ORIGINAL ARTICLE

MICROBIOLOGICAL SPECTRUM OF UROPATHOGENS CAUSING URINARY TRACT INFECTIONS IN DIABETICS

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Background: Patients with diabetes mellitus (DM) are at increased risk of infections most commonly urinary tract infections. The objective of this study was to determine the spectrum of bacterial pathogens causing UTI in diabetic patients. **Methods:** A descriptive, cross-sectional study was carried out in the Department of Pathology, Abbas Institute of Medical Sciences, Muzaffarabad, from Jan to Dec 2019. A total of 292 patients with DM were enrolled in the study after informed consent regardless of the presence or absence of UTI symptoms. Patients with underlying renal pathology or chronic renal disease, pregnancy and antimicrobial therapy were excluded. Urine samples were taken and isolates were identified on the basis of colony morphology, gram staining, and biochemical reactions like catalase, coagulase, DNAse, oxidase test and Analytical Profile Index 20E strips (BioMerieux) as required. **Results:** A total of 292 patients were included. Mean age of the patients was 40.95±8.95 years. Out of these, 120 (58.90%) were male and 172 (41.10%) were females. The frequency of bacterial pathogens causing UTI was 37 (12.67%) for *E. coli*, 13 (4.45%) for *Klebsiella* spp, 5 (1.71%) for *Proteus* spp, 7 (2.40%) for *Pseudomonas* spp, 9 (3.08%) for *Staphylococcus aureus*, 7 (2.40%) for *Enterococci*, and in 214 (73.29%) patients there was no growth of any organism. **Conclusion:** *E. coli* is the most common bacterial pathogens causing UTI in diabetic patients.

Keywords: Diabetics, Urinary Tract Infections, UTI, Pathogens, Frequency

Pak J Physiol 2021;17(4):31–4

INTRODUCTION

Urinary tract infection (UTI) is common health problems in community as well as nosocomial setup and is usually caused by bacteria.[1] These infections range from asymptomatic bacteriuria (ASB) on one hand to acute pyelonephritis and gram-negative septicaemia on the other hand. Diabetes is one of the potential reasons of UTI[2], as sugar in the urine makes for a fertile breeding ground for bacteria and there is alteration in the immunity due to granulocyte dysfunction[3]. UTI is the most common infection among patience with DM and is responsible for considerable morbidity, particularly if it is untreated or unrecognized.[4] UTI in diabetic patients may lead to severe kidney damage and renal failure. Risk factors in patients are obesity, female gender, glycosuria, low immunity, and bladder dysfunction associated with DM.[5–7]

The spectrum of UTI in diabetics ranges from ASB to lower UTI (cystitis), pyelonephritis, and severe urosepsis. Mostly these patients are prone to resistant pathogens as the cause of their UTI, including extended-spectrum β-lactamase-positive Enterobacteriaceae[8], fluoroquinolone-resistant uropathogens[9], carbapenem-resistant Enterobacteriaceae, and vancomycin-resistant Enterococci.

Prevalence of UTI among diabetics is reported to be 25.3%.[10] The most frequently isolated uropathogens reported are *Escherichia coli, Klebsiella* spp, *Staph. aureus* and *Enterobacter*.[11]

There is no such data reported from northern areas of Azad Kashmir, especially for diabetics. The objective of this study was to evaluate microbiological spectrum of uropathogens causing urinary tract infections in diabetic patients of Muzaffarabad and surrounding areas.

METHODOLOGY

This descriptive, cross-sectional study was carried out in the Department of Pathology, Abbas Institute of Medical Sciences (AIMS), Muzaffarabad, from January to December 2019. A total of 292 adults (above 16 years) patients (both male and female) with DM who attend the outpatient and inpatient departments of AIMS were enrolled in the study after informed consent regardless of the presence or absence of UTI symptoms. Patients with underlying renal pathology or chronic renal disease, pregnancy, and antimicrobial therapy were excluded.

