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Causative Uropathogenic Bacteria and Their Antimicrobial Susceptibility Patterns in Diabetic Patients with Urinary Tract Infection in Ilam, Western of Iran

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ABSTRACT

Introduction: In fact, individuals with diabetes mellitus (DM) are highly susceptible to developing urinary tract infections (UTIs). This study aimed to evaluate the etiologic agents of UTIs and their patterns of antimicrobial resistance among diabetic patients with UTIs in Ilam, western Iran.

Materials & Methods: A cross-sectional study was conducted from June 2020 to December 2023 in Ilam, in western Iran. A total of 3,362 patients with DM and UTI were evaluated for common uropathogens. Bacterial identification was performed using colony morphology, Gram staining, and standard biochemical tests. The antibiotic susceptibility pattern was determined using the Kirby-Bauer disk diffusion method on Muller-Hinton agar. Data analysis was performed using SPSS software version 16.0. Fisher's exact test and Pearson's chi-square test were used to assess the association between the variables (p-value <0.05 as significant).

Results: Among the diabetic samples, 0.8% (27/3,362) were positive for uropathogens, with a higher prevalence in females (77.8%) than in males (22.2%) (p-value = 0.03). All positive cases were older than 44 years (p-value = 0.02). Gram-negative bacteria (GNB) were more common (70.3%) than gram-positive bacteria (GPB) (29.7%) (p-value = 0.001). *Escherichia coli* was the predominant pathogen (63%), followed by coagulase-negative *staphylococci* (25.9%), *Citrobacter* spp. (3.7%), *Klebsiella* spp. (3.7%), and coagulase-positive *staphylococci* (3.7%). Cefazolin and cephalexin showed the highest efficacy against GNB (both 10.5% resistance), whereas norfloxacin (63.2%), ampicillin (57.9%), and nalidixic acid (52.6%) exhibited higher resistance rates. Among GPB, co-trimoxazole, gentamycin, cefazolin, and amoxicillin/clavulanic acid were more effective (100% sensitive), whereas oxacillin (37.5%) showed poor efficacy.

Conclusion: This study highlights the prevalence and resistance patterns of uropathogens in diabetic patients with UTIs, emphasizing the need for targeted antibiotic therapy and continuous monitoring.

Keywords: Urinary Tract Infections, Drug Resistance, Diabetes

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Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases, affecting millions of individuals annually (1). The infection can impact the upper urinary tract, which is known as pyelonephritis, or the lower urinary tract, commonly called cystitis (2). Despite their generally low mortality rate, UTIs impose a significant financial burden on healthcare systems, leading to increased costs for diagnosis and treatment (3(3). In particular, the epidemiology, species distribution, and antimicrobial resistance patterns of uropathogens vary across different regions and populations, highlighting the need for localized studies to guide treatment strategies (4). (4). can be caused by a various range of bacterial pathogens originating from both community and healthcare settings. *Escherichia coli* is recognized as the most predominant causative agent of UTIs, accounting for a significant portion of cases worldwide. Her important uropathogenic bacteria include *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus saprophyticus, Enterococcus faecalis, Pseudomonas aeruginosa, Enterobacter, Serratia*, and Group-B *Streptococcus* (5, 66). Among the populations at increased risk of UTIs, individuals with diabetes mellitus (DM) are particularly vulnerable (7). Diabetes is a chronic metabolic disorder characterized by impairments in insulin function and glucose metabolism, leading to complications in various organ systems (8). Notably, in 2011, diabetic patients accounted for approximately 10% of all emergency department visits in the United States related to infections, with 30% of these cases being UUTIs. Furthermore, the hospitalization rate for UTIs among diabetics was estimated to be around 200,000 cases annually, with treatment costs significantly higher than those for non-diabetic individuals, ranging from 1.2 to 1.5 times more (9). Unfortunately, the incidence of diabetes is rising alarmingly, and it is estimated that by 2030, the number of cases will reach approximately 552 million. The management of UTIs in diabetic individuals is particularly challenging due to the heightened risk of serious complications. Left untreated or inadequately managed, UTIs can progress to renal papillary necrosis, renal abscesses, and bacteremia, posing severe health risks (10). ven the growing diabetic population, the effective diagnosis and treatment of UTIs in this group are critical for preventing complications and improving patient outcomes (11). One of the major concerns in treating UTIs, particularly in diabetic individuals, is the increasing trend of antimicrobial resistance. The World Health Organization (WHO) has identified antibiotic resistance in uropathogens as a global healthcare crisis, warning that ineffective treatments may lead to longer hospital stays, higher medical costs, and increased mortality rates (12, 113). The resistance patterns of uropathogens vary significantly, especially among diabetic patients who are at a greater risk of developing multidrug-resistant infections due to frequent antibiotic exposure (14). Empirical antibiotic therapy is often initiated before pathogen identification; however, delays in accurate diagnosis can contribute to inappropriate treatment choices and increased resistance development (15). To address these challenges, this study investigated the etiological agents responsible for UTIs in diabetic patients and evaluated antimicrobial resistance patterns of these pathogens.

