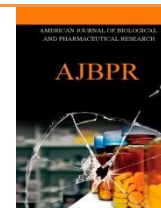




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### INCIDENCE OF BACTERIAL UROPATHOGENS AMONG DIABETIC PATIENTS

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#### ABSTRACT

Urinary Tract Infections (UTIs) occur more frequently in diabetic than in non-diabetic patients and have been proven to be the primary cause of renal failure. This study was to investigate the prevalence of UTIs in clinically diagnosed patients with diabetes and to determine the Uropathogens responsible for UTIs as well as the antimicrobial susceptibility pattern. A total of 50 diabetic patients, i.e. 25 female and 25 male, with symptomatic UTI were included in this study. All urine samples were processed in the lab following standard laboratory protocol. Among the study cases, 48% male and 72% female diabetic patients had positive growth from urine. The commonest Uropathogen obtained was *Escherichia coli* (48%) followed by the *Klebsiella pneumonia* (28%), *Pseudomonas* (16%), *Staphylococcus aureus* (16%) and *Proteus* (12%) respectively. The antibiotic susceptibility for the five predominant species of bacteria revealed high resistance to Penicillin, Erythromycin, Ampicillin and susceptibility to Ciprofloxacin. The prevalence rate of UTI was high in diabetic female than male patients. Gram negative pathogens especially *E. coli* was predominate among the causative agents of UTIs. Ciprofloxacin found to be containing broad spectrum activity against both gram positive and gram negative pathogens.

#### INTRODUCTION

Human urine can support bacterial growth due to its favorable chemical composition [1]. Urinary tract is normally sterile, when bacteria moves from rectum or vagina to urethra and multiplying within urinary tract and cause Urinary tract infection (UTI). UTIs are the frequent infections observed in clinical practice and results in a significant morbidity and high medical costs. UTIs are among the most common bacterial infection up to 50% of woman report having had at least one UTI in their lifetime [2,3]. UTI manifestation includes pain, fever and

discomfort but is easily treated unless it spreads to the kidneys. The clinical manifestations of UTI depend on portion of the urinary tract involved, the etiologic organisms, the severity of the infection and the patient's ability to mount an immune response to it [4]. Normally  $10^5$  microorganisms/ml of urine from midstream collection indicate an UTI [5]. Most UTIs are caused by facultative anaerobes usually originating from the bowel flora.

Diabetes mellitus has a long-term effect on genitourinary system and diabetics are more prone to Urinary tract infection (UTI) and particularly to upper Urinary tract (UT) [6,7]. Diabetes mellitus alters the genitourinary system where UTI can be caused due to severe complications ranging from dysuria (pain of burning sensation during urination) organ damage to sometimes even death due (pyelonephritis) [8].

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Patients with diabetes have a 10-fold increased risk of UTI when compared to non-diabetics [9] and diabetics have a longer hospitalization than non-diabetics [10]. Diabetes has long been considered to be a predisposing factor for urinary tract infection. In females, the urinary tract has an important association with the reproductive organs because of its proximity [11]. Women with diabetes have higher risk of UTI because of changes in immune system. Any other disorder that suppresses the immune system raises the risk of urinary infection. The increased frequency of UTIs in diabetic patients is likely due to several factors. Suggested host-related mechanisms are: (a) the presence of glycosuria; (b) defects in neutrophil function and (c) increased adherence to uroepithelial cells [12]. Based on the facts addressed above, the present work was intended to study the prevalence of bacterial Uropathogens among diabetic patient was performed.

## MATERIALS AND METHODS

### Sample collection

A total of 50 urine samples were collected during the months from January to April 2014 at Rajah Muthiah Medical College and Hospital, Chidambaram. Sterile wide mouth universal containers into which a clean catch (midstream urine) of about 10–20 ml urine were collected from the diabetic patients. Diagnosis of diabetes was made based on the WHO criteria [13]. The urine samples were transported in cooler boxes to microbiology laboratory, Annamalai University for bacterial investigation within 4–6 hrs of collection. Until culture time, the urine samples were stored at 2–8°C in refrigerator.

### Identification of Uropathogens from urine samples

The urine samples were cultured on Blood agar, MacConkey agar and Cysteine Lactose Electrolyte Deficient Agar (CLED) and the plates were incubated at 37°C for 24 h. The plates containing more than 10<sup>5</sup> CFU/ml colonies were selected as significant growth [14]. Bacterial species were diagnosed according to colony morphology and color on CLED media, the results of confirmatory biochemical tests (Indole, Methyl red, Voges-proskauer, Simmon's Citrate, Semisolid manitol and Oxidase test, Coagulase, catalase, novobiocin sensitivity test) according to Morello *et al.* (2006) [15].

