

Clinical and microbiological profile of urinary tract infection in male diabetic patients

Abstract

The possibility of diabetic patients to develop various severe and fatal infections has been rising continuously. In diabetic patients urinary tract infection is very common. Due to the recurrent consumption of antibiotics and the usage of broad spectrum antibiotics, antibiotic resistant pathogens may develop in the urinary tract of diabetic patients. If untreated, UTI can lead to serious complications. Urine samples were taken from the Health Centre of Punjab University. Urine samples were plated on CLED media. Then identification of microorganisms was done by using the method of gram staining and by biochemical testing while some were identified by using API kit. After this, the sensitivity and resistance pattern of these uro-pathogens against different antibiotics were also checked by disk agar diffusion method. Physical and biochemical parameters of diabetic patients were also checked. Our results suggest that the presence of surplus glucose in the urine promotes the growth of pathogens that leads to UTI in diabetic patients. Improved glycemic control in diabetics may help in controlling the UTIs. The assessment of physical and biochemical parameters will be useful in the early diagnosis and prediction of diabetes mellitus. Sensitivity testing before antibiotic therapy may also help in the treatment and to prevent the resistance of antibiotics.

Keywords: urinary tract infection (UTI), CLED (Cysteine Lactose Electrolyte Deficient), API kit (Analytical profile index)

Volume 8 Issue 1 - 2020

Nusair Malik,¹ Arwa Faisal,² Snobia Munir,² Samreen Riaz²

¹Department of Medicine, Health center university of the Punjab, Pakistan

²Department of Microbiology and Molecular Genetics, University of the Punjab, Pakistan

Correspondence: Snobia Munir, Research Scholar at Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan, Tel 92-3158269694, Email snobiamuni@yahoo.com

Received: December 31, 2019 | **Published:** January 22, 2020

Introduction

In the past few decades, diabetes has become prevalent in adults especially in Pakistan. The prevalence in diabetes might be due to changes in lifestyle.¹ Recent studies have shown that about 422 million individuals in the world are suffering from diabetes mellitus.² The chances of developing infections are very high in patients of diabetes. Skin infections, skin rashes, pneumonia (infection in lungs), infection in tissues and urinary tract infections are very common in patients of diabetes which can lead to serious outcome. Among all these infections, urinary tract infections seem to be very common.³ UTI affect all age groups but it seems to be more common in patients suffering from diabetes as compared to those who are non-diabetic.⁴ UTI can be treated by taking antibiotics. Sensitivity testing before starting antibiotic therapy is necessary to check the resistivity and sensitivity pattern of uro-pathogens.⁵ The research was carried out to find the uro-pathogens and to determine their sensitivity and resistance pattern against different antibiotics. It was also checked that why UTI is more common in diabetic patients as compared to non-diabetic patients. The physical and biochemical parameters of diabetic patients were also checked and these parameters were compared with the parameters of healthy control subject.

Materials and methods

Clinical sampling

Urine samples were collected from the Health Centre of University of the Punjab, Lahore from August 2018 to February 2019 from diabetic patients that were suspected to be suffering from UTI. Urine samples were collected in dry, sterilized and leak proof plastic bottles. These

bottles were sterilized by autoclaving them for about 15 minutes at 121°C (15 lbs pressure) and then the samples were collected from patients. Samples were 100 in number. Samples were instantly shifted to laboratory where they were plated on CLED media and then they were further processed. If delayed, samples were kept at 4°C and then they were examined within 2 hours of collection.

Laboratory investigation of urine samples

One drop of urine was taken on glass slide and its smear was made. Then the cover slip was set on the smear and then it was observed under microscope (40X). 500 ul urine samples were plated on these petri plates. Petri plates were put on incubation incubated at 37°C for about 24 hours. After incubation, morphology of well isolated colonies was noted and total colonies' number was counted. Then well isolated colonies of bacteria were streaked on N-agar plates.

Identification of bacteria

After that bacterial colonies were identified by various biochemical tests. While some were identified by API kit.

