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Original Research Article

Antimicrobial susceptibility pattern of pathogens from urinary isolates of diabetic patients in a tertiary care hospital in South Tamil Nadu

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ABSTRACT

Introduction: Since diabetes has several negative effects on the urinary tract and host immune system, urinary tract infections (UTIs) pose a serious health issue for diabetic people. The majority of individuals with defective genitourinary tracts get complicated UTIs. To avoid morbidity and significant life-threatening conditions linked to co-morbid diabetes and UTIs, quick diagnosis and treatment are required. Effective care of these patient groups will be facilitated by knowledge of the many bacterial agents that cause UTI in diabetes patients and non-diabetic patients, as well as their susceptibility profiles.

Materials and Methods: This cross sectional study, which was conducted in a tertiary care hospital Virudhunagar, South Tamil Nadu, is aimed to compare the prevalence of UTI, the causative bacteria, and their antibiotic susceptibility profiles in diabetic patients (168) and non- diabetics (150). The existence of urinary tract bacterial pathogens was determined by microbiologic analysis of a urine sample that was collected aseptically. The Kirby Bauer technique was used to screen the isolates for drug susceptibility in order to determine their patterns of antibiotic resistance.

Results: Urinary tract bacterial infections were found in 37.5% and 36.6% of samples from diabetes and non-diabetic people, respectively. *E. coli, Klebsiella, Pseudomonas aeruginosa, Proteus, Citrobacter freundii, Acinetobacter* species, *Enterococcus, Staphylococcus aureus*, and *Coagulase Negative Staphylocossus* (*CoNS*) were the organisms present in the urine samples in the following proportions for the diabetic and non-diabetic individuals, respectively: 34.92% and 29.09%, 12.69% and 10.9%, 7.93% and 12, 6.34% and 5.45%, 3.17% and 1.81%, 3.17% and 0, 22.2% and 16.36%, 9.52% and 14.54% and 0 and 9.09%. Nevertheless, the difference in the percentages of isolated bacteria was not statistically significant (p-value = 0. 856). The majority of the antibiotics tested on bacteria isolated from diabetic and non-diabetic people were extremely effective, particularly Meropenem, Amikacin, Gentamicin, Piperacillin – tazobactam and Nitrofurantoin for gram negative bacteria and Vancomycin and Amikacin for gram - positive bacteria.

Conclusion: The current study's findings highlight the necessity of doing sensitivity testing before beginning antibiotic therapy for UTI since they might aid in the right selection of antibiotics, ensure that they are used effectively, and thus avoid antibiotic resistance.

1. Introduction

A collection of metabolic disorders known as diabetes mellitus (DM) are defined by hyperglycemia characterized by abnormalities in insulin production, insulin action, or both.[1] More than 366 million people were already affected

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by DM, and by 2030, and the same is projected to increase to 552 million, posing a severe danger to both developed and developing nations' public health.[2] In India, there are currently an estimated 77 million diabetics, and by 2045, they are expected to be over 134 million.[3]

Urinary tract infection (UTI), is mostly linked to diabetes mellitus (DM). This is due to the fact that diabetes alters the natural host system, which may lead to the development of UTI.[4] They comprise of increased microbial adherence to uroepithelial cells as well as granulocyte dysfunction, which may be caused by an abnormal intracellular calcium metabolism.[5]*Escherichia coli, Proteus species*, *Klebsiella species*, *Pseudomonas aeruginosa, Enterococcus species, Staphylococcus aureus*, and *Coagulase negative staphylococc*i (CoNS) are the bacteria most frequently linked to UTI in diabetics.[6,7]

The first step in treating UTI patients is frequently empirical. The antibiotic resistance pattern of the urinary pathogens were used to direct the course of treatment. To enhance recommendations for empirical antibiotic therapy, continuous monitoring of resistance patterns is important due to the developing and ongoing phenomena of antibiotic resistance. Thus, a study was conducted on patients from a tertiary care hospital in south Tamil Nadu to identify the causes of UTIs and their patterns of resistance to routinely prescribed medicines. Around 40 million Indians, the majority of whom are uninformed of the disease's care and its repercussions, are facing the pandemic of diabetes mellitus, whose prevalence is constantly growing. In this study, we have determined which microorganisms cause urinary tract infections and how to treat them. The needed antibiotic sensitivity for this inquiry can either prevent or not prevent pathogen development in the urinary system.