Patients were asked to provide a midstream urine sample according to clean-catch procedure. Sample was collected in sterile containers and processed within 1 hour of collection. Five ml of urine centrifuged at 3,000 rpm for 5 minutes was examined microscopically to detect WBCs, RBCs and bacteria. Urine analysis was done with dipstick test. Urine sample was inoculated on Cysteine lactose electrolyte deficient (CLED) agar with Bacteriuritest® strip (Mast Diagnostic) dipped in urine up to a defined mark (the strip picks 0.2 µL of urine). Plates were incubated for

18–24 hours at 37 °C under aerobic conditions and the outcome was judged as significant/non-significant growth or contaminated. Urine culture plates showing $>10^5$ colony-forming units (CFU)/ml of single bacterial species were considered as significant bacteriuria. Lower bacterial counts were considered insignificant and growth of more than two types of organisms was considered as contamination. MDR bacteria was defined as isolates resistant to >2 antimicrobial agents.

Isolates were identified on the basis of colony morphology gram staining and biochemical reactions like catalase, coagulase, DNAse (in case of gram-positive organism) and oxidase test and Analytical Profile Index (API) 20E strips (BioMerieux) in case of gram-negative rods. For the Quality Control, *E coli* ATCC 51153, *Staph aureus* ATCC 51153 and *Pseudomonas aeruginosa* ATCC 27858 bacterial strains were used. The Kirby-Bauer disc diffusion method was used to determine the antimicrobial susceptibility of isolates on Muller-Hinton agar using 0.5 McFarland standard, and disposable sterile swabs. Antimicrobial susceptibility and resistance was determined by isolate growth zone diameter according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Data was analysed using SPSS-16. Descriptive frequencies and percentages were computed for qualitative variables such as gender, marital status, level of education, type of DM, socioeconomic status, symptomatic and asymptomatic UTI, and bacterial pathogens. Mean and standard deviation were computed for qualitative variables. Stratification was done with regard for age, symptomatic and asymptomatic UTI, gender, type of DM and duration of diabetes mellitus to control effect modifiers. Post stratification Chi-square test was applied, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

A total of 292 patients were included in this study. Mean age of the patients was 40.95±8.95 years. Majority of the patients (155, 53.08%) were >40 years of age (Table-1).

Out of 292 patients, 120 (58.90%) were male and 172 (41.10%) were females. The frequency of bacterial pathogens causing UTI was 37 (12.67%) for *E. coli*, 13 (4.45%) for *Klebsiella* spp, 5 (1.71%) for *Proteus* spp, 7 (2.40%) for *Pseudomonas* spp, 9 (3.08%) for *Staphylococcus aureus*, 7 (2.40%) for *Enterococci*, and in 214 (73.29%) patients there was no growth of any organism (Table-2).

Table-1: Age distribution of patients (n=292)

Age (Years)	No. of Patients	Percentage
16–40	137	46.92
>40	155	53.08
Total	292	100.0

Table-2: Frequency of uropathogens causing UTI in diabetic patients

Bacterial pathogens	No. of Patients	Percentage
E. coli	37	12.67
Kleibsella spp	13	4.45
Proteus spp	5	1.71
Pseudomonas spp	7	2.40
Staphylococci	9	3.08
Enterococci	7	2.40
No organism	214	73.29

DISCUSSION

Patients with diabetes are susceptible to infections (about four times more than non-diabetics)[12,13], which might be ascribed to their abnormalities in immune function.[14,15] Among the infections, urinary tract infections (UTIs) occur more frequently in diabetic patients[16,17] because of urine glucose excretion and chronic neurologic bladder dysfunction.[18] Furthermore, the prevalence of asymptomatic bacteriuria in diabetic patients is three times higher than in normal people; however, whether the symptomatic UTIs are preceded by ASB is unknown.[19,20]

UTIs may cause serious complications in diabetic patients, such as emphysematous cystitis, renal failure, bacteremia and papillary necrosis.[21,22] UTIs can also cause systemic inflammation and oxidative stress that elevate blood glucose and increase insulin resistance. Although the incidence of UTIs is higher and the severity of UTIs is more than anyone thought of previously, less than half of the UTI patients seek treatment in Asia.[23]

Escherichia coli is the frequent uropathogen in UTIs. However, the incidences of UTIs in different races and ethnicities are different. In previous studies, it was reported that the isolation of *E. coli* in European patients with UTIs was decreasing in the past 15 years.[24,25] The isolation rate of *E. coli* was 26% in Japanese patients with UTIs and 55.1% in Indian patients.[26,27]