Biochemical tests

1. Gram's staining 2. Catalase test 3. DNase test 4. Oxidase Test 5. Motility Test 6. Indole test 7. Urease Test 8. Methyl Red test 9. Voges-Proskauer test 10. Citrate utilization Test 11. Triple Sugar Iron Test

API kit

This API (Analytical Profile Index) -20E test strip is used to identify the enteric gram negative rods. API-20E test strip consists of

wells in which dehydrated substrates is present. Bacterial suspension is used to rehydrate this. This strip consists of 20 tests.

Antibiotic sensitivity test

Resistance and sensitivity pattern of all organisms was checked against different antibiotics by Kirby Bauer method. Mueller Hinton agar was used for this. By using cotton swab colony was streaked on plate. Different antibacterial discs were placed on the plate. Plates were put on incubation at 37°C for 24. After incubation, zones of inhibition were recorded in mm.

Statistical analysis

The statistical analysis was done with SPSS statistical software package (version 22.0). Physical parameters including age, weight, height and body mass index (BMI) was calculated. The biochemical parameters which we assessed include sugar, HbA1c, cholesterol, LDL, VLDL, HDL, triglycerides, ALT, ALP, uric acid, creatinine and bilirubin. Significance of difference between mean analysis of control and diabetic groups was determined.

Table 2 Identification of gram negative organisms

Organism	Biochemical Reactions					TSI			
	Oxidase	Citrate	Motility	Indole	Urease	Slope	Butt	H2S	Gas
<i>E.coli</i>	-	-	+	+	-	Y	Y	-	+
<i>Klebsiella</i>	-	+	-	-	+	Y	Y	-	+
<i>Proteus</i>	-	+	+	-	+	R	Y	+	+
<i>Pseudomonas</i>	+	+	+	-	D	R	R	-	-

Table 3 Identification of gram positive organism

Organism	Identification Tests			
	Gram Staining	Catalase Test	DNase Test	Novobiocin sensitivity
<i>Staphylococcus aureus</i>	Gram +ve cocci	+	S	+

Our results showed that *Escherichia coli* was the most common and the most recurrently occurring uro-pathogen which means that *Escherichia coli* is the principal agent which causes urinary tract infection in the patients of diabetes. While other pathogens that were found to cause urinary tract infection were *Klebsiella*, *Pseudomonas*, *Staphylococcus aureus*, *Candida* and *Proteus* spp (Table 4).

Sensitivity pattern of pathogens were checked against different antibiotics by disk diffusion method. It shows that *E.coli* was

Results

From the urine cultures, gram positive and gram negative organisms were identified by staining technique and it showed that more gram negative pathogens (82.3%) were present in the urine of diabetic patients as compared to gram positive pathogens (17.6%). Moreover, the number of lactose fermenter (80.6%) is also high in the urine samples (Table 1).

Table 1 Frequency of different pathogens in UTI

Organism	Strains (N=300)	Percentage
Gram Positive	53	17.60%
Gram negative	247	82.30%
Lactose fermenter	242	80.60%
Non-lactose fermenter	58	19.30%

Gram positive and gram negative pathogens were further identified by biochemical tests and by using API kit and their results are shown in Table 2 and Table 3.

resistant towards nalidixic acid followed by cephalexin, cefixime and pipemidic acid. *Klebsiella* was resistant towards cephalexin, cefixime, nalidixic acid, pipemidic acid and amoxicillin. *Pseudomonas* was resistant towards cephalexin, cefixime, nalidixic acid, and amoxicillin. *Proteus* was resistant towards cephalexin, cefixime, nalidixic acid, nitrofurantoin and pipemidic acid. *Staphylococcus aureus* was resistant towards cephalexin, cefixime, ciprofloxacin, ceftazidime, nalidixic acid, and pipemidic acid (Table 5 and Figure 1).