2. Materials and Methods

A cross-sectional study was conducted at the Department of Microbiology at a tertiary care Hospital, South Tamil Nadu. The study was carried out from September 2022 to February 2023. A total of 318 patients were screened and their samples were collected. A clean catch midstream urine specimens after a mild antiseptic wash were collected in a sterile container. The urine samples were directly inoculated into CLED agar (Cysteine lactose electrolyte Deficient agar). The organisms were identified by a test panel consisting of Gram stain, colony morphology and colony counts on solid media. The resultant isolates were confirmed by Standard culture methods and biochemical properties as per CLSI standards.

2.1. Antimicrobial susceptibility testing

According to CLSI guidelines, anti-microbial susceptibility testing was carried out using the Kirby-Bauer Disc Diffusion technique. Organisms were prepared in 0.9% saline and adjusted to match 0.5 McFarland standard with a spectrophotometer. All organisms were tested for Antimicrobial susceptibility pattern on Muller-Hinton agar. The Antibiotic Discs (Hi media) used were Amikacin (30 μg), Amoxicillin-Clavulate (20/10 μg), Ceftazidime (30 μg), Cefaperazone-sulbactum (75/10 μg), Ciprofloxacin (5 μg), Levofloxacin (5 μg), Norfloxacin (10 μg), Nitrofurantoin (800 μg), Piperacillin-tazobactum (100/10 μg), Gentamicin (10 μg), Meropenam (10 μg), Ampicillin (10 μg), Co-trimoxazole (1.25/23.75 μg), Penicillin (10U), Linezolid (30 μg), High level Gentamicin (120 μg), Vancomycin (30 μg) and Tetracycline (30 μg). Interpretation of the zone diameter was based upon CLSI guidelines.[8]*Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) were included as quality control strains following the protocol described by CLSI guidelines.

2.2. Statistical analysis and software used

The statistical software programme SPSS was used for all statistical analyses (version 16.0). The frequencies of isolates and whether there was a statistically significant difference between isolates from diabetic patients and non-diabetic people were used to summarize the data. Statistical significance was defined as a p-value < 0.05.

3. Results

The microbiological examination of the urine cultures, which were further described, allowed for the identification of the gram-negative and gram-positive bacteria from the urine culture (Table 1).

In this study, a total of 318 participants, 168 patients with diabetes and 150 patients without had their urine samples collected. There were 41 female and 22 male diabetes patients, compared to the 39 female and 16 male non-diabetic patients (Table 1).

Table 1: Total participants who were culture positive/negative

Status	Total	Culture +	Culture -	Male	Female
Diabetic	168	63	105	22	41
Non-diabetic	150	55	95	16	39

Urinary tract bacterial infections were found in 37.5% and 36.6% of samples from diabetic and non-diabetic people, respectively (Table 2). For diabetics and non-diabetics, the organisms were in the following proportions, respectively: *E. coli* (34.92% and 29.09%), *Klebsiella* (12.69% and 10.9%), *Pseudomonas aeruginosa* (7.93% and 12.72%), *Proteus* (6.34% and 5.45%), *Citrobacter freundii* (3.17% and 1.81%), *Acinetobacter* species (3.17% and 0), *Enterococcus* (22.2% and 16.36%), *Staphylococcus aureus* (9.52% and 14.54%) and *CoNS* (0 and 9.09%).

Table 2: Bacterial isolates identified from diabetic and non-diabetic patients

Isolates	Diabetic group		Non-diabetic group		p -value
	n	%	n	%	
Escherichia coli	22	34.92	16	29.09	0.856
Klebsiella species	8	12.69	6	10.9	
Pseudomonas aeruginosa	5	7.93	7	12.72	
Proteus species	4	6.34	3	5.45	
Citrobacter freundii	2	3.17	1	1.81	
Acinetobacter species	2	3.17	0	0	
Enterococcus species	14	22.2	9	16.36	
Staphylococcus aureus	6	9.52	8	14.54	
CoNS	0	0	5	9.09	

Table 3: Antimicrobial susceptibility pattern of the Gram negative isolates from diabetic patients

