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Occurrence and characterization of urinary tract infection in patients with and without diabetes mellitus with special reference to *Escherichia coli*

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Abstract

Introduction: The urinary tract is the second most common site for infection next to respiratory tract and urinary tract infection (UTI) occurs more frequently in persons with diabetes mellitus. The factors affecting UTI in diabetic patients are not well documented and there is lack of adequate surveillance information and characterization pertaining to urinary pathogens in diabetic cases in developing countries. Hence, the study aimed to look into the characterization and antibiotic susceptibility pattern of pathogens associated with UTI among diabetic and non-diabetic patients with special reference to *Escherichia coli*.

Materials and Method: A total of 1695 non-repetitive urine samples were screened prospectively from symptomatic cases of diabetic and non-diabetic individuals by semi-quantitative calibrated loop culture technique between 2014 and 2016.

Result: In our study, out of 1695 urine samples screened, 446 (24.9%) urine samples showed significant bacteriuria and *Escherichia coli* was accounted for 280 (62.7%) bacterial isolates. Though *E.coli* was the predominant organism, its occurrence was significantly high in non-diabetic subjects compared to diabetic subjects (70.6% Vs 55.2%, $p<0.001$). While *Staphylococcus aureus* (6.5% Vs 2.7%; $p< 0.001$) and *Candida* species (7% Vs 2.7%; $p <0.001$) were the more frequent cause of UTI in diabetic subjects. Among diabetic cases, 61% of the UTI cases were having long term diabetes of more than 10 years. The *E.coli* strains isolated from both groups showed increased resistance to ampicillin (86.7%), ceftazidime (63.2%), ciprofloxacin (52.5%) and trimethoprim/sulfamethoxazole (50%).

Conclusion: *E.coli* was predominant organisms isolated from UTI in both diabetic and non-diabetic groups. Microorganisms encountered in UTI did not differ by diabetic status except for *Candida* species and *Staphylococcus aureus*, which were more frequently isolated from diabetic patients. Among diabetics, age of the patient, long duration and poor glycemic control were associated risk factors in the development of UTI.

Keywords: Urinary Tract Infection, Diabetes mellitus, *Escherichia coli*

Introduction

The urinary tract is second most common site of bacterial infection next to the respiratory tract, and a chief source of human discomfort.[1] Several innate immune mechanisms play a vital role to curtail UTI, including urine voiding, mucus shedding and epithelial cell sloughing. When uropathogenic *E.coli* (UPEC) crosses these physical barriers by attaching to the uroepithelium with fimbriae, a robust innate immune response is generated and a number of factors like antimicrobial peptides, Tamm-Horsfall Protein, cytokines and chemokines are produced in mammalian bladders.[2] However, even with these remarkable host defenses and ever-increasing antibiotic usage, UTI remains one of the most common infections.[3,4]

It has been noted that UTI occurs more frequently in patients with diabetes mellitus[5] and they are at a higher risk of development of asymptomatic bacteriuria to symptomatic UTI.[6] Factors related to increased risk of urinary infections in diabetic patients are decreased antibacterial activity due to glucosuria, defects in neutrophil function, increased adherence to uroepithelial cells and compromised antioxidant system.[7] The acute pyelonephritis is approximately 10 times more common in diabetic population than in non-diabetics.[8] Though factors affecting UTI in diabetic patients have been well studied in the developed nations, there is lack of adequate surveillance information and characterization pertaining to pathogens isolated from UTI patients with and without diabetes mellitus in developing countries. The current study aims in the characterization and antibiotic susceptibility pattern of pathogens associated with UTI among diabetic and non-diabetic patients with special reference to *Escherichia coli*.

Materials and Method

A total of 5800 non-repetitive urine samples were collected from patients attending a tertiary care center of Southern India, suffering from UTI [defined as a combination of the following symptoms: (i) bacteria with $\geq 10^4$ CFU/ml mid stream urine, (ii) the presence of white blood cells (WBCs), with ≥5 WBCs per high-power field, (iii) and the presence of clinical signs or symptoms of UTI in the host, including dysuria and frequency or urgency of urination] were collected. Of which 1695 samples were included in the study and analyzed further, as they were from true diabetic and non-diabetic category (whose diabetic status was confirmed clinically and diagnostically).

