Muhammad Fayaz et. al Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

Muhammad Fayaz¹, Shah Khalid¹, Fath Ur Rahman², Abdullah¹, Shawkat Ali³, Mehrin Sherazi¹, Fawad Ahmed⁴, Bushra Jamil¹, Naeem Khan¹, Amir Zaib¹, Hayat Khan⁵*

- ¹Department of Health & Biological Sciences, Abasyn University, Peshawar, 25000, Pakistan
- ²Rufaida College of Nursing, Peshawar,, Pakistan
- ³Ahmed Medical Institute, Peshawar, Pakistan
- ⁴Mehboob Medical Institute Hayatabad peshawar
- ⁵Department of Microbiology, University of Swabi,KPK, Pakistan
- *Corresponding Email: hayatkhan@uoswabi.edu.pk

ABSTRACT

Diabetes mellitus is a group of metabolic disorder characterized by high blood sugar (glucose) levels that result from defects in insulin secretion, or its action, or both.Diabetes mellitus (DM) type II is caused by the failure in beta-cell role and insulin-resistance. This study aimed to comparatively identify and antibiogram analysis of bacterial pathogens associated with Urinary tract infection (UTI) of type II diabetic and healthy individuals. Among total samples (200), 41(20.5%) were positive for bacterial infections, 34 (34%) were positive in diabetic patients and only 07 (7%) were positive in non-diabetic healthy individuals. The most common bacterial isolates from diabetic patient were Escherichia coli 15 (44.11%), followed by Staphylococcus aureus 12 (35.29%), Pseudomonas aeruginosa 02(5.88%), Klebsiella spp. 03(8.82%)and Proteus spp were 02(5.88%) while among healthy control, E.coli 04(57.14%) and S. aureus 03(42.85%) were determined to be associated with UTI. Furthermore, the diabetic patients were also screened for viral hepatitis by immune chromatographic technique (ICT). A total of 05(5%) samples were found positive, among them 02(2%) were HBS Ag positive and 03(3%) with anti-HCV respectively. Antibiotics i.e. Amoxicillin, Ciprofloxacin, Levofloxacin, Amikacin, Sulfamethoxazole, Clarithromycin, Ceftriaxone, Moxifloxacin, Streptomycin and Tigecycline were evaluated against bacterial isolates. Among antibiotics Amikacin (93.33%), Ciprofloxacin (80%) proved effective while Amoxicillin (93.33%) and Streptomycin (73.33%) were found in active against Staphylococcus aureus. P. aeruginosa isolates were found susceptible to Ciprofloxacin and Amoxicillin, however, relatively low activity was observed for the Amikacin, Clarithromycin, Ceftriaxone, Streptomycin, and Tigecycline.Ciprofloxacin, Amikacin, Levofloxacin and Moxifloxacin were comparatively effective than Ceftriaxone, Sulfamethoxazole Streptomycin against UTI isolates of Klebsiella spp. Isolates of Proteus spp from UTI were found comparatively more susceptible towards Ciprofloxacin, Ceftriaxone and levofloxacin. E. coli isolates proved relative resistant towards Sulfamethoxazole, Streptomycin and Tigecycline. These finding highlight the importance of controlling glycemia in diabetic patients to reduce the UTI regardless of age and gender.