Antibiotics	Pattern	*Escherichia coli* n=22	*Klebsiella species* n=8	*Pseudomonas aeruginosa* n=5	Proteus species n=4	Citrobacter freundi n=2	Acinetobacter species n=2	Total (43)
AMC	S	2(9.09)	0	2(40)	1(25)	0	0	5(11.62)
	R	20(90.91)	8(100)	3(60)	3(75)	2(100)	2(100)	38(88.37)
MRP	S	19(86.36)	8(100)	5(100)	4(100)	1(50)	1(50)	38(88.37)
	R	3(13.64)	0	0	0	1(50)	1(50)	5(11.62)
AK	S	22(100)	7(87.5)	5(100)	4(100)	2(100)	2(100)	42(97.67)
	R	0	1(12.5)	0	0	0	0	1(2.33)
GEN	S	17(77.27)	7(87.5)	2(40)	3(75)	2(100)	2(100)	33(76.75)
	R	5(22.73)	1(12.5)	3(60)	1(25)	0	0	10(23.25)
CIP	S	6(27.27)	2(25)	2(40)	1(25)	0	0	11(25.58)
	R	16(72.73)	6(75)	3(60)	3(75)	2(100)	2(100)	32(74.41)
NX	S	8(36.36)	2(25)	2(40)	0	0	0	12(27.9)
	R	14(63.64)	6(75)	3(60)	4(100)	2(100)	2(100)	31(72.09)
CTX	S	0	1(12.5)	0	0	0	0	1(2.325)
	R	22(100)	7(87.5)	5(100)	4(100)	2(100)	2(100)	42(97.67)
PIP	S	19(86.36)	8(100)	5(100)	4(100)	2(100)	1(50)	39(90.69)
	R	3(13.64)	0	0	0	0	1(50)	4(9.3)
CPZ	S	7(31.82)	1(12.5)	0	2(50)	0	0	10(23.25)
	R	15(68.18)	7(87.5)	5(100)	2(50)	2(100)	2(100)	33(76.74)
AMP	S	0	0	0	0	0	0	0
	R	22(100)	8(100)	5(100)	4(100)	2(100)	2(100)	43(100)
COT	S	7(31.82)	4(50)	2(40)	2(50)	0	1(50)	16(37.2)
	R	15(68.18)	4(50)	3(60)	2(50)	2(100)	1(50)	27(62.79)
NIT	S	16(72.73)	4(50)	2(40)	NA	2(100)	0	24(55.81)
	R	6(27.27)	4(50)	3(60)	NA	0	2(100)	15(34.88)

NA: Not Applicable; AMC: Amoxicillin-clavulanate, MRP: Meropenem, AK: Amikacin, GEN: Gentamicin, CIP: Ciprofloxacin, NX: Norfloxacin, CTX: Cefotaxime, PIP: Piperacillin- tazobactam, CPZ: Cefoperazone + Sulbactam, AMP: Ampicillin, COT: Co-trimoxazole, NIT: Nitrofurantoin

Nevertheless, there was no statistically significant difference between the proportions of bacteria extracted (p-value = 0.856).

Meropenem, Amikacin, Gentamicin, Piperacillin-Tazobactam and Nitrofurantoin recorded the highest potency for the antibiotic pattern for gram negative isolates from the diabetic patients, with *Klebsiella, Pseudomonas aeruginosa, Citrobacter freundii* and *Acinetobacter* isolates being 100% susceptible to these antibiotics (Table 3). Among the 12 drugs tested, *Citrobacter freundii* all shown 100% sensitivity to four antibiotics. Vancomycin and Amikacin showed the highest potency against the gram-positive isolates from the diabetic individuals, with *Enterococcus* and *Staphylococcus aureus* following the case after (Table 4).

Gram negative isolates from non-diabetic people had antibiotic patterns that were almost identical to those of diabetes patients (Table 5). *Citrobacter freundii,* one of the individual isolates, had great sensitivity (100%) to antibiotics tested, including Meropenem, Amikacin Gentamicin, Piperacillin, and tazobactam. Vancomycin and Amikacin showed the highest potency in the antibiotic pattern for gram positive isolates from diabetic individuals, with isolates of *Enterococcus, Staphylococcus aureus*,

Table 4: Antimicrobial susceptibility pattern of the Gram-Positive isolates from diabetic patients