These patients were categorized into the diabetic group and the non-diabetic group based on the following definitions:

- The diabetic group consisted of patients with prior diagnosis of diabetes mellitus (complicated or uncomplicated), with a blood HbA1C level above 6.5% and having at least traces of sugar (≥ 0.8 mmol/l) in urine.
- Patients who had no clinical history of diabetes mellitus, with blood HbA1C level less than 6.5% and no detectable sugar level (0 – 0.8 mmol/l) in the urine were included in the non-diabetic group.
- Any patient with the history of antibiotic intake and on immunosuppressive drugs in the preceding 2 weeks, having obstructive uropathy and showing impaired blood and/or urine glucose level with HbA1C <6.5% were excluded from the study.

The study was carried out with the approval from Institutional Ethical Committee and informed consent was obtained from the subjects willing to participate. Clean-catch midstream urine samples were collected in sterile disposable (Uricol, Hi Media) container and immediately transported to the Microbiology Laboratory and processed in one hour.

Primary Identification: The semi-quantitative standard calibrated loop (Hi-Media metal loop SS-2, measuring 2.2 mm diameter and holding capacity of calibrated 0.005 ml of urine) technique is used to culture a fixed volume of uncentrifuged urine. The total bacterial count per ml of urine will be the number of bacterial colonies multiplied by 200 (conversion factor). The presence of more than 10^5 CFU/ml (colony forming unit/ml) of single species of bacteria is considered as significant bacteriuria which indicates active infection. The urine samples were inoculated on Blood agar, Mac Conkey's agar and CLED agar. The inoculated plates were incubated at 37ºC for overnight. The organisms isolated in significant number (10^5CFU/ml) were identified by colony characteristics, Gram staining and standard biochemical tests.[9]

Antimicrobial susceptibility test: Antibiotic Susceptibility test was done by Kirby–Bauer's Disc Diffusion method in accordance with CLSI guidelines using antibiotic discs: ampicillin (10µg), amoxicillin/clavulanic acid (20/10µg), Co-trimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), levofloxacin (5µg), netilmicin (30µg), ceftazidime (30 µg), cefotaxime (30 µg), and gentamicin (30µg). *Escherichia coli* ATCC 25922 was used as control.[10,11]

Statistical Analysis: Statistical analysis was performed by Software Package used for Statistical Analysis (SPSS) version 16.0. The results were analyzed with a descriptive statistics wherever appropriate. The *chi*-square and Fisher's exact test were used to evaluate the statistical significance of differences in the results. A *p*-value of <0.05 was considered statistically significant.

Results

In the present study, a total of 1,695 samples categorized in diabetes mellitus (DM) and non-DM groups were screened, and of which 446 (24.9%) samples showed significant bacteriuria. *Escherichia coli* accounted for 62.7% bacterial isolates. Out of 280 *E.coli* isolates, 154 (70.6%) isolates were from non-DM and 126 (55.2%) isolates were from the urine of DM patients (Table 1). There was a significant difference (p<0.001) in the isolation rate of *E.coli* between non-DM and DM subjects.

Table 1: Urinary Tract Infection in DM and non-DM cases

Sl. No	UTI Category	Samples included	Significant bacteriuria	% of samples with significant bacteriuria	*E.coli**	% of *E.coli* isolated
1	Non-DM	985	218	22.1	154	70.6
2	DM	710	228	30.9	126	55.2
3	Total	1,695	446	24.9	280	62.7

DM – Diabetes mellitus

p-value (DM Vs Non-DM)* - < 0.001 significant bacteriuria

Fisher's exact test

Primary identification of organisms responsible for urinary tract infection: Though *E.coli* was the predominant organism in both the groups, its occurrence was significantly high in non-DM subjects compared to DM subjects (70.6% Vs 55.2%, *p*<0.001), while *Staphylococcus aureus* (6.5% Vs 2.7%; *p< 0.001*) and *Candida* species (7% Vs 2.7%; *p <0.001*) were the more frequent cause of UTI in DM than in non DM subjects (Table 2). There was no significant difference (*p*>0.05) in the occurrence of *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterococcus faecalis and CONS*.

