ORIGINAL ARTICLE

Evaluating the antibiotic resistance and frequency of adhesion markers among *Escherichia coli* isolated from type 2 diabetes patients with urinary tract infection and its association with common polymorphism of mannose-binding lectin gene

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Abstract

The present paper aims to determine the frequency and antibiotic resistance patterns of pathogenic bacteria, the virulence factor profile of *Escherichia coli* and mannose-binding lectin (MBL) gene polymorphism in individuals with diabetes mellitus (DM) and urinary tract infection (UTI). The population under study was 130 individuals with type 2 diabetes mellitus (T2DM) and UTI. The patients' clinical characteristics and urine and blood samples (5 mL) were collected. Antibiotic resistance was determined using a disc diffusion method, and the results were interpreted according to CLSI. The presence of virulence genes was detected by multiplex PCR. To detect the MBL gene polymorphism, PCR and restriction fragment length polymorphism methods were applied. The predominant Gram-negative and Gram-positive bacteria included *E. coli* and *Streptococcus* spp.viridans group, respectively. Women were more susceptible to the incidence of UTI than men. The *E. coli* isolates showed a high level of resistance to amoxicillin-clavulanic acid (87.35%), and nitrofurantoin and ceftizoxime were the most effective antimicrobial agents for *E. coli*. Cefotaxime and ceftizoxime were the most effective antimicrobial agents for *Enterobacter* spp., norfloxacin and ciprofloxacin were the most effective antimicrobial agents for *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. *papGII* (52.87%) and *papEF* (1.14%) had the highest and lowest frequency among examined genes in *E. coli* isolates, respectively. The GG genotype had the highest frequency among patients with T2DM and UTI. Results showed that the detection of *E. coli* in individuals with an AA genotype, codon 54 of the MBL gene, can play an important role in the molecular diagnosis and timely treatment of bacterial infections in individuals with diabetes.

Keywords: Antibiotic resistance, diabetes, *Escherichia coli*, mannose-binding lectin gene, urinary tract infections
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Introduction

Diabetes mellitus (DM) as a chronic and metabolic disorder is associated with high levels of blood glucose and reduced insulin production and is considered a global challenge for health-care specialists [1,2]. Type 2 diabetes mellitus (T2DM) is a heterogeneous class of disease, and >90% of individuals with DM have T2DM [3]. T2DM occurs as a result of deficient insulin action and inadequate insulin secretion rather than insufficient use of insulin. Individuals with T2DM are at high risk of moderate or severe infections, including gangrenous cholecystitis, foot infections, soft-tissue infections and urinary tract infections (UTI) [1,4,5]. As the result of persistent neurological bladder dysfunction and urine glucose excretion, UTI are the most prevalent bacterial infection detected in individuals with DM [6]. In comparison with individuals without DM, the risk of a