Antibiotics	Pattern	Enterococcus (n=14)	Staphylocossus Aureus (n=6)	CoNS (n=0)	otal (n=20)
		3(21.43)	1(16.7)	0	4(20)
	R	11(78.57)	5(83.3)	0	16(80)
LE		9(64.28)	3(50)	0	12(60)
	R	5(35.71)	3(50)	0	8(40)
CIP		7(50)	3(50)	0	10(50)
	R	7(50)	3(50)	0	10(50)
HLG		7(50)	NA	0	7(35)
	R	7(50)	NA	0	7(35)
LZ		6(42.85)	1(16.6)	0	7(35)
	R	8(57.15)	5(83.4)	0	13(65)
NX		3(21.43)	0	0	3(15)
	R	11(78.57)	6(100)	0	17(85)
NIT		5(35.71)	4(66.7)	0	9(45)
	R	9(64.29)	2(33.3)	0	11(55)
VAN		14(100)	6(100)	0	20(100)
	R	0	0	0	0
TE		4(28.57)	1(16.7)	0	5(25)
	R	10(71.43)	5(83.3)	0	15(75)
PIP		12(85.71)	NA	NA	12(60)
	R	2(14.29)	NA	NA	2(10)
AK		NA	6(100)	0	6(30)
	R	NA	0	0	0
CTX		NA	2(33.3)	0	2(10)
	R	NA	4(66.67)	0	4(20)
COT		NA	3(50)	0	3(15)
	R	NA	3(50)	0	3(15)
GEN		NA	3(50)	0	3(15)
	R	NA	3(50)	0	3(15)

NA: Not Applicable; P: Penicillin, LE: Levofloxacin, CIP: Ciprofloxacin, HLG: High level gentamicin, LZ: Linezolid, NX: Norfloxacin, NIT: Nitrofurantoin, VAN: Vancomycin, TE: Tetracycline, AK: Amikacin, CTX: Cefotaxime, COT: Co-trimoxazole, GEN: Gentamicin

and CoNS being completely sensitive to these antibiotics (Table 6). Gram positive isolates from non-diabetic patients had patterns for vancomycin and amikacin that were comparable to those of diabetic patients.

4. Discussion

One of the most prevalent illnesses found in daily practice when treating patients is urinary tract infections. The urethra, bladder, and kidney are all involved. Infections with *Escherichia coli* were found in 80–90% of these cases.[9] When accompanied with anatomical or neurological diseases of the urinary system at any age, UTIs are one of the serious pregnancy complications that frequently results in mortality.[10] The most common causes of urinary tract infection include both intrinsic (such urinary blockage and pregnancy) and extrinsic risk factors (like catheterization and other invasive treatments).[11,12]

The urine samples were collected and analysed in the microbiology laboratory in the tertiary care hospital in south Tamil Nadu, where all diabetic and non-diabetic patients samples were examined. To enhance effective empirical therapy, this study offers useful information to compare and track the level of antibiotic resistance across uropathogens. Globally, rising antibiotic resistance has been observed.[13]

In this investigation, urinary tract bacterial pathogens were found in 37.5% and 36.6% of patients who were diabetes and non-diabetic, respectively. In contrast to the similarities shown in the present study, prevalence was substantially greater in diabetics than in non-diabetics. This is more or less comparable to the investigation by Owusu et al. (2022),[14] where the total prevalence of urinary tract infections among diabetic patients was 28%. This can be because the research populations were different.

Escherichia coli was found to be the most often growing organism in both diabetes and non-diabetic subjects in a research that was identical to the one from India (34.92% and 29.09%).[15] A greater propensity for Escherichia coli adherence has been seen in diabetic individuals with poor glycemic control.[16] Patients with diabetes had a little greater percentage prevalence of uropathogens than non-diabetics, who had a prevalence of 36.6%; however, this difference was not statistically significant. This result, however, emphasizes the prevalent similarities between the two groups examined. The prevalence of diabetic patients also contrasts with the stated non-diabetic prevalence in

Table 5: Antimicrobial susceptibility pattern of the gram-negative isolates from non-diabetic patients