Table 2: Microorganisms encountered in urinary tract infection

Sl. No.	Organisms	Non-DM		DM		Total		p-value
		n=218	%	n=228	%	n=446	%	
1	*Escherichia coli*	154	70.6	126	55.2	280	63.1	<0.001
2	*Klebsiella pneumoniae*	36	16.5	31	13.5	67	15	0.74
3	*Klebsiella oxytoca*	1	0.4	0	0	1	0.2	-
4	*Proteus mirabilis*	4	2.3	9	3.9	13	2.9	=1.0
5	*Proteus vulgaris*	3	1.3	0	0	3	0.6	-
6	*Pseudomonas aeruginosa*	6	3.6	13	5.7	19	4.2	=0.19
7	*Staphylococcus aureus*	4	2.7	15	6.5	19	4.2	<0.001
8	*CONS*	3	1.3	9	3.9	12	2.6	=0.16
9	*Enterococcus faecalis*	3	1.3	3	1.3	6	1.3	=1.0
10	*Candida albicans*	4	2.7	16	7	18	4	<0.001
11	*Candida non albicans group*	0	0	6	2.6	6	1.3	-

CONS – Coagulase negative *Staphylococci*

As per Table 3, Of 126 urinary tract infections by *E.coli* in DM cases, predominant group was (IDDM) Insulin Dependent Diabetes Mellitus (42.8%), followed by (NIDDM) Non-Insulin Dependent Diabetes Mellitus (38%), Gestational diabetes (17.4%) and juvenile diabetes (1.5%). The preponderance of DM cases treated with insulin alone (46%) over combined treatment (insulin and oral hypoglycemic) (17.4%), oral hypoglycemic (23.8%) and diet alone (12.6%) was recorded. In the study, more than 50% of the urinary infections occurred in individuals with DM for 6-20 years duration and 31.7% of urinary infections occurred in individuals with DM for < 1 year duration, which included 17.4% of gestational DM

Table 3: Occurrence of UTI by *E. coli* in different types and duration of DM

Sl. No.	Type of Diabetes	*E. coli* (n=126)	%
1	IDDM (Type I DM)	54	42.8
2	NIDDM (Type II DM)	48	38
3	Gestational DM	22	17.4
4	Juvenile DM (Type I DM)	2	1.5
Type of Diabetic Treatment			
1	Diet alone	16	12.6
2	Insulin alone	58	46
3	Oral Hypoglycemic	30	23.8
4	Both Insulin & Hypoglycemic	22	17.4
Duration of Diabetes			
1	< 1 yr	40	31.7
2	1-5 yrs	8	6.3
3	6-10 yrs	45	35.7
4	11-20 yrs	26	20.6
5	>20 yrs	7	5.5

IDDM - Insulin Dependent Diabetes Mellitus
NIDDM - Non Insulin Dependent Diabetes Mellitus

Antimicrobial Susceptibility Test: In the present study, *E.coli* strains isolated from UTI cases showed increased resistance to ampicillin (86.7%), ceftazidime (63.2%), ciprofloxacin (52.5%) and co-trimoxazole (50%), and least resistance to imipenem (0.7%), netilmicin (22.5%), gentamicin (28.9%) and nitrofurantoin (37.5%). There was no significant difference (p <0.05) in the resistance pattern of *E.coli* to antibiotics between non-DM and DM cases (Fig. 1).

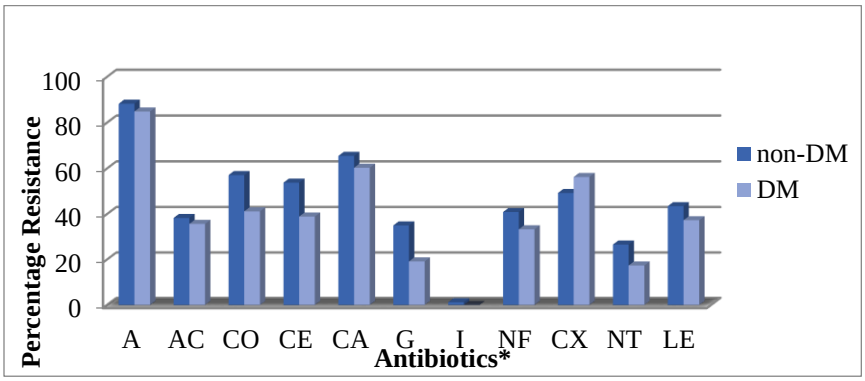

Fig. 1: Antimicrobial susceptibility test showing resistance pattern of *E.coli*

* A (ampicillin), AC (Amoxycillin+clavulanic acid), CO (Co-trimaoxazole), CA (ceftazidime), G (gentamicin), I (imipenem), NF (nitrofurantoin), CX (ciprofloxacin), NT (netilmicin), LE (levofloxacin)