### Antibiotic sensitivity test

Antibiotic susceptibility testing was carried out using the Kirby–Bauer disc diffusion technique on Muller–Hinton agar and commercial antibiotic discs were used for antimicrobial testing [16]. The antibiotic discs used were:

Ofloxacin(Of), Nalidixic acid(Nx), Ciprofloxacin(C), Ampicillin(A), Gentamicin(G), Amikacin(Ak), Penicillin G(P) and Erythromycin(E). The antibiotic disc impregnated culture plates were incubated at 37°C for overnight. The diameter of the zone of inhibition was measured and recorded as resistant or susceptible according to the National Committee for Clinical Laboratory Standards [17] interpretative criteria.

## RESULTS AND DISCUSSION

Of 50 urine samples examined, 25 were from male and 25 were from female diabetic patients. The prevalence of UTI among the female diabetic patients was 18(72%) which was higher when compared to prevalence in male 12(48%) (Table 1). Our study showed similar with other reports stating the high prevalence of UTI in females [18,19]. It is stated that UTI is predominantly a disease of the females due to a short urethra and proximity to the anal opening.

Number of isolates in diabetic males and females included *Escherichia coli* 12(48%), *Klebsiella* sp. 7(28%), *Pseudomonas* sp. 4(16%), *Staphylococcus aureus* 4(16%) and *Proteus* sp. 3(12%) (Table 2). Bacteriological studies usually reveal the involvement of gram negative enteric organism that commonly causes urinary tract infections, such as *E. coli*, *Klebsiella* sp. and *Proteus* sp. [20]. Similarly, the predominant number of pathogens isolated in our study was gram negative bacilli rather than gram positive pathogens. In another study from India, it was found that *E. coli* was the most commonly grown organism (64.3%) followed by *Staphylococcus aureus* [9,21]. Lloyds *et al.* (1998) [22] have shown that *Enterococci* sp. accounted for 35% of urinary tract isolates. The antibiotic sensitivity patterns of the isolates to various antimicrobial agents are shown in Table 3. The results shown that the most of the bacterial isolates were highly sensitive to Ciprofloxacin, Nalidixic acid and Ofloxacin and poorly effective to Amikacin and Gentamycin. All the UTI pathogens were highly resistant to Ampicillin and Penicillin. The resistance of *E. coli* to Cefotaxime is attributed also to  $\beta$ -lactamase enzyme production by these bacteria and resistance to Trimethoprim is due to Dihydrofolate reductase gene produced [23]. Bacterial resistance to Aminoglycosides (Amikacin and Gentamycin) are mediated by enzymatic modification of various sites on the antibiotic, alter the target ribosome, decrease the drug uptake and due to drug efflux. The resistance is usually transferable, especially among members of the family *Enterobacteriaceae* which are the predominant organisms implicated in UTI [24].

**Table 1. Prevalence of UTI from diabetic patients over the sex distribution**

S. No	Sex	Number of cases examined	Number of cases positive	Percentage (%)
1.	Male	25	12	48
2.	Female	25	18	72



**Table 2. Frequency of pathogen in UTI (N = 50)**

S. No	UTI pathogens	Sex				Total number of isolates	Percentage (%)
		Male		Female			
		No.	%	No.	%		
1.	<i>Escherichia coli</i>	4	33.3	8	67	12	48
2.	<i>Klebsiella pneumoniae</i>	3	42.9	4	57.14	7	28
3.	<i>Pseudomonas sp.</i>	2	50	2	50	4	16
4.	<i>Staphylococcus aureus</i>	2	50	2	50	4	16
5.	<i>Proteus sp.</i>	1	33.3	2	67	3	12

**Table 3. The antibiotic susceptibility pattern of the bacterial isolates**

S. No	UTI pathogens	Antimicrobial agents tested							
		Ak	Cf	Nx	G	Of	Am	P	E
1.	<i>Escherichia coli</i>	S	S	S	I	S	R	R	R
2.	<i>Klebsiella pneumoniae</i>	I	S	S	I	S	R	R	R
3.	<i>Pseudomonas sp.</i>	I	S	S	S	I	R	R	R
4.	<i>Staphylococcus aureus</i>	S	S	I	S	S	I	R	R
5.	<i>Proteus sp.</i>	S	S	I	S	S	I	R	R

Notes: Amikacin(Ak), Ciprofloxacin(C), Nalidixic acid (Nx), Ampicillin(A), Gentamicin(G), Ofloxacin(Of), Penicilin G(P), and Erythromycin(E). S – Sensitive, I – Intermediate, R – Resistant.

## CONCLUSION

In summary, the prevalence of UTI was high in women with diabetes than in men. *Escherichia coli* was commonly isolated, the UTI pathogens were highly sensitive to Ciprofloxacin, Ofloxacin and Nalidixic acid. Thus, the study may be concluded that the surveillance of

Uropathogens and antibiotic sensitivity pattern of the same is essential to set up the appropriate treatment system to manage UTIs. The study also implies the fact that monitoring and maintenance of glucose levels in both blood and urine, personal hygiene is mandatory to prevent UTIs among diabetic individuals.

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