Keywords: Blood sugar, UTI, Antimicrobial, HBS Ag, antibiotic

Muhammad Fayaz et. al Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

Tob Regul Sci. ™ 2022;8(1): 3531-3542 DOI: doi.org/10.18001/TRS.8.1.266

INTRODUCTION

Diabetes is a condition linked with abnormal sugar level (high) in the blood. The body have some mechanism like production of insulin which maintains glucose level normal. Abnormality in insulin production, resulted diabetes caused. There are two types of diabetes, type I (insulindependent) and type II (non-insulin-dependent) [1]. Symptoms of diabetes include; polyuria, polydipsia, fatigue, hunger, skin problems, reduces wound healing, fungal infections, and sensation or numbness in the toes or planter. Abnormal production of insulin affects muscles cells and fat tissues leading to a condition called insulin resistance. This problem usually occurs in type II diabetes. Similarly, due to zero production of insulin in type 1 diabetes patient's destruction of beta cells in the pancreas^[2, 3]. Diabetes mellitus (DM) type II is caused by the failure function of beta-cell and insulin-resistance. Obesity is the major cause for the diabetes mellitus (type II) and is thought to confer improved risk for type II diabetes through the mechanism of related insulin resistance. Diabetes mellitus (type II) is a metabolic disorder and is cause by imperfection in insulin discharge or insulin action) [4, 5]. Type II diabetes is also called non-insulin dependent diabetes mellitus (NIDDM), or acute onset diabetes mellitus (AODM). Type II diabetic patients insufficient produce insulin. Due to increase in insulin resistance, the insulin release from pancrease is also defective. However, glucogenesis process becomes compromised [6]. Diabetes is the most common metabolic disorder as declared by the health practitioners. In USA about 23.6 million people have been diagnosed with diabetes. The factors that are involved in the occurrence of diabetes are growing age and low socioeconomic status of the population and obesity. Owing to the current trends, the cases of diabetes will be double by 2030 [7]. Currently, 6.9 million patients are affected with diabetes mellitus and expecting to increase to 11.2 million people by 2025. It is a more challenging problem for researchers, doctors and policy makers in Pakistan. The prevalence of type 2 diabetes in Pakistanis11.77%. The prevalence of type II is higher in male (11.2%) as compared to females (9.19%). It is the need of the day that Pakistan include diabetes preventive measure in health policy to reduce as much as possible the burden of this chronic disease [8, 9]. The most commonly involved microorganism in UTI is Escherichia coli followed by enterococcus and Pesudomonas spp. In free diabetic male and females, the prevalence of microbes in UTI are: Escherichia coli (31.4 and 58.2%), Enterococcus spp., (9.4 and 6.5%) and Pseudomonas spp.(17.2 and 4.7%), on other hand, the prevalence of pathogens involved in UTI in diabetic patients are; Escherichia coli (32.5 and 54.1%), Enterococcus spp., (9.4 and 8.3%) and Pseudomonas spp, (8.5 and 3.9%) respectively. Diabetes mellitus type II patients are at high risk of infection that include or the most common among these is urinary tract infection. In urine higher sugar level, may enhance the growth of infectious bacterial species [10, 11]. The current research work was focused on the comparative analysis of UTI in type II diabetic patients and healthy individuals as UTI is one of the most severe form of

Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

infection diabetes. For this purpose, isolation of pathogens involved in UTI and screened with various antibiotics was carried out. In the current study the Possible outcomes is also lead us to explore whether the advanced sources of treatment are required due to emerging resistance to previously considered potent antibiotics. Diabetic samples were also processed for the detection of HBs Ag and anti HCV among diabetic patients.

MATERIAL AND METHODS

Study area and Samples Collection

This study was conducted at the Research Laboratory of Microbiology and Biotechnology, Abasyn University Peshawar. Urine sample were collected from various tertiary care hospitals such as Lady Reading Hospital Peshawar (LRH) and Al-khidmat Hospital Peshawar. A total of 200 individuals (100 each of diabetic and healthy group) of different age groups and genders were included in the study. Informed consent was signed from all the subjects.

Urine analysis

After sample collection physical examination was performed for the color, odor, and urine sample was collected in a sterile container and then the samples were preceded to various biochemical examinations. The biochemical examination was performed on a uric 3V urine strip (ACON laboratories USA) for the detection of sugar, albumin and pH of urine.

Pure Culture

The sample were brought to the microbiology laboratory, Abasyn university Peshawar and processed for culturing and identification of the organisms. For obtaining a pure culture and clear morphology, subculture was performed on MacConkey Agar, Blood Agar, Mueller-Hinton Agar and Nutrient agar plates and then incubated at 37°C for 24 hours. The same procedure was performed on fresh media for obtaining pure culture.

Biochemical test

All plates were further processed for colony morphology and differentiation by gram staining and biochemical testing(Catalase test, Coagulase test, Cytochrome Oxidase test, indole test, urease test, triple sugar iron test) to identify isolated bacteria

Blood examination

The HBs rapid test is a lateral flow chromatographic immunoassay based on the principle of the double antibody-sandwich technique. The membrane is pre-coated with anti-HBs Ag antibodies on the test line region of the test. During testing, Hepatitis B surface antigen in the serum or plasma specimen reacts with the particle coated with anti-HBs Ag antibodies on the membrane and generates a coloured line. The presence of this coloured line in the test region indicates a positive result, while its absence indicates a negative result. The HCV antibodies rapid test is a lateral flow chromatographic immunoassay based on the principle of the double antigensandwich technique.

Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of isolates was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Merck, Germany), according to Clinical Laboratory Standards Institute (CLSI) 2016 guidelines. Inoculum was prepared by suspending a single well isolated colony from overnight blood agar or MacConkey agar plates in distilled water to the final turbidity of a 0.5 McFarland standard.

The bacterial suspension was spread over the agar homogeneously and antimicrobial discs such Amoxicillin(AMC), Ciprofloxacin(CIP), Levofloxacin(LEV), Amikacin(AMK), Clarithromycin (CLR), Sulfamethoxazole(SXT), Streptomycin(STM), Tigecycline(TGC), Ceftriaxone(CFT) Moxifl oxacin(MXF) were placed on the agar plate in order to find out the bacterial susceptibility to antibiotics. The plates were incubated for 18 to 22 hours at 37°C. Antibiotic sensitivity assay for each antibiotic against the test bacteria was repeated thrice. Zone of inhibition was measured via scale. The result was then measured by using scale in mm and the interpretive criteria used are given in a tabular form. The diameter of zone of inhibition was measured for all and interpreted as recommended by CLSI 2016 guidelines.

RESULTS

Collection of clinical samples and processing

Urine samples (100 each) from diabetic type II and non-diabetic healthy individuals were collected. All processed specimens were then subjected for isolation identification and antibiogram analysis of bacterial pathogens. Patients were interviewed for clinical history. Out of 100 samples 34 urine samples were found positive for the bacterial isolates among the diabetic subject whereas only 07 samples proved positive for the bacterial strains among 100 healthy control individuals. Percent incidence of pathogenic bacterial isolates was 34% and 07% respectively for the diabetic and healthy individuals. The diabetic patient was also screened for HBs Ag and HCV through ICT method, out of 100 samples 2% HBs Ag positive and 3% HCV were found positive. Bacterial isolates in urine samples of diabetic included were *E. coli*15 (44.11%), *S. aureus* 12 (35.29%), *P. aeruginosa* 02 (5.88%), *Klebsiella spp.* 03(8.82%), and *Proteus spp* were 02 (5.88%) whereas is *E. coli* 4 (57.15%) and *S. aureus* 3 (42.85%) were identified in the urine of healthy individuals (Table.1).

Table. 1: Percent frequencies of bacterial positive samples in type II diabetic and healthy individuals.

S No.	Bacterial	diabetic	Percent	Healthy	Percent (%)	Total Number	
	Isolates	Patients	(%)	Control		Bacterial	
						Isolates	
1	E. coli	15	44.11	04	57.15	19	
2	S. aureus	12	35.29	03	42.85	15	
3	P. aeruginosa	02	5.88	0	0	02	

4	Klebsiella spp.	03	8.82	0	0	03	
5	Proteusspp.	02	5.88	0	0	02	
	Total	34	100	07	100	41	

Gender wise frequency distribution of positive samples among the diabetic and Healthy control

Among the healthy individuals, females 5(71.42%) were found relatively more prone to infection as compared to males 2(28.57%). However, the frequency of infected males and female was same in case of diabetic patients.

Age wise distribution of Bacterial infection among the Diabetic and Control Groups.

The UTI was relatively more frequent in age group of 30 to 45 years 18(52.94%) among the diabetic and control groups. Frequencies of UTI infections in other age groups among the diabetic and healthy control groups have been show in table 2.

Table. 2: Age wise frequency distribution of bacterial infections among the diabetic and Healthy control

S. No	A ~~	Diabetic type-	Non-diabetic (Healthy
No	Age	II	individual)
1	30-45	18 (52.94%)	5 (71.42%)
2	46-60	16 (47.05%)	2 (28.57%)
3	Above 60	0 (0%)	0 (0%)

Correlation of Therapeutic response and glycemia

Therapy response towards various antidiabetic drugs was also evaluated in the type II diabetic patients. Glycated haemoglobin method (HbA1c) was used for the categorization of diabetic patients into controlled and uncontrolled glycemic groups. Frequency of bacterial infection was relatively low incontrolled glycemic group 14 (41.17%) as compared to uncontrolled diabetic patients 50 (58.82%). Similarlythe response towards various antidiabetic drugs was also evaluated in correlation with controlled and uncontrolled glycemic parameter (Table 3). UTI infection is relatively more common in an uncontrolled glycemic group with relatively poor therapeutic outcome.

Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

Table.3: Correlation of bacterial infection and therapeutic outcome of various drugs with the Glycemia.

		Control	Uncontrolled
		glycemic group	glycemic group
Frequency Bact	erial Infection	41.17%	58.82%
HbA1c		5.68±0.91	8.44±1.33
Anti-diabetic	Insulin	Yes	No
drugs	Metformin	No	Yes
	Glimepiride	No	Yes

Prevalence HCV and HBVin diabetic patients

The diabetic patients were also tested for the infection of hepatitis B and C infection through immune-chromatographic test (ICT) method. Diabetic samples were also screened for the HCV and HBV infection and it was found that 2% and 3% diabetic patients were infected with HBV and HCV respectively. The percent frequencies of HBV and HCV infection among the diabetic patients.

HBs Ag=Hepatitis B Surface Antigen

Antibacterial activity against S. aureus

All bacterial isolates were evaluated for their sensitivity and resistance towards a panel of selected antibiotics *.S. aureus* showed differential susceptibility to Ciprofloxacin (80%), Levofloxacin (80%), Amikacin (93.33%), Sulfamethoxazole (60%), Clarithromycin (66.66%), Ceftriaxone (73.33%), Moxifloxacin (53.33%), and Tigecycline (66.66%). Similarly it showed more resistance towards Streptomycin (73.33%) and Amoxicillin (93.33%). Efficacy of each antibiotic has been shown in term of zone of inhibition along with standard deviation of triplicate (Table.4).

Table.4: Antibacterial activities of various antibiotics against different *S. aureus* isolates in term of Mean Zone of Inhibition (mm)

S.	AMC	CIP	AMK	SXT	STM	TGC	CFT	CLR	LEV	MXF
N	MEAN	MEA	MEA	MEA	MEA	MEA	MEA	MEA	MEA	MEA
о.	±SD	N ±SD	N ±SD	N ±SD	N	N ±SD	N ±SD	N ±SD	N ±SD	N ±SD
	(mm)	(mm)	(mm)	(mm)	±SD	(mm)	(mm)	(mm)	(mm)	(mm)
					(mm)					
1	21±2.64	20.3±1	21.3±2	25±1	0±0	21.3±0	25.3±1	9±0	23.6±2	28±1
		.5	.8			.57	.52		.3	
2	24.6±0.	12.3±2	20.6±0	20.3±1	12±1	8±1	13.6±2	10±0	11±2	13.6±2
	57	.52	.57	.5			.5			.3