Table 4 Prevalence of UTI pathogens

Organism	No.of pathogens (N=300)	Percentage
<i>E.coli</i>	245	81.60%
<i>Klebsiella</i>	21	7%
<i>Pseudomonas</i>	17	5.66%
<i>Staphylococcus aureus</i>	7	2.33%
<i>Candida</i>	6	2%
<i>Proteus</i>	4	1.39%

Table 5 Sensitivity pattern of different pathogens against different antibiotics

Antibiotic	Sensitivity E.coli (mm)	Sensitivity Klebsiella (mm)	Sensitivity Pseudomonas (mm)	Sensitivity Proteus (mm)	Sensitivity Staphylococcus aureus (mm)
Amoxicillin(AMC)	75.1	54.9	38.7	99.9	99
Cephalexin (CL)	27.6	18.7	13	0	0
Cefixime (CFM)	39.8	14.3	38	0	0
Ciprofloxacin (CIP)	73.9	68.9	90	100	0
Ceftazidime (CAZ)	65.2	67.6	82	99.9	0
Nalidixic acid (NA)	21.2	22.7	35	0	0
Nitrofurantoin (F)	98	97.6	70	0	99
Pipemidic acid (PIP)	52.4	22	66	0	0
Cefizox (ZOX)	84.7	91	70	100	100
Imipenem (IMP)	98.9	97.2	96	99	99