Materials and methods

Study Design and Participants

The study population included Ilamian outpatients who were introduced to perform the urine culture test from June 2020 to December 2023. The inclusion criterion in the current study was individuals diagnosed with DM, and it excluded non-diabetic individuals. In addition, only diabetic patients from Ilam were included in this study. Criteria for the diagnosis of diabetes: a case was classified as diabetic if the fasting blood sugar level was ≥126 U/L mg/dL and/or the hemoglobin A1c (HbA1c) level was ≥6.5% (16), as measured by the biochemical auto-analyzer Sat 450, Italy.

Sample Size

Since the incidence of uropathogens among patients with DM in Ilam was not previously studied, to provide a reliable dataset for statistical analysis, all patients that met our inclusion criteria were included in this study without restriction on sample size. The urine samples were collected from medical laboratories affiliated with Ilam University of Medical Sciences during the study period. In total, 3,362 urine samples meeting the inclusion criteria were analyzed for the presence of uropathogens.

Measurements & Validity and Reliability

Sample collection and culture

Midstream urine specimens were collected in sterile culture containers following standard procedures. All specimens were cultured on MacConkey agar and blood agar media within two hours of collection and then immediately incubated aerobically at 37ºC overnight. Urine cultures were considered positive if they contained ≥105 CFU/ml of a single identified bacterial species. Cultures with fewer than 105 CFU/ml and samples from patients already undergoing antibiotic treatment were excluded from the study (17). Bacterial identification was done using standard microbiological techniques, including bacterial colony appearance, Gram staining, and conventional biochemical tests for both gram-positive and gram-negative isolates separately (18). Antibiotic susceptibility of isolated organisms was evaluated according to the Kirby-Bauer disc diffusion method. Microbial suspensions were prepared in sterile tubes using freshly grown bacteria, adjusted to turbidity equal to a 0.5 McFarland standard, and then cultured on Muller-Hinton agar medium. Antibiotic discs were located around the plate's outer edge, and then the diameter of the inhibition zone was measured after overnight incubation at 37ºC according to CLSI 2023. The urine culture method demonstrated 95% sensitivity and 85% specificity, making it a reliable method for diagnosing urinary pathogens (19). Similarly, the Kirby-Bauer disk diffusion method exhibited acceptable agreement with reference methods (sensitivity, >90%; specificity, >80%), thereby ensuring correct identification of susceptibility patterns (20, 21).

Ethical consideration

Ethical approval for this study was granted by the Ethics Committee of Ilam University of Medical Sciences, assigning reference number IR.MEDILAM.REC.1403.250. The authors have no conflicts of interest to disclose. Written informed consent for participation was not necessary from the participants.

Statistical and Data Analysis

All data were analyzed using SPSS 16.0. Fisher's exact test and Pearson's chi-square test were used to assess the association between different variables and antibiotic sensitivity. A *p*-value of less than 0.05 was determined for the statistically significant.