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UTI is three-to four-fold higher in individuals with DM [7]. Different spectra of severe complications of UTI in individuals with T2DM range from asymptomatic bacteriuria, cystitis, emphysematous cystitis, pyelonephritis, renal papillary necrosis, renal abscesses, to severe urosepsis [5]. Moreover, UTI are associated with end-stage renal disease or impaired renal function among paediatric patients, leading to several abnormalities in patients with increased risk of pyelonephritis, increased premature delivery and high fetal mortality among pregnant women [8–10]. In general, DM can increase the level of urine glucose and pH, so the urine becomes an appropriate microenvironment for harmful bacteria to grow and reproduce [11]. Individuals with DM are more susceptible to resistant bacterial pathogens due to their complicated UTI, including vancomycin-resistant enterococci, fluoroquinolone-resistant uropathogens, extended-spectrum β-lactamase-positive *Enterobacteriaceae* and carbapenem-resistant *Enterobacteriaceae* [5]. Among different pathogenic bacteria, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, Group B streptococci and *Candida* species are frequently isolated from individuals with DM with UTI [4,12,13]. However, in DM and non-DM patients, *E. coli* is the pathogen most conducive to UTI [6]. *Escherichia coli* causes severe infections in individuals with any underlying condition such as DM. According to the results of studies in Japanese and Indian patients with UTI in 2014, the isolation rates of *E. coli* were 26% and 55.1%, respectively [14,15]. However, among European patients with UTI, isolation of this bacterium has fallen over the past 15 years [16,17]. Moreover, most uropathogens such as *E. coli* isolated from individuals with DM are resistant to several classes of antibiotics. Hence, choosing appropriate antibiotics probably decreases and prevents bacterial resistance [17]. The results of several published studies revealed that individuals with DM were susceptible to the vital risk factor in the multidrug-resistant property of uropathogens [4,18]. On the one hand, the number of studies evaluating the antibiotic resistance in individuals with DM is limited. On the other hand, the frequency of virulence markers in these bacteria is high; *E. coli* is characterized by several virulence factors, including iron acquisition/transport system, adhesive organelles, flagella and haemolysin, to survive in and successfully colonize the urinary tract [19,20]. Data concerning the frequency of different pathogens in UTI among individuals with DM, the frequency of virulence markers and antibiotic resistance profiles of *E. coli* isolates are helpful for physicians and health-care professionals to treat the infection. Individuals with T2DM are prone to *E. coli* infections. Resistance to the antibiotics used in treating these infections can cause many problems. However, the presence of virulence factors is also involved in colonization and disease severity, and the polymorphism of mannose-binding lectin (MBL), which is an important component of innate immunity, may play a role in the susceptibility and diagnosis of *E. coli*. The present study aims to determine the frequency of pathogenic bacteria that cause UTI; identify their antibiotic resistance profile; determine the frequency of virulence markers in *E. coli* isolates and determine MBL gene polymorphism and its association with DM and UTI.

Materials and methods

Study population, clinical samples and bacterial identification

A total of 130 individuals with T2DM and UTI referred to Hakim laboratory in Tehran, Iran between January 2018 and December 2019 cooperated in this retrospective study. The average age of the study population was 45 years. The exclusion criteria for patient selection include recent hospitalization history, patients with urinary catheters, immunocompromised patients and use of antibiotics over the last 14 days. All individuals with DM with a UTI and positive urine cultures were included in this study. Patients with T2DM were selected in line with the standard definition of the American Diabetes Association. Overall, 130 urine and blood samples (5 mL) were collected from the selected patients. The positive urine samples were subcultured on specific media (all media from Merck, Darmstadt, Germany), namely eosin methylene blue agar, blood agar plates, MacConkey agar and mannitol salt agar; then, Gram-staining of bacterial colonies was performed. The isolation and identification of different bacterial strains of positive cultures were performed using conventional biochemical tests such as IMVIC test (indole, methyl red, Voges Proskauer, and citrate), urease test, catalase and oxidase tests, coagulase test, CAMP test (Christie-Atkins-Munch-Peterson), growth on Kligler iron agar, SIM (sulphide indole motility), bile esculin agar, growth on 6% NaCl and DNase test.

Antibiotic susceptibility test

The susceptibility of the isolated pathogens was determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Merck) and the vancomycin susceptibility test was carried out by a microdilution method for staphylococci. The following antibiotics are considered in the study: trimethoprim-sulfamethoxazole, nalidixic acid, nitrofurantoin, norfloxacin, ciprofloxacin, amoxicillin-clavulanic acid, ceftizoxime, gentamicin, cefixime, ceftazidime, ceftriaxone, vancomycin and cefotaxime. The antibiotic discs were purchased from MAST company (Merseyside, United Kingdom). CLSI criteria were considered in interpreting the results. Bacterial isolates,

including *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* ATCC 25923 and *E. coli* (ATCC 25922), were used as quality control strains.

