antibiotic sensitivity patterns of pathogenic bacteria. The data were categorized viz., gender and age.

Patients were instructed to collect mid stream urine sample after washing the perineal area for females and penile area for males. All urine specimens were obtained aseptically in well labeled,

screw capped universal containers and transported promptly to the laboratory. In any case when it was not possible to reach the laboratory in time, the specimens were kept in refrigerator not more than 6 h.

Urine cultures were done by inoculating urine samples on Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar plates (Yengkokpam *et al*., 2007) using a calibrated loop (0.01 mL). After incubation at 37°C for 18-24 h, the identification of the responsible pathogen was done.

Isolated colonies after purification were initially Gram stained. By using Bergey's Manual of Determinative Bacteriology, the isolates were biochemically characterized and identified. All the bacterial isolates were preserved on nutrient agar slants at 4°C and subcultured periodically.

Antibiotic sensitivity test was done by the disc diffusion test (Bauer *et al*., 1960). The antibiotics used were rifamipicin, oxytetracycline, erythromycin, trimethoprim, nalidixic acid, chloramphenicol, kanamycin and streptomycin and the test was performed by employing dried filter paper discs impregnated with specific concentration of antimicrobial agents on Muller Hinton agar according to the National Committee for the Clinical Laboratory Standards (NCCLS, 1993; Bauer *et al*., 1966) as well as the manufacturer instructions about the clear zones of growth inhibition around the disks.

RESULTS AND DISCUSSION

A total of 44 mid stream urine samples from diabetic patients were collected between the age range of 30-80 years which consists of 30 male (68%) and 14 female (32%) and was found that 68% of the patients were having asymptomatic bacteriuria.

When the data were classified into different age and gender groups, it appeared that the cases of Urinary Tract Infections (UTIs) were more in men than women and a total of 38 strains were isolated. Colony characteristics on CLED agar and MacConkey agar were observed initially and Gram's staining reactions showed that most of the isolates were of Gram negative organisms and biochemical characterization revealed that Gram negative organisms were mainly responsible for urinary tract infections than the Gram positive ones (Table 1).

The most frequent causative agents of UTI were *Escherichia coli* accounting for 39.4% of the isolates followed by *Staphylococcus* (18.4%), *Klebsiella* (15.7%), *Enterococcus* (13.1%), *Proteus* (7.8%), *Pseudomonas* and *Candida* (2.6% each) (Table 1, Fig. 1).

The antibiotic sensitivity pattern has been determined by the zone of inhibition and classified as resistant, moderately sensitive and sensitive. A total of 8 antibiotics were tested against the isolates (Table 2) and it was observed that trimethoprim had best inhibitory activity against all

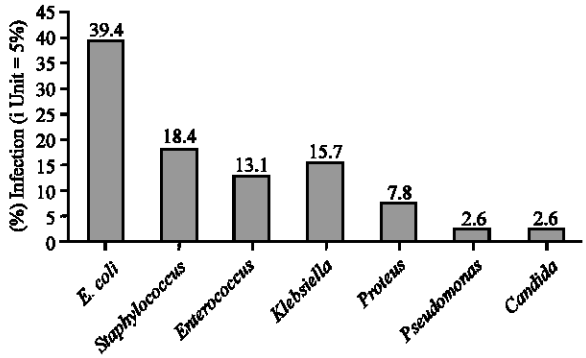

Fig. 1: Percent Infection in UTI

Table 1: Morphological and biochemical characterization of urinary isolates

		Biochemical characteristics								
Strain	Microscopic appearance	Ind	MR	VP	Cit	Ure	Gel	Cat	Oxi	Organism
DP1	G-ve rod	-	-	+	+	+	+	+	-	*Klebsiella*
DP1a	G-ve rod	+	+	-	-	++	+	+	-	*Proteus*
DP4	G+ve cooci	-	+	+	-	-	+	+	-	*Staphylococcus*
DP4a	G-ve rods	-	-	+	+	+	+	+	-	*Klebsiella*
DP5	G-ve rods	+	+	-	-	+	+	+	-	*Proteus*
DP6	G+ve cooci	-	-	+	ND	ND	-	-	-	*Enterococcus*
DP6a	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP7	G+ve cooci	-	+	+	-	-	+	+	-	*Staphylococcus*
DP7a	G-ve rod	-	-	+	+	+	+	+	-	*Klebsiella*
DP8	G-ve rod	-	-	-	-	-	+	+	+	*Pseudomonas*
DP8a	G+ve cocci	-	+	+	-	-	+	+	-	*Staphylococcus*
DP10	G+ve cooci	ND	-	+	ND	ND	-	-	-	*Enterococcus*
DP10a	G+ve cocci	-	+	+	-	-	+	+	-	*Staphylococcus*
DP11	G-ve rod	-	-	+	+	+	+	+	-	*Klebsiella*
DP13	G-ve rod	-	-	+	+	+	+	+	-	*Klebsiella*
DP14	G+ve cooci	-	+	+	-	-	+	+	-	*Staphylococcus*
DP14a	G+ve cocci	ND	-	+	ND	ND	-	-	-	*Enterococcus*
DP16	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP17	G+ve cocci	ND	-	+	ND	ND	-	-	-	*Enterococcus*
DP18	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP19	G-ve rod	-	-	+	+	+	+	+	-	*Klebsiella*
DP19a	G-ve rod	+	+	-	-	+	+	+	-	*Proteus*
DP20	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP25	G+ve cocci	-	+	+	-	-	+	+	-	*Staphylococcus*
DP26	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP28	G-ve rod	ND	-	+	ND	ND	-	-	-	*Enterococcus*
DP31	G+ve cocci	-	+	+	-	-	+	+	-	*Staphylococcus*
DP33	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP34	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP35	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP36	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP37	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP38	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP39	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP41	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP42	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP43	G-ve rod	+	+	-	-	-	-	+	-	*E. coli*
DP44	Ovoid cells	ND	ND	ND	ND	ND	ND	ND	ND	*Candida*