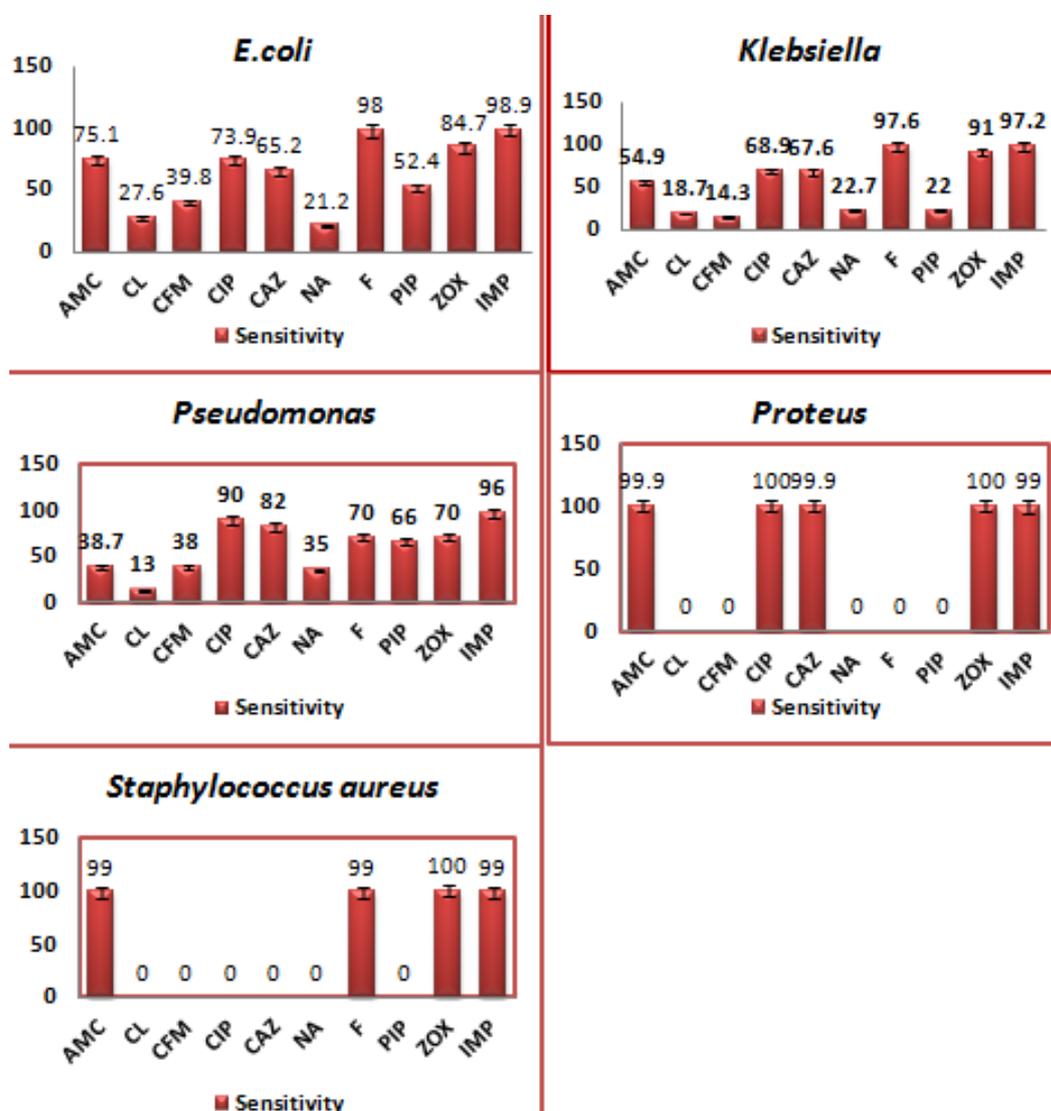


Figure 1 Graphical representation of sensitivity pattern of different pathogens against different antibiotics.

Table 6 shows the physical and biochemical parameters of normal and diabetic male patients. The difference or standard error in the mean age, weight, BMI, blood fasting sugar, bilirubin, uric acid, VLDL, HDL, triglycerides, HbA1c, ALT and ALP was statistically significant. While the difference or standard error in the mean height, creatinine, cholesterol, LDL was not statistically significant (Table 6).

Table 6 Physical and biochemical parameters of normal and diabetic male patients

Parameters	Normal (N= 100)	Diabetic (N=100)
Age (Years)	43.6±2.4	61.02***±1.57
Height (m)	1.6002±0.065	1.6101*±0.009
Weight (kg)	58.3±2.1	70.68***±0.793
BMI (kg/m ²)	23.9±0.8	27.42***±0.38
Blood Fasting Sugar (mg/dL)	95.3±2.99	202.2***±6.67
Bilirubin (mg/dL)	0.351±0.0079	0.762**±0.012
Creatinine (mg/dL)	0.89±0.032	0.9**±0.017
Uric acid (mg/dL)	13.5±0.09	13.9***±0.17
HbA1c (%)	5.38±0.27	9.88***±0.231
Cholesterol (mg/dL)	155±10.1	157.1***±5.786
LDL (mg/dL)	94.08±2.15	104.4***±4.22
VLDL (mg/dL)	16±1.2	31.4***±1.32
HDL(mg/dL)	43.48±0.62	45.6***±0.645
Triglycerides (mg/dL)	140.244±21.29	231.9***±9.62
ALT(U/L)	30.7±5.5	42.9***±2.00
ALP(U/L)	108.02±1.251	202.9***±9.00

±sign indicates the standard error. (P > 0.05) = non-significant. (P < 0.001)*= Highly significant. Number of "*" asterisks show the level of significant difference

Discussion

Due to destructive immune system type 2 diabetic patients have increased chances to develop UTI.⁶ The main reason for the prevalence of UTI in diabetics may be the nerve damage due to high glucose level in blood. Due to nerve damage, the bladder might not be able to sense the urine. As a result, urine stays in bladder for quite long time and thus increases the probability of infection.⁷ High level of glucose in urine also promotes bacterial growth in urine.⁸ In our research, urine samples of diabetic patients suffering from UTI were collected and inoculated on CLED media. As compared to gram positive pathogens, more gram negative pathogens are present in the urine of diabetic patients.⁹ Table 1 also showed that 82% were gram negative pathogens while 18% were gram positive pathogens in the urine samples of male patients. Table 1 showed that 80.60% were lactose fermenter while 19.30% were non-lactose fermenter.

The most common pathogen found in the urine samples of diabetics is *E.coli*. Along with *E.coli* other pathogens are also prevalent including *Pseudomonas*, *Klebsiella*, enterococci, streptococci, and *Proteus mirabilis*.¹⁰ According to Aguiar and Vignesh, UTI is the most frequently occurring infection in the patients of diabetes. The most dominant pathogens isolated were gram negative bacilli rather

than gram positive pathogens. According to Vignesh et.al *Escherichia coli* were isolated from 30.2% of the subjects, *Klebsiella pneumonia* from 22.2%. *Pseudomonas aeruginosa* was isolated from 7.9%, *Proteus mirabilis* from 3.2%, *Staphylococcus aureus* from 3.2%. In our study, *E.coli* was the most common pathogen followed by *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus* (Table 4). In another study from India, it was found that *E. coli* was the most commonly grown organism.^{11,12}