Results

The overall prevalence of UTIs was 0.8% (27/3,362), and all positive cases were older than 44 years (*p*-value= 0.02). Females 21 (77.8%) UTI was significantly higher than in males 6 (22.2%) (*p*-value= 0.03). Among the 27 isolates obtained, the frequency of gram-negative bacteria (GNB) was higher than that of gram-positive bacteria (GPB) (*p*-value= 0.001). GNB accounted for 19 (70.3%) of the isolates, while 8 (29.7%) GPB were isolated. *E. coli* was the most predominant isolated bacteria, accounting for 63% (17/27) of all isolates. Additional information is provided in Table 1.

Table 1. Incidence of uropathogenic bacteria among diabetic patients with UTIs.

Isolated uropathogens	Number (%)
Gram-negative	19 (70.3%)
Escherichia coli	17 (63%)

Citrobacter species	1 (3.7%)
Klebsiella species	1 (3.7%)
Gram-positive	8 (29.7%)
Coagulase-negative staphylococci	7 (25.9%)
Coagulase-positive staphylococci	1 (3.7%)
Total	27 (100%)

The tested GNB were highly resistance to norfloxacin (63.2%), ampicillin (57.9%), and nalidixic acid (52.6%) but demonstrated low-level of resistance to cefazolin and cephalexin (both 10.5%) (Table 2).

Table 2. Resistance profiling of Gram-negative bacteria isolated from patients with UTIs.

Antibiotics	*E. coli* n=17	*Citrobacter* spp. n=1	*Klebsiella* spp. n=1	Total n=19	*p*-value (Fisher's exact test)
SXT	5 (29.4%)	0	0	5 (26.3%)	0.14
CP	5 (29.4%)	1 (100%)	1 (100%)	7 (36.8%)	0.21
CN	1 (5.9%)	0	1 (100%)	2 (10.5%)	0.48
NA	8 (47.0%)	1 (100%)	1 (100%)	10 (52.6%)	0.06
NOR	10 (58.8%)	1 (100%)	1 (100%)	12 (63.2%)	0.01
CTX	1 (5.9%)	1 (100%)	1 (100%)	3 (15.8%)	0.33
AN	1 (5.9%)	1 (100%)	1 (100%)	3 (15.8%)	0.33
AM	9 (52.9%)	1 (100%)	1 (100%)	11 (57.9%)	0.01
CZ	1 (5.9%)	0	1 (100%)	2 (10.5%)	0.48
AMC	2 (11.8%)	1 (100%)	1 (100%)	4 (21.1%)	0.22

SXT: Co-trimoxazole; CP: Ciprofloxacin; CN: Cephalexin; NA: Nalidixic acid; NOR: Norfloxacin; CTX: Cefotaxime; AN: Amikacin; AM: Ampicillin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid

Among the tested GPB, 100% were sensitive to co-trimoxazole, gentamycin, cefazolin, and amoxicillin/clavulanic acid, whereas oxacillin (37.5%) presented lower efficacy (Table 3).

Table 3. Resistance profiling of Gram-positive bacteria isolated from patients with UTIs.

Antibiotics	CoPS n=1	CoNS n=7	Total n=8	*p*-value (Fisher's exact test)
SXT	0	0	0	0.14
CP	1 (100%)	0	1 (12.5%)	0.29
CN	1 (100%)	1 (14.3%)	2 (25%)	0.08

NA	1 (100%)	0	1 (12.5%)	0.29
GM	0	0	0	0.14
FM	1 (100%)	0	1 (12.5%)	0.29
OXA	1 (100%)	1 (14.3%)	2 (25%)	0.08
AMX	0	3 (42.9%)	3 (37.5%)	0.01
CZ	0	0	0	0.14
AMC	0	0	0	0.14

SXT: Co-trimoxazole; CP: Ciprofloxacin; CN: Cephalexin; NA: Nalidixic acid; GM: Gentamycin; FM: Nitrofurantoin; OXA: Oxacillin; AMX: Amoxicillin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; CoNS: Coagulase-negative *staphylococci*; CoPS: Coagulase-positive *staphylococci*