Antibiotics	Pattern	*Escherichia coli* n=16	*Klebsiella species* n=6	*Pseudomonas aeruginosa* n=7	*Proteus species* n=3	*Citrobacter freundii* n=1	*Acinetobacter species* n=2	Total (35)
AMC	S	3(18.75)	0	3(42.86)	2(66.6)	0	0	8(22.86)
	R	13(81.25)	6(100)	4(57.14)	1(33.3)	1(100)	2(100)	27(77.14)
MRP	S	15(81.25)	6(100)	7(100)	3(100)	1(100)	1(50)	32(91.43)
	R	1(6.25)	0	0	0	0	2(100)	3(8.57)
AK	S	15(93.75)	6(100)	7(100)	3(100)	1(100)	0	32(91.43)
	R	1(6.25)	0	0	0	0	2(100)	3(8.57)
GEN	S	13(81.25)	5(83.3)	5(71.42)	2(66.7)	1(100)	0	26(74.29)
	R	3(18.75)	1(16.7)	2(28.58)	1(33.3)	0	2(100)	9(25.71)
CIP	S	7(43.75)	1(16.7)	5(71.42)	2(66.7)	0	0	15(42.85)
	R	9(56.25)	5(83.3)	2(28.58)	1(33.3)	1(100)	2(100)	20(57.14)
NX	S	8(50)	1(16.7)	5(71.42)	1(33.3)	0	0	15(42.85)
	R	8(50)	5(83.3)	2(28.58)	2(66.7)	1(100)	2(100)	20(57.14)
CTX	S	3(18.75)	2(33.3)	0	1(33.3)	0	0	6(17.14)
	R	13(81.25)	4(66.7)	7(100)	2(66.7)	1(100)	2(100)	29(82.86)
PIP	S	11(68.75)	6(100)	6(85.71)	3(100)	1(100)	0	27(77.14)
	R	5(31.25)	0	1(14.29)	0	0	2(100)	8(22.86)
CPZ	S	9(56.25)	3(50)	2(28.58)	2(66.7)	1(100)	0	17(48.58)
	R	7(43.75)	3(50)	5(71.42)	1(33.3)	0	2(100)	18(51.42)
AMP	S	0	0	0	0	0	0	0
	R	16(100)	6(100)	7(100)	3(100)	1(100)	2(100)	35(100)
COT	S	6(37.5)	2(33.3)	3(42.86)	1(33.3)	1(100)	0	13(37.14)
	R	10(62.5)	4(66.7)	4(57.14)	2(66.7)	0	2(100)	22(62.85)
NIT	S	9(56.25)	5(83.3)	1(14.29)	NA	1(100)	0	16(45.71)
	R	7(43.75)	1(16.7)	6(85.71)	NA	0	2(100)	16(45.71)

NA: Not Applicable; AMC: Amoxicillin -clavulanate, MRP: Meropenem, AK: Amikacin, GEN: Gentamicin, CIP: Ciprofloxacin, NX: Norfloxacin, CTX: Cefotaxime, PIP: Piperacillin- tazobactam, CPZ: Cefoperazone + Sulbactam, AMP: Ampicillin, COT: Co-trimoxazole, NIT: Nitrofurantoin

Ghana, Nigeria, and India.

Gram-negative enteric organisms including *Escherichia coli, Klebsiella* species, and *Proteus* species, which frequently cause urinary tract infections, are typically implicated in bacterial research.[15,17] Similarly, Gram-negative pathogens were more frequently isolated than Gram-positive ones. *Escherichia coli* was found among the diabetic patients, with *Klebsiella, Pseudomonas aeruginosa, Proteus species, Citrobacter freundii, Acinetobacter species, Enterococcus species, Staphylococcus aureus*, and *CoNS* being the next most common bacteria. *Escherchia coli* was discovered to be the most often isolated uropathogen from both diabetic and non-diabetic people in a study by Prakash and Saxena (2013),[18] Shah et al. (2019),[19] and Owusu et al. (2022)[15] that was carried out in India, Iran, and Ghana.