Discussion

The factors related to increased risk of urinary infections in diabetic patients were decreased antibacterial activity due to glucosuria, defects in neutrophil function, increased adherence to uroepithelial cells and compromised antioxidant system.[12] Even though the prevalence of diabetic bladder dysfunction increases with the duration of diabetes, it can also occur silently and early in the course of disease.[13] Zhanel *et al.* (1995) correlated host factors of the patient with bacteriuria and found bacteriuric women were significantly more likely to have non-insulin dependent diabetes mellitus, longer duration of diabetes, and neuropathy than non bacteriuric women.[14]

In the present study, the increased prevalence of UTI by *E.coli* was seen in type II DM (42.8%) compared to type I DM (38.0%) with no statistically significant difference ($p>0.05$). Also the increased frequency of UTI (61.8%) was recorded in patients with diabetes for more than 5 years duration.

Out of 126 diabetic UTI cases studied, 58 (46%) cases were on insulin therapy, 30 (23.8%) on oral hypoglycemic drugs, 22 (17.4%) cases were on combination therapy and 16 (12.6%) of the cases were only on diet restriction. However, Schneeberger and associates reported that diabetic (pre-menopausal and post-menopausal) women with UTI were predominantly on oral hypoglycemic agents compared to DM patients with insulin therapy.[15]

Ghanghesh and associates have observed no difference in the isolation rate of uropathogens between DM and non-DM cases, when compared to the age of the patients ≤ 40 years with that of age > 40 years.[16] However, in the present study, 72.2% of DM cases and 29.9% of non-DM cases above 40 yrs of age were suffered with UTI. Conversely, 27.8% of DM and 70.1% of non-DM cases below 40 yrs of age suffered with UTI. The difference in the rate of isolation of uropathogens between the two groups was statistically highly significant ($p<0.001$).

Though *E.coli* was the predominant organism in both the groups, its occurrence was significantly high in non DM subjects compared to DM subjects (70.6% Vs 55.2%, $p<0.001$), the reduced *E.coli* occurrence in non DM was associated with significantly higher isolation rate of *Staphylococcus aureus* (6.5% Vs 2.7%; $p< 0.001$) and *Candida* species (7% Vs 2.7%; $p <0.001$) in DM cases. Our data supports the observation by Raffel *et al.* (1981), who have shown that diabetic rats with elevated glucosuria are more susceptible to *Candida albicans* and *Staphylococcus aureus* infections.[17] Furthermore, the presence of Gestational DM could have also influenced a slightly higher isolation of *Staphylococcus aureus* and *Candida* species. It is known that gram positive bacteria and yeast are frequently isolated in pregnant women with DM.[18,19]

Antibiotic resistance among UPEC was observed against commonly used drugs, predominantly to ampicillin (86.7%), followed by ceftazidime (63.2%), ciprofloxacin (52.5%), and trimethoprim/ sulphamethoxazole (50%). The greater prevalence of resistance of *E.coli* to common antibiotics has also been reported by other workers.[20,21,22] Chitnis *et al* have observed similar finding among Gram negative bacilli that the maximum number of isolates were resistant to ampicillin (83.2%) and the lowest to netilmicin (24.3%).[20] In our study, except for few antibiotics (trimethoprim/sulphamethoxazole, cefotaxime and gentamicin), we found no significant difference in the resistance pattern of *E.coli*, regardless of whether they were isolated from DM or non-DM patients with UTI. A study by Bonadio *et al* in Italy reported that the resistance of uropathogens to antibiotics was similar in patients with and without DM.[23]

In conclusion, *E.coli* was predominant organism isolated from UTI in both DM and non-DM groups. However, there was decreasing trend of UTI caused by *E.coli* in DM cases compared to non-DM cases. Microorganisms encountered in UTI did not differ by diabetic status except for *Candida* species and *Staphylococcus aureus*, which were more frequently isolated from diabetic patients. Among diabetics, age of the patient, long duration and poor glycemic control were associated risk factors in the development of UTI.

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