Discussion

This study highlights key findings related to the patterns of antimicrobial resistance among diabetic patients with UTIs in Ilam. The investigation revealed several noteworthy trends and factors associated with uropathogen prevalence and antibiotic resistance, particularly in this vulnerable population. The incidence of UTIs among diabetic patients in this study was low, with only 0.8% of individuals testing positive for uropathogen. This rate is lower compared to other reports, such as those by Alemu et al. (11.6%) (22) and Worku et al. (9.8%) among diabetic patients (23). Moreover, all diabetic patients in this study with UTIs were older than 44 years, reaffirming prior research suggesting that UTIs are more common among elderly individuals with diabetes (24, 25). Factors such as age-related bladder dysfunction, immune system alterations, and increased comorbidities in older adults could contribute to this trend (26). In the present study, in line with the results of other reports (27, 28), we observed that diabetes was more prevalent in females. This may be explained by anatomical and physiological factors, such as shorter urethra length and proximity to the rectal area in females, which facilitate bacterial ascension. In males, protective antibacterial effects from prostate secretions may contribute to lower infection rates (26, 29). In this study, 70.3% of uropathogens were GNB, while 29.7% were GPB. This is in agreement with other studies that reported GNB are more common among diabetic patients with UTIs (30, 31). We observed that *E. coli* (63%) was the predominant isolated bacteria among diabetic patients. Similar results were reported for diabetic patients from previous studies (14, 32). This is principally due to its ability to easily bind to glycoconjugate receptors on the urinary tract epithelial cells and its high colonization rate in the urogenital tract (33, 34). Other isolated pathogens included coagulase-negative *staphylococci*, *Citrobacter* spp., *Klebsiella* spp., and coagulase-positive *staphylococci*, albeit in much smaller proportions. The analysis of antimicrobial susceptibility revealed that among GNB, cefazolin and cephalexin (both 10.5% resistance) demonstrated the highest efficacy, while norfloxacin, ampicillin, and nalidixic acid exhibited lesser performance, with resistance rates of 63.2%, 57.9%, and 52.6%, respectively. It may result from its extensive use in clinical settings, leading to selective pressure favoring resistant strains (35, 36). For GPB, several antibiotics, such as co-trimoxazole, gentamicin, cefazolin, and amoxicillin/clavulanic acid, were mostly effective (100% sensitive), indicating their potential as first-line treatments. In contrast, resistance to oxacillin (37.5%) was relatively high. This emphasizes the growing challenge posed by antimicrobial resistance, which can complicate the management of UTIs in diabetic patients. The findings underscore the importance of localized studies to understand the regional variability in antibiotic resistance patterns. Differences in socio-geographical characteristics, sample size, study period, level of antibiotic resistance, diagnostic methods, diabetes duration, presence of long-term complications, and type of diabetes can influence the prevalence and resistance profiles of uropathogens (1, 37-40). To the best of our knowledge, this study is the first to investigate the antibiotic resistance of uropathogens in diabetic individuals in Ilam. However, our study also included several limitations, such as (i) the study only assessed 27 diabetic patients with positive uropathogens, which may not provide sufficient representation to generalize findings; (ii) the study focused only on Iranian diabetic patients, which may limit the applicability of findings to other regions; (iii) access to the patients' medical data was not possible because of the lack of a registry system for outpatients; and (iv) although phenotypical antibiotic resistance patterns were evaluated, the underlying molecular mechanisms causing resistance were not studied.

Conclusion

In this study, we evaluated the antibiotic resistance patterns of uropathogenic bacteria causing UTIs in diabetic patients from Ilam. The findings indicated that UTIs were more prevalent in elderly diabetic females and predominantly involved GNB, with *E. coli* being the most common isolate. The study highlighted significant resistance to commonly used antibiotics such as norfloxacin, ampicillin, nalidixic acid, and oxacillin, emphasizing the growing challenge of antimicrobial resistance. For future studies, we suggest (i) examining the relationship between the duration of diabetes, glycemic control, susceptibility to UTIs, and resistance patterns and (ii) comparing antibiotic resistance patterns between uropathogens isolated from hospitalized diabetic patients and those from outpatient settings.

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Conflict of interest

The authors declare no conflicts of interests.

Authors’ contributions

Conceptualized and designed the study, Investigation, Methodology, Formal Analysis, Writing– Review & Editing: SH, Investigation, Methodology, Formal Analysis, Funding Acquisition: MK, Visualization, Project Administration, Writing– Review & Editing, Funding Acquisition: FM.

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