DNA extraction and multiplex PCR

Genomic DNA was extracted from *E. coli* strains cultured in brain–heart infusion medium using the DNA extraction kit (GeNet Bio Company, Daejeon, Korea; Cat. no. K-3000) according to the manufacturer's instructions and, then, preserved at −80°C. Multiplex PCR was conducted for *papA*, *papC*, *papEF*, *papGI*, *papGII* and *papGIII* genes using specific primer pairs, followed by DNA sequencing [21]. The primers used in this study are listed in Table 1. Multiplex PCR was conducted in a final volume of 25 µL reaction mixture containing 12 µL of 2 × Master Mix (Cinnagene, Tehran, Iran; Cat. no. PR901638) including 0.4 mmol/L dNTPs, 1 × PCR buffer, 0.08 IU *Taq* DNA polymerase and 3 mmol/L $MgCl_2$, 0.5 µM of each primer (10 mM), 3 µL of template DNA and 9 µL of sterile distilled water. The multiplex PCR were performed in the following conditions: one cycle at 94°C for 4 minutes, followed by 35 cycles at 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes with a final extension at 72°C for 10 minutes following the last cycle. *Escherichia coli* ATCC 25922 and distilled water were used as positive and negative controls, respectively. Finally, the PCR products were screened on a 2% agarose gel stained with DNA safe stain (Sinaclon), then visualized and photographed under UV light.

DNA extraction from serum samples and PCR

DNA was extracted from serum samples using the DNA extraction kit (High Pure PCR Template Preparation Kit; Roche, Mannheim, Germany, Cat. no. 11769828001) according to the manufacturer's guidelines. The primer sequence used for the MBL gene is shown in Table 1 [22]. The PCR was performed in a 25-µL reaction mixture containing 11 µL of 1 × Master Mix (Cinnagene, Tehran, Iran; Cat. no. PR901638) that includes 0.4 mmol/L dNTPs, 1 × PCR buffer, 0.08 IU *Taq* DNA polymerase and 3 mmol/L $MgCl_2$, 2 µL of each primer (10 mM), and 4 µL of template DNA and 6 µL of sterile distilled water. The PCR was performed in the following conditions: one cycle at 95°C for 10 minutes, followed by 35 cycles at 95°C for 30 seconds, 55°C for 40 seconds and 74°C for 1 minute with a final extension at 74°C for 10 minutes following the last cycle.

TABLE 1. Primers used for the detection of the virulence markers and the MBL gene

Primers name		Sequence (5'→3')	PCR product size (bp)
papA	F	ATGGCAGTGGTGTTTTGGTG	720
	R	CGTCCCACCATACGTGCTCTTC	
papC	F	GTGGCAGTATGAGTAATGACCGTTA	200
	R	ATATCCTTTCTGCAGGGATGCAATA	
papEF	F	GCAACAGCAACGCTGGTTGCATCAT	336
	R	AGAGAGAGCCACTCTTATACGGACA	
papGI	F	TCGTGCTCAGGTCCGGAATTT	461
	R	TGGCATCCCCCAACATTATCG	
papGII	F	GGGATGAGCGGGCCTTTGAT	190
	R	CGGGCCCCCAAGTAACTCG	
papGIII	F	GGCCTGCAATGGATTTACCTGG	258
	R	CCACCAAATGACCATGCCAGAC	
MBL 54	F	GAGGCTTAGACCTATGGGGCTAG	329
	R	CCCCTTTTCTCCCTTGGTGT	

Determining the MBL gene polymorphism by RFLP

To identify the MBL gene polymorphism, the restriction fragment length polymorphism (RFLP) method with a specific enzyme was implemented. Briefly, a 20-µL RFLP mixture consists of 2 µL of 10 × *Ban*I restriction enzyme buffer, 0.5 µl *Ban*I restriction enzyme (5 units), 10 µL PCR product (0.1–0.5 µg) and 7.5 µL sterile distilled water. In the next step, the RFLP mixture was vortexed and incubated at 37°C for 3 hours. Moreover, to prevent the enzymatic reaction, microtubes were incubated at 65°C for 20 minutes. Finally, PCR products were screened on 2.5% agarose gel, stained with DNA-safe stain (Sinaclon), and visualized on a UV transilluminator.

Statistical analysis

The patient information was added to the statistical package SPSS v.23.0 (SPSS Inc., Chicago, IL, USA) and analysed using χ^2 test.