A favorable finding indicates that several antibiotics that are often administered in Tamil Nadu, India, are effective against UTI isolates from diabetic patients and non-diabetic people. This information would aid in the efficient management of UTI in such patients to prevent diabetes-UTI complications. In our study, gram negative isolates (*Klebsiella species, Pseudomonas aeruginosa, Citrobacter freundii* and *Acinetobacter species*) were nearly 100% susceptible to antibiotics such as Meropenem, Amikacin, Gentamicin, Piperacillin, and tazobactam. This may have been used since the patients were all from the same population and hence were not exposed to highly resistant bacteria or unnecessary medications.[20]

The sensitivity pattern were also extensively studied. In cases of sensitivity, diabetic UTI *Escherchia coli* of 100% to Amikacin, *Klebsiella* of 100% to Meropenem and Piperacillin- tazobactam, *Pseudomonas aeruginosa* of 100% to Meropenem, Amikacin and Piperacillin-tazobactam *Proteus* of 100% to Meropenem, Amikacin and Piperacillin- tazobactam, *Citrobacter freundii* of 100% to Amikacin, Gentamicin, Piperacillin- tazobactam and Nitrofurantoin, *Acinetobacter* of 100% to Amikacin and Gentamicin, *Enterococcus* of 100% to Vancomycin and *Staphylococcus aureus* of 100% to Vancomycin and Amikacin. Both diabetic and non-diabetic individuals had high susceptibility to the frequently used antibiotic Amikacin. In environments with limited resources, amikacin OPAT (Out-patient parenteral antibiotic therapy) is a viable treatment alternative for non-bacteremic UTIs brought on by ESBL- *Escherichia coli.*[21] As a result, the research area's empirical medicine of choice for the prospective treatment of UTI is Amikacin.

Table 6: Antimicrobial susceptibility pattern of the Gram-Positive isolates from non-diabetic patients

Antibiotics	Pattern	*Enterococcus* (n=9)	*Staphylocossus Aureus* (n=8)	*CoNS* (n=5)	Total (n=22)
P	S	3(33.3)	2(25)	2(40)	7(31.81)
	R	6(66.7)	6(75)	3(60)	15(68.19)
LE	S	7(77.8)	5(62.5)	1(20)	13(59.09)
	R	2(22.2)	3(37.5)	4(80)	9(40.91)
CIP	S	5(55.6)	5(62.5)	2(40)	12(54.54)
	R	4(44.4)	3(37.5)	3(60)	10(45.46)
HLG	S	5(55.5)	NA	NA	5(22.73)
	R	4(44.4)	NA	NA	4(18.18)
LZ	S	3(33.3)	2(25)	4(80)	9(40.91)
	R	6(66.7)	6(75)	1(20)	13(59.09)
NX	S	1(11.1)	2(25)	2(40)	5(27.72)
	R	8(88.9)	6(75)	3(60)	17(77.28)
NIT	S	7(77.8)	5(62.5)	3(60)	15(68.19)
	R	2(22.2)	3(37.5)	2(40)	7(31.81)
VAN	S	9(100)	8(100)	5(100)	22(100)
	R	0	0	0	0
TE	S	4(44.4)	2(25)	2(40)	8(36.36)
	R	5(55.6)	6(75)	3(60)	14(63.64)
PIP	S	8(88.9)	NA	NA	8(36.36)
	R	1(11.1)	NA	NA	1(4.54)
AK	S	NA	7(87.5)	5(100)	12(54.54)
	R	NA	1(12.5)	0	1(4.54)
CTX	S	NA	3(37.5)	2(40)	5(27.72)
	R	NA	5(62.5)	3(60)	8(36.36)
COT	S	NA	4(50)	2(40)	6(27.27)
	R	NA	4(50)	3(60)	7(31.81)
GEN	S	NA	4(50)	4(80)	8(36.36)
	R	NA	4(50)	1(20)	5(27.72)

NA: Not Applicable; P: Penicillin, LE: Levofloxacin, CIP: Ciprofloxacin, HLG: High level gentamicin, LZ: Linezolid, NX: Norfloxacin, NIT: Nitrofurantoin, VAN: Vancomycin, TE: Tetracycline, AK: Amikacin, CTX: Cefotaxime, COT: Co-trimoxazole, GEN: Gentamicin

5. Conclusion

This study has demonstrated similarities between diabetic and non-diabetic patients at the tertiary care hospital, Virudhunagar, South Tamil Nadu in terms of UTI prevalence, the causing bacteria, and their antibiotic susceptibility patterns. It's a great finding that the tested antibiotics were largely effective against the bacterial isolates. These people's UTI will be managed with the use of the study's data. To develop accurate information for the best empirical therapy for diabetic patients with urinary tract infections, continuous monitoring of the susceptibility pattern of urine pathogens is crucial.

6. Source of Funding

None.

7. Conflict of Interest

None.

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