Muhammad Fayaz et. al Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

				25.0	1 (1	0 1	20 /	0 0	20.1	15.2.0
3			23.6±1	25±0	16±1	9±1	30±4	9±0	20±1	
	12	.53	.53							.57
4	37±1	22±1	24.7 ± 0	22.3 ± 1	0 ± 0	21±1	40.3 ± 0	36.6±0	23±1.7	28±1.7
			.58	.52			.6	.57	3	
5	24±0	17.3±0	24±1	0±0	0±0	18±1	25.3±1	30.3±1	22.6±1	28.3±1
		.57					.15	.5	.15	.15
6	17±0	9.3±0.	17.6±0	9± 0	0±0	14±1	15.3±0	0±0	14.3±0	18.3±1
		57	.57				.57		.57	.5
7	20.6±0.	0±0	0±0	0±0	0±0	0±0	18±1	0±0	0±0	0±0
	57									
8	12.3±0.	25±1.7	19.3±6	9.3±2.	12.6±	17.6±0	23.6±1	38.6±4	24.6±2	19±2
	57		.1	1	1.5	.57	.53	.6	.1	
9	14.6±1.	21±1	30±5.5	14.3±1	15±2	13.6	28.6±1	38.6±4	29.3±0	15.6±0
	15		1	.15		±2.1	.52	.93	.6	.57
10		26.3±1	1 23±3.8							
10		26.3±1 .15					25.3±0		20.3±0	
10	14.6±1.	.15		15.6±1	16.6± 2.5	19.3±0 .57	25.3±0 .57	38.6±1 .15	20.3±0	15.3±1 .52
	14.6±1.	.15	23±3.8	15.6±1	16.6± 2.5	19.3±0 .57	25.3±0 .57	38.6±1 .15	20.3±0 .57	15.3±1 .52
11	14.6±1. 52 9.3±0.5	.15 26.3±0 .57	23±3.8 41.6±2 .3	15.6±1	16.6± 2.5 25.3± 0.6	19.3±0 .57 11.3±0 .57	25.3±0 .57 32.3±0	38.6±1 .15 28±4.9	20.3±0 .57 25.6±0 .57	15.3±1 .52 25±1
11	14.6±1. 52 9.3±0.5	.15 26.3±0 .57	23±3.8 41.6±2 .3	15.6±1 .53 0±0	16.6± 2.5 25.3± 0.6	19.3±0 .57 11.3±0 .57	25.3±0 .57 32.3±0 .6	38.6±1 .15 28±4.9	20.3±0 .57 25.6±0 .57	15.3±1 .52 25±1
11	14.6±1. 52 9.3±0.5	.15 26.3±0 .57 31.6±0	23±3.8 41.6±2 .3 35.3±3 .46	15.6±1 .53 0±0	16.6± 2.5 25.3± 0.6 7.3±1.	19.3±0 .57 11.3±0 .57 18.3±0 .6	25.3±0 .57 32.3±0 .6	38.6±1 .15 28±4.9 31.3±1	20.3±0 .57 25.6±0 .57 35.3±0	15.3±1 .52 25±1 26.6±0
11	14.6±1. 52 9.3±0.5 7 11±0	.15 26.3±0 .57 31.6±0 .58	23±3.8 41.6±2 .3 35.3±3 .46	15.6±1 .53 0±0 11±1 13.6±0	16.6± 2.5 25.3± 0.6 7.3±1.	19.3±0 .57 11.3±0 .57 18.3±0 .6	25.3±0 .57 32.3±0 .6 22±1 40.6±0	38.6±1 .15 28±4.9 31.3±1 17.6±1	20.3±0 .57 25.6±0 .57 35.3±0 .57	15.3±1 .52 25±1 26.6±0 .57
11 12 13	14.6±1. 52 9.3±0.5 7 11±0	.15 26.3±0 .57 31.6±0 .58 32.3±1 .15	23±3.8 41.6±2 .3 35.3±3 .46 36±3.6	15.6±1 .53 0±0 11±1 13.6±0	16.6± 2.5 25.3± 0.6 7.3±1. 15 0±0	19.3±0 .57 11.3±0 .57 18.3±0 .6	25.3±0 .57 32.3±0 .6 22±1 40.6±0 .57	38.6±1 .15 28±4.9 31.3±1 17.6±1 2.4	20.3±0 .57 25.6±0 .57 35.3±0 .57 34±1	15.3±1 .52 25±1 26.6±0 .57 28±1
11 12 13	14.6±1. 52 9.3±0.5 7 11±0	.15 26.3±0 .57 31.6±0 .58 32.3±1 .15 38.3±0	23±3.8 41.6±2 .3 35.3±3 .46 36±3.6	15.6±1 .53 0±0 11±1 13.6±0 .57 13.6±0	16.6± 2.5 25.3± 0.6 7.3±1. 15 0±0	19.3±0 .57 11.3±0 .57 18.3±0 .6 19.3±0	25.3±0 .57 32.3±0 .6 22±1 40.6±0 .57	38.6±1 .15 28±4.9 31.3±1 17.6±1 2.4	20.3±0 .57 25.6±0 .57 35.3±0 .57 34±1	15.3±1 .52 25±1 26.6±0 .57 28±1
11 12 13 14	14.6±1. 52 9.3±0.5 7 11±0 15±1 23.3±0.	.15 26.3±0 .57 31.6±0 .58 32.3±1 .15 38.3±0 .6	23±3.8 41.6±2 .3 35.3±3 .46 36±3.6 25.3±1 .5	15.6±1 .53 0±0 11±1 13.6±0 .57 13.6±0	16.6± 2.5 25.3± 0.6 7.3±1. 15 0±0	19.3±0 .57 11.3±0 .57 18.3±0 .6 19.3±0	25.3±0 .57 32.3±0 .6 22±1 40.6±0 .57 32±1	38.6±1 .15 28±4.9 31.3±1 17.6±1 2.4	20.3±0 .57 25.6±0 .57 35.3±0 .57 34±1 32.3±0 .57	15.3±1 .52 25±1 26.6±0 .57 28±1
11 12 13 14	14.6±1. 52 9.3±0.5 7 11±0 15±1 23.3±0. 57	.15 26.3±0 .57 31.6±0 .58 32.3±1 .15 38.3±0 .6	23±3.8 41.6±2 .3 35.3±3 .46 36±3.6 25.3±1 .5	15.6±1 .53 0±0 11±1 13.6±0 .57 13.6±0 .57	16.6± 2.5 25.3± 0.6 7.3±1. 15 0±0	19.3±0 .57 11.3±0 .57 18.3±0 .6 19.3±0 .6 21±0	25.3±0 .57 32.3±0 .6 22±1 40.6±0 .57 32±1	38.6±1 .15 28±4.9 31.3±1 17.6±1 2.4 17±1	20.3±0 .57 25.6±0 .57 35.3±0 .57 34±1 32.3±0 .57	15.3±1 .52 25±1 26.6±0 .57 28±1 20.3±1 .52