(+) Positive; (-) Negative; ND - Not Done

the isolates proving its broad spectrum activity. However, nalidixic acid, chloramphenicol and kanamycin had significantly inhibited the growth of isolates, thus suggesting that use of these antibiotics for urinary tract infections. Rifampicin had controlled the growth of *E. coli, Staphylococcus* and *Enterococus* but failed to control *Klebsiella, Proteus* and *Pseudomonas*. Erythromycin has showed significant effect on most of the isolates and streptomycin exhibited moderate response against all the isolates. Most of the isolates recorded growth in the presence of oxytetracycline and a moderate sensitivity was observed by *Staphylocoocus* (Table 3).

Table 2: Antibiotic assay of urinary isolates

Organism	Rif	Oxy	Ery	Tri	Nal	Chl	Kan	Str
Klebsiella DP1	S	R	R	S	S	S	S	S
*Proteus*DP1a	S	R	R	S	S	S	S	R
Staphylococcus DP4	R	MS	MS	S	SR	R	R	R
Klebsiella DP4a	S	R	R	S	S	S	S	S
Proteus DP5	S	R	R	S	S	S	S	R
Enterococcus DP6	R	R	R	S	S	S	R	S
E. coli DP6a	R	R	R	S	S	S	S	R
Staphylococcus DP7	R	MS	MS	S	R	R	R	R
Klebsiella DP7a	S	R	R	S	S	S	S	S
Pseudomonas DP8	S	R	R	S	R	R	S	MS
Staphylococcus DP8a	R	MS	MS	S	R	R	R	R
Enterococcus DP10	R	R	S	S	S	S	R	S
Staphylococcus DP10a	R	MS	MS	S	R	R	R	R
Klebsiella DP11	S	R	R	S	S	S	S	S
Klebsiella DP13	S	R	R	S	S	S	S	S
Staphylococcus DP14	R	MS	MS	S	R	R	R	R
Enterococcus DP14a	R	R	S	S	S	S	R	S
E. coli DP16	R	R	R	S	S	S	S	R
Enterococcus DP17	R	R	S	S	S	S	R	S
E. coli DP18	R	R	R	S	S	S	S	R
Klebsiella DP19	S	R	R	S	S	S	S	S
Proteus DP19a	S	R	R	S	S	S	S	R
E. coli DP20	R	R	R	S	S	S	S	R
Staphylococcus DP25	R	MS	MS	S	R	R	R	R
E. coli DP26	R	R	R	S	S	S	S	R
Enterococcus DP28	R	R	S	S	S	S	R	S
Staphylococcus DP31	R	MS	MS	S	R	R	R	R
E. coli DP33	R	R	R	S	S	S	S	R
E. coli DP34	R	R	R	S	S	S	S	R
E. coli DP35	R	R	R	S	S	S	S	R
E. coli DP36	R	R	R	S	S	S	S	R
E. coli DP37	R	R	R	S	S	S	S	R
E. coli DP38	R	R	R	S	S	S	S	R
E. coli DP39	R	R	R	S	S	S	S	R
E. coli DP41	R	R	R	S	S	S	S	R
E. coli DP42	R	R	R	S	S	S	S	R
E. coli DP43	R	R	R	S	S	S	S	R
Candida DP44	R	R	R	S	S	S	S	R

Rif: Rifampicin; Oxy: Oxytetracycline; Ery: Erythromycin; Tri: Trimethoprim; Nal: Nalidixic acid; Chl: Chloramphenicol; Kan: Kanamycin; Str: Streptomycin. R: Resistance; S: Sensitive, MS: Moderately sensitive

Urine is the commonest sample to be received in a microbiology laboratory. A large spectrum of organisms has been reported from patients of UTI with *E. coli* and *Klebsiella* sp. being the most common. It is stated that UTI is predominantly a disease of the females due to a short urethra and proximity to the anal opening and our findings revealed that there was a male preponderance for this infection.