Results

Clinical features, serum findings and pathogen distribution

A total of 130 individuals with T2DM and UTI cooperated in this study, and the results showed that women were more susceptible to the incidence of UTI than men. In this study, 89% of patients were female and 11% were male (sex ratio 12%, $p < 0.001$). The incidence of uropathogens in individuals with DM is shown in Table 2. Gram-negative and Gram-positive bacteria comprised 86.1% (n = 112) and 13.9% (n = 18) of the total bacteria, respectively. The isolated Gram-negative bacteria included *E. coli* (87/112; 77.6%), *Enterobacter* spp. (17/112; 15.1%), *Citrobacter* spp. (2/112; 1.7%), *Acinetobacter* spp. (2/112; 1.7%), *Klebsiella* spp. (2/112; 1.7%), *Proteus* spp. (1/112; 0.9%) and *Pseudomonas aeruginosa* (1/112; 0.9%), respectively. Furthermore, the most predominant isolated Gram-positive bacteria included *Streptococcus* spp. viridans group (6/18; 33.3%), *Staphylococcus epidermidis* (4/18; 22.2%), *Staphylococcus saprophyticus* (4/18; 22.2%) and *Enterococcus* spp. (3/18; 16.6%).

Antimicrobial susceptibility profile

The resistance rates of the isolated Gram-negative bacteria to the commonly used antimicrobials are shown in Table 3.

TABLE 2. The incidence of the uropathogens in diabetic patients

Pathogens	Men, *n*/*N* (%)	Women, *n*/*N* (%)	Patients, *n*/*N* (%)
Escherichia coli	8/130 (6.1)	79/130 (60.7)	87/130 (66.9)
Enterobacter spp.	2/130 (1.5)	15/130 (11.5)	17/130 (13)
Streptococcus spp.viridans group	1/130 (0.7)	5/130 (3.8)	6/130 (4.6)
Haemolytic streptococcus	0/130 (0)	1/130 (0.7)	1/130 (0.7)
Staphylococcus epidermidis	0/130 (0)	4/130 (3)	4/130 (3)
Staphylococcus saprophyticus	1/130 (0.7)	3/130 (2.3)	4/130 (3)
Citrobacter spp.	0/130 (0)	2/130 (1.5)	2/130 (1.5)
Acinetobacter spp.	1/130 (0.7)	1/130 (0.7)	2/130 (1.5)
Enterococcus spp.	1/130 (0.7)	2/130 (1.5)	3/130 (2.3)
Klebsiella spp.	0/130 (0)	2/130 (1.5)	2/130 (1.5)
Proteus spp.	0/130 (0)	1/130 (0.7)	1/130 (0.7)
Pseudomonas spp.	0/130 (0)	1/130 (0.7)	1/130 (0.7)
Total	14/130 (10.7)	116/130 (89.2)	130/130 (100)

Escherichia coli, as the most conducive pathogen of UTI, showed a high level of resistance to amoxicillin-clavulanic acid (87.35%), trimethoprim-sulfamethoxazole (55.17%) and nalidixic acid (55.17%), respectively. In addition, *E. coli* was found to have low levels of resistance to nitrofurantoin (2.29%) and ceftizoxime (19.54%), respectively. Cefotaxime (0%) and ceftizoxime (11.76%) were the most effective antimicrobial agents for *Enterobacter* spp. However, the resistance level to trimethoprim-sulfamethoxazole (47.05%) was high. *Klebsiella* spp. (100%) showed a high level of resistance to amoxicillin-clavulanic acid. Norfloxacin, ciprofloxacin, gentamicin and cefotaxime were the most effective antimicrobial agents for *Klebsiella* spp. *Acinetobacter* spp. showed a high level of resistance to ciprofloxacin and gentamicin (50%). Overall, among Gram-positive bacteria, *Enterococcus* spp. was 66.66% resistant to norfloxacin and gentamicin (high-level 120 µg). Moreover, norfloxacin (0% and 0%) and ciprofloxacin (0% and 0%) were the most effective antimicrobial agents for *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, respectively. On the whole, vancomycin was found to be an effective antibiotic against Gram-positive bacteria and exhibited a low level of resistance. A vancomycin susceptibility test was performed using the microdilution method for staphylococci. The resistance rates of the isolated Gram-positive bacteria to the commonly used antimicrobials are shown in Table 4.