Key: AMC=Amoxicillin, CIP= Ciprofloxacin, LEV= Levofloxacin, AMK= Amikacin, CLR= Clarithromycin,SXT= Sulfamethoxazole,STM= Streptomycin,TGC= Tigecycline,CFT= Ceftriaxone, MXF= Moxifloxacin

Antibacterial susceptibility profile of P. aeruginosa

Antibacterial activity of a panel of antibiotics against *P. aeruginosa* isolates have been demonstrated in the figure 3.2 and table.3.7. The percent frequency of resistant and susceptible *P. aeruginosa* has been shown diagrammatically in figure whereas the antibacterial potential of antibiotics in term of zone of inhibition along with standard deviation (SD) against all *P. aeruginosa* isolates has been tabulated in Figure 2.

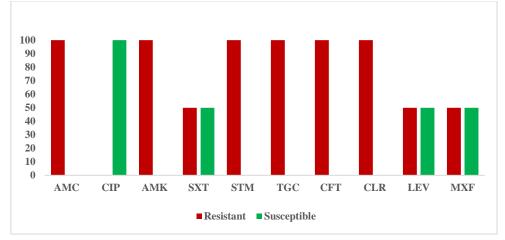


Figure.2. Frequency distribution of resistant and susceptible *P. aeruginosa* towards a panel of antibiotic

Antibacterial susceptibility profile of Klebsiella species

Antibacterial activity of a panel of antibiotics against *Klebsiella spp* isolates have been demonstrated in the figure 3. The percent frequency of resistant and susceptible *P. aeruginosa* has been shown diagrammatically in figure whereas the antibacterial potential of antibiotics in term of zone of inhibition along with standard deviation (SD) against all *Klebsiella spp* isolates.

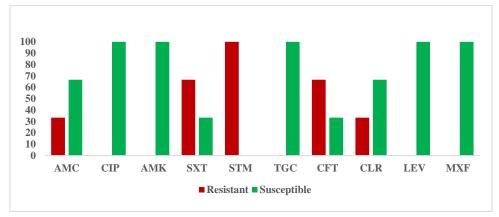


Figure . 3. Percent frequency distribution of resistant and susceptible *Klebsiella spp* towards a panel of antibiotics

Antibacterial susceptibility profile of *E. coli*

Antibacterial activity of a panel of antibiotics against *E. coli* isolates have been demonstrated in the figure 4. The percent frequency of resistant and susceptible *P. aeruginosa* has been shown diagrammatically in figure whereas the antibacterial potential of antibiotics in term of zone of inhibition along with standard deviation (SD) against all *E. coli* isolates.

Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of

Diabetic and Control Group