In this study, we have tried to determine whether there are differences in the bacteriologic patterns of UTI and in the antibiotic sensitivity patterns of the pathogens concerned with diabetic patients. The study was carried-out on a large series of elderly adult diabetic patients. However,

Table 3: Susceptibility patterns of uropathogenic organisms to different antibiotics

Antibiotic	*E. coli*	*Staphylococcus*	*Enterococcus*	*Klebsiella*	*Proteus*	*Pseudomonas*
Rifampicin	R	R	R	S	S	S
Oxytetracycline	R	MS	R	R	R	R
Erythromycin	R	MS	S	R	R	R
Trimethoprim	S	S	S	S	S	S
Nalidixic acid	S	R	S	S	S	R
Chloramphenicol	S	R	S	S	S	R
Kanamycin	S	R	R	S	S	S
Streptomycin	R	R	S	S	R	MS

R: Resistance; S: Sensitive, MS: Moderately sensitive

due to the frequency and severity of urinary tract infections in diabetic patients, prompt diagnosis and early therapy is warranted and therefore the challenges will be to provide long term care, controlling of elevated blood sugar and maintenance of normal health hygiene to prevent complications in diabetic patients.

Diabetes mellitus has for a long time been associated with increase in prevalence of bacteriuria compared with patients without diabetes (Sullivan *et al*., 1961; Harding *et al*., 2002). The prevalence of asymptomatic bacteriuria is higher in women with diabetes than in women without diabetes (Geerlings *et al*., 2000; Bhushan and Tiwari, 2001). However, the present investigation reveals that diabetic men are more prone to UTI than women which give contradictory results against the previous studies.

E. coli was the most frequent uropathogen isolated, was responsible for UTI in 39% of diabetic which was in accordance with the findings of previous studies where *E. coli* being the predominating isolates (Oduyebo *et al*., 2001; Adeyeba *et al*., 2007; Bashir *et al*., 2008; Mohammadi *et al*., 2010; Khleifat *et al*., 2006). However, a low proportion of *E. coli* isolates in elderly adult patients with asymptomatic UTI in both diabetic and non diabetic patients was reported (Bonadio *et al*., 2006) and *Klebsiella* was found to be the commonest followed by *E. coli, P. aeruginosa* and *S. aureus* in UTI (Bajaj *et al*., 1999).

In view of the emerging drug resistance amongst bacteria, therapy should only be advocated, as far as possible, after culture and sensitivity has been performed. This would not only help in the proper treatment of the patients but would also discourage the indiscriminate use of the antibiotics and prevent further development of bacterial drug resistance. On a phenotypic level there are two ways of fighting development and spread of drug resistant bacteria. The first is to reduce the use of antimicrobial agents to decrease the selection of resistant bacteria and the second is to improve hygienic measures to prevent the spread of resistant bacteria. It is necessary to develop new antimicrobials and therapeutic agents having high effectiveness with no side effects (Gul *et al*., 2004).

Antibiotic activity has described the assessment of resistance patterns of urinary isolates to commonly used antibiotics to evaluate the options for empirical treatment of UTI and was observed that the use of trimethoprim would cover the majority of urinary tract pathogens followed by nalidixic acid, chloramphenicol and kanamycin but earlier results showed that ampicillin (wide spectrum penicillin) and fluoroquinolones had a very significant role in the therapy of urinary infections (Lazarevic *et al*., 1998; Naber, 2000). It was reported that trimethoprim and sulphamethoxazole combination was not found to be effective for the treatment of urinary tract infections (Gupta *et al*., 2002) but trimethoprim sulphamethoxazole and nalidixic acid possess high

efficacy against urinary isolates (Falakaflaki *et al*., 2007; Manikandan *et al*., 2011). The present investigation proved that use of trimethoprim as a single agent for empirical treatment of a suspected UTI has covered the majority of urinary pathogens.

It was observed that oxytetracycline has failed to control the growth of all isolates throughout the study. The other antibiotics erythromycin, rifampicin and streptomycin have exhibited moderate effect on the growth of isolates. It was found that nalidixic acid, chloramphenicol and kanamycin had played a significant role in controlling the growth of urinary isolates. All the isolates have failed to grow in the presence of trimethoprim and thus proved which can be used to treat urinary tract infections effectively. The antibiotic sensitivity pattern showed that most isolates were sensitive to trimethoprim while they are resistant to oxytetracycline which indicates that increased consumption of a particular antibiotic can be a pathway to its resistance.

CONCLUSION

The results of antibiotic susceptibility pattern on the casual agents in this study revealed that most of the isolates were resistant to oxytetracycline which is relatively cheaper and more frequently prescribed. The resistance rates among common uropathogens continue to evolve and appear to be increasing too many commonly used antimicrobial agents such as rifampicin, erythromycin and streptomycin. Continued surveillance of resistance rates among uropathogens is needed to ensure appropriate recommendations for the treatment of the infections.

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