TABLE 3. Antimicrobial resistance of isolated Gram-negative bacteria in diabetic patients

Antibiotics		Isolated bacteria						
		Escherichia coli (*n* = 87)	*Enterobacter* spp. (*n* = 17)	*Citrobacter* spp. (*n* = 2)	*Acinetobacter* spp. (*n* = 2)	*Klebsiella* spp. (*n* = 2)	*Proteus spp.* (*n* = 1)	*Pseudomonas aeruginosa* (*n* = 1)
SXT	R (%)	55.17	47.05	0	0	50	0	–
NA	R (%)	55.17	35.29	0	–	0	0	–
NI	R (%)	2.29	29.41	50	–	50	–	–
NOR	R (%)	35.63	23.52	0	–	0	0	0
CIP	R (%)	36.78	23.52	0	50	0	0	0
AUG	R (%)	87.35	–[a]	–	–	100	100	–
ZOX	R (%)	19.54	11.76	0	–	0	0	–
GM	R (%)	31.03	17.64	0	50	0	0	0
CFM	R (%)	31.03	41.17	0	–	50	0	–
CAZ	R (%)	26.46	23.52	0	0	50	0	0
CRO	R (%)	26.46	17.64	0	0	50	0	–
CTX	R (%)	37.93	0	0	0	0	0	–

Abbreviations: AUG, amoxicillin-clavulanic acid; CAZ, ceftazidime; CFM, cefixime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; GM, gentamicin; NA, nalidixic acid; NI, nitrofurantoin; NOR, norfloxacin; SXT, trimethoprim-sulfamethoxazole; ZOX, ceftizoxime.
[a]This antibiotic was not used for this bacterium.

TABLE 4. Antimicrobial resistance of isolated Gram-positive bacteria in diabetic patients

Antibiotics		Isolated bacteria				
		Streptococcus spp.viridans group (*n* = 6)	Haemolytic streptococcus (*n* = 1)	*Staphylococcus epidermidis* (*n* = 4)	*Staphylococcus saprophyticus* (*n* = 4)	*Enterococcus* spp. (*n* = 3)
SXT	R (%)	–[a]	–	50	25	–
NI	R (%)	–	–	–	0	33.33
NOR	R (%)	–	–	0	0	66.66
CIP	R (%)	–	–	0	0	66.66
GM	R (%)	–	–	25	25	66.66[b]
VA	R/NS[c] (%)	16.66[c]	0[c]	25[d]	25[d]	0

Abbreviations: CIP, ciprofloxacin; GM, gentamicin; NI, nitrofurantoin; NOR, norfloxacin; SXT, trimethoprim-sulfamethoxazole; VA, vancomycin.
[a]This antibiotic was not used for this bacterium.
[b]High-level (120 µg) gentamicin.
[c]NS, non-susceptible.
[d]MIC test.

Frequency of adhesion genes and MBL gene polymorphisms

Multiplex PCR was performed on *papA*, *papC*, *papEF*, *papGI*, *papGII* and *papGIII* genes. The results of the multiplex PCR show that the frequencies of the examined genes in 87 *E. coli* isolates were as follows: *papGIII*, 51.72%; *papGII*, 52.87%; *papGI*, 20.68%; *papEF*, 1.14%; *papC*, 27.58% and *papA*, 12.64%. In this study, the conventional PCR and RFLP methods with *Ban*I restriction enzyme for the detection of MBL codon 54 genetic polymorphism (AA, GA, GG genotypes) were applied. The product sizes of restriction enzyme *Ban*I are as follows: AA genotypes, 329 bp; AG genotype, 329 bp, 245 bp and 84 bp; and GG genotype 84 bp and 245 bp.

The results indicate that the frequency of the examined genotypes is as follows: AA genotype, 12%; GA genotype, 20%; and GG genotype, 68%. In short, the GG genotype has the highest frequency among individuals with T2DM and UTI.