Prevalence of UTI among diabetics is reported to be 25.3% in a recently published article.[10] Another study has revealed *Escherichia coli* in 13%, *Klebsiella* 13%, *Staph aureus* 9% and *Enterobacter* in 5%.[11] Fifty-seven percent diabetic patients yielded no growth.[11] Geerling has reported the members of the family *Enterobacteriaceae* (i.e., *Proteus, Klebsiella, Enterobacter,* and *Citrobacter* species), *Pseudomonas* species, *Enterococcus* species, *Streptococci, Staphylococci,* and *C. albicans.*[28] The emergences of resistant bacterial strains in hospitals pose a continued challenge to treat and control the spread of infections. Moreover, indiscriminate use of antibiotics often results in increased resistance of urine pathogens to most commonly used antimicrobial agents.[29]

Saber H, *et al*, in a study with a total of 288 diabetics (196 females and 92 males), and 63 non

diabetic patients (43 females and 20 males) with symptomatic UTI found that 43.8% diabetic patients and 42.9% non-diabetic patients had positive growth from urine. Same study shows rate of isolation of *Escherichia coli* in diabetics as 61.8% compared to non-diabetics (77.8%). Frequency of other organisms isolated in diabetic and non-diabetic patients in their study were respectively: *Klebsiella* spp 6.9% *vs* 3.7%, *Enterococcus* 12.2% *vs* 3.7%, *Pseudomonas* species 3.8% *vs* 0%, *Candida* species 4.6% *vs* 3.7%, *Staphylococcus aureus* 4.6% *vs* 7.4%.[30]

In our study, the organism associated with UTI was predominantly *E. coli.* The main reason for this is that the *E. coli* being the normal flora of gut gets easy access for UTI. These findings are similar to those observed by Boyko *et al*[31] on 218 diabetic postmenopausal women indicating that the prevalence of *E. coli* was 74.4% and that of *Klebsiella* spp. was 7%. Another case-control study, conducted in New Delhi, India, that evaluated the prevalence of UTI and renal scarring in 155 patients with diabetes, also found that *E. coli* was the most commonly involved organism (64.3%), followed by *Staphylococcus aureus* (21.4%) and *Klebsiella pneumoniae* (14.3%).[32]

Another study revealed that *E. coli* (49%) and *Enterococcus* species (35%) were the most prevalent pathogens followed by *Klebsiella pneumonia* (11%) and *Proteus mirabilis* (8%).[3] This finding is similar to other findings which indicate that gram negative bacterium, particularly *E. coli* remains the commonest pathogen isolated in patients with UTI.[33,34] In a study from Nepal, it was found that *Escherichia coli* was the most commonly grown organism (54.5%), followed by *Staphylococcus aureus* (17.3%), *Enterococcus* spp (9.4%) and *Klebsiella* spp (7.5%).[35]

In a study among 328 diabetic patients' urine samples, 34 (10.37%) showed culture positivity while out of same number of non-diabetic urine samples, 55 (16.77%) showed culture positivity. *E. coli* was found to be the most common isolated pathogen in diabetic and non- diabetic patients at 61.7% and 67.3% respectively. Other causative organisms of UTI in diabetic were *Klebsiella peumoniae* (14.70%), *Staph aureus* (11.77%), *Staph saprophyticus* (8.82%), *Pseudomonas aeruginosa* (2.94%), and in non-diabetic patients *Klebsiella pneumoniae* (5.45%), *Citrobacter freundii* (5.45%), *Klebsiella oxytoca* (3.63%), *Proteus mirabilis* (3.63%), *Providencia* spp. (3.63%), *Staph. aureus* (5.45%), *Staph saprophyticus* (1.82%), *Pseudomonas aeruginosa* (1.82%) and *Enterococcus faecalis* (1.82%).[36]

CONCLUSION

E. coli is the most common bacterial pathogen causing UTI in diabetic patients in diabetic patients of Muzaffarabad and surrounding areas.

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Received: 24 Jun 2021 **Reviewed:** 20 Oct 2021 **Accepted:** 21 Oct 2021

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MAK: Data collection, statistical analysis, final drafting
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SM: Literature review, proof reading, drafting
AM: Sample processing, literature review

Conflict of interest: None declared
Funding: None