The main goal of treating UTI is to eliminate pathogens. So investigating the bacterial resistance and sensitivity is of utmost importance. The sensitivity and resistance pattern of different pathogens against different antibiotics has been shown in above table. In our research, 100 were control and 100 were diabetic patients. All these people were male. Their physical and biochemical parameters were compared. Physical parameters are age, weight, height and BMI while biochemical parameters are blood fasting sugar, uric acid, creatinine, bilirubin, cholesterol, triglycerides, HDL, LDL, VLDL, HbA1c, ALT, ALP. The difference or standard error in the mean age, weight, BMI, blood fasting sugar, bilirubin, uric acid, VLDL, HDL, triglycerides, HbA1c, ALT and ALP was statistically significant. While the difference or standard error in the mean height, creatinine, cholesterol, LDL was not statistically significant. The assessment of such physical and biochemical parameters will be useful in the early diagnosis and prediction of diabetes mellitus. So it is essential to evaluate these parameters by linking them with diabetes.

Conclusion

It is concluded from the studies that patients of diabetes mellitus suffer from UTI at some point in their life because the presence of surplus glucose in the urine promotes the growth of uro-pathogens that leads to UTI in diabetic patients. The most common pathogen that leads to UTI is *E.coli*. While the frequent usage of antibiotics and broad spectrum antibiotics may cause the production of antibiotic resistant pathogens in urinary tract. The mean values of physical and biochemical parameters of diabetics are high as compared to normal control subjects. The evaluation of these parameters can help to diagnose diabetes mellitus. Improved glycemic control in diabetics may help in controlling the UTIs.

Acknowledgments

University of the Punjab, Lahore provided funds. We acknowledge Dr. Nauser Malik, F.P.G.M.I., Department of Medicine, Health Center University of the Punjab, Lahore, for collection of samples.

Conflicts of interest

The author(s) declare that the publication of this article has no conflict of interest.

References

1. Blas E, Kurup AS. *Equity, social determinants and public health programmes*. World Health Organization; 2010. 303 p.
2. Bommer C, Sagalova V, Heesemann E, et al. Global economic burden of diabetes in adults: projections from 2015 to 2030. *Diabetes Care*. 2018;41(5):963–970.
3. Soo Park B, Lee SJ, Wha Kim Y, et al. Outcome of nephrectomy and kidney-preserving procedures for the treatment of emphysematous pyelonephritis. *Scand J Urol Nephrol*. 2006;40(4):332–338.

4. Mnif MF, Kamoun M, Kacem FH, et al. Complicated urinary tract infections associated with diabetes mellitus: Pathogenesis, diagnosis and management. *Indian J Endocrinol Metab.* 2013;17(3):442–445.
5. Huttner A, Gambillara V. The development and early clinical testing of the ExPEC4V conjugate vaccine against uropathogenic *Escherichia coli*. *Clinical Microbiology and Infection.* 2018;24(10):1046–1050.
6. Saleem M, Shah M. Detection of Siderophore production in Uropathogenic *Escherichia coli* in patients with Type 2 Diabetes Mellitus. *Int J Med Microbiol Trop Dis.* 2017;3(4):176–177.
7. Sewify M, Nair S, Warsame S, et al. Prevalence of urinary tract infection and antimicrobial susceptibility among diabetic patients with controlled and uncontrolled glycemia in Kuwait. *Journal of Diabetes Research.* 2016;2016:6573215.
8. Johnsson KM, Ptaszynska A, Schmitz B, et al. Urinary tract infections in patients with diabetes treated with dapagliflozin. *Journal of Diabetes and its Complications.* 2013;27(5):473–478.
9. Aswani SM, Chandrashekar U, Shivashankara K, et al. Clinical profile of urinary tract infections in diabetics and non-diabetics. *Australas Med J.* 2014;7(1):29–34.
10. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. *Dis Mon.* 2003;49(2):71–82.
11. Shifflett T. *Diabetes and Urinary Tract Infections – Things You Need To Know.* The diabetes council; 2018.
12. Woodford HJ, George J. Diagnosis and management of urinary infections in older people. *Clin Med.* 2011;11(1):80–83.