Discussion

In general, individuals with T2DM are more susceptible to UTI and their complications caused by uropathogens than the general population [6]. UTI are the most frequent bacterial infections around the globe [1]. Among pathogenic microorganisms, *Enterobacteriaceae*, especially *E. coli* and *Klebsiella* spp., are the most frequently isolated microorganisms in men and women with UTI [9]. The current study was carried out to determine the frequency of the pathogenic bacteria that cause UTI, antibiotic susceptibility profile of the isolated pathogens, virulence markers of *E. coli* as the predominant pathogen in UTI, and MBL gene polymorphism among patients with T2DM in Tehran, Iran. The results of the current study revealed that most of the UTI occurred as the result of Gram-negative bacteria. In the urinary tract, Gram-negative bacteria, especially *E. coli*, can bind to the glycoconjugate receptors of epithelial cells and initiate the infection [1]. However, in a study carried out in China in 2017, the frequency of *E. coli* was suggested to be the same in patients regardless of the presence or absence of DM [3]. *Proteus* spp. and *Pseudomonas aeruginosa* have lower frequencies among Gram-negative bacteria isolated from patients; this result is consistent with a study conducted in India [23]. In the present study, *Streptococcus* spp. viridans group and haemolytic streptococci had the highest and lowest frequencies among Gram-positive bacteria isolated from patients, respectively, which was not consistent with studies conducted in Pakistan and Nepal [1,24]. In general, several factors such as type specimen for detecting the pathogen, applied detection methods, and specimen size can affect the frequency of the isolated pathogens [25,26]. Among the different antibiotics tested against *E. coli* isolates, nitrofurantoin and ceftizoxime were the most effective antibiotics; this is consistent with the rates reported in Pakistan [1], India [27] and France [28]. These results show the restricted use of amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, nalidixic acid, and use of nitrofurantoin and ceftizoxime for *E. coli*, at least in the study area. *Escherichia coli* showed a high level of resistance to amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole and nalidixic acid. This result is consistent with the results of a study conducted in Nepal in 2019 [29]. In studies on *E. coli*, which causes UTI in Iran, the highest resistance is related to ampicillin, amoxicillin and tetracycline. This indicates that the widespread use of commonly used antibiotics has led to resistance. However, these studies have shown that antibiotics such as imipenem, nitrofurantoin and amikacin are the most effective treatments. According to studies conducted in Iran, it is recommended not to use penicillin, first-generation cephalosporins and trimethoprim-sulfamethoxazole in primary *E. coli* infections [30].

Enterobacter spp. showed the lowest and highest rates of resistance to cefotaxime and trimethoprim-sulfamethoxazole, respectively. Based on the acquired information, appropriate antibiotic selection is required to facilitate the treatment of UTI caused by pathogenic bacteria. Consequently, primary knowledge about antibiotic resistance profiles in UTI caused by uropathogens can be of help when making a precise therapeutic choice [25]. *Enterobacter* is a causative agent of nosocomial infections, and these strains are often resistant to quinolones, third-generation cephalosporins and penicillins because of previous treatment. The use of fourth-generation cephalosporins and carbapenems is recommended for treatment. Aminoglycosides are also a good choice if carbapenems use is limited [31].

Among the tested antibiotics, the results of the present study revealed that vancomycin is an effective antibiotic against *Enterococcus* spp., *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*, which is consistent with the rates reported in China [6]. A vancomycin susceptibility test was done using the microdilution method for staphylococci. In the present study, the presence of *papGIII*, *papGII*, *papGI*, *papEF*, *papC* and *papA* genes among *E. coli* was analysed with multiplex PCR assays. The results indicated that *papGII* and *papGIII* had the highest frequencies, whereas *papEF* had the lowest frequency among *E. coli* strains. This was not consistent with studies conducted in South Korea [21], Pakistan [32] and Egypt [33]. The presence of these genes in *E. coli* isolates possibly contributed to biofilm formation, which could support their persistence [34,35]. MBL is the main molecule of the innate immune system and an acute-phase serum protein in the family that identifies pathogenic bacteria by its carbohydrate recognition domain [36]. The

results of conventional PCR and RFLP methods on MBL gene polymorphism revealed that, among different genotypes, the GG genotype showed the highest frequency among patients with T2DM and UTI.

In conclusion, the results of the present study revealed that *E. coli* was the main pathogen in patients with T2DM and UTI and, also, nitrofurantoin was an effective antibiotic against *E. coli* infection. In addition, the results showed that the detection of *E. coli* in individuals with the AA genotype, codon 54 of the MBL gene, could play an important role in the molecular diagnosis and timely treatment of bacterial infections in individuals with diabetes.

Author contribution

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data. They played an active role in drafting the article or revising it critically to achieve important intellectual content, gave the final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Conflict of interest

All of the authors declare that there are no commercial, personal, political nor any other potentially conflicting interests related to the submitted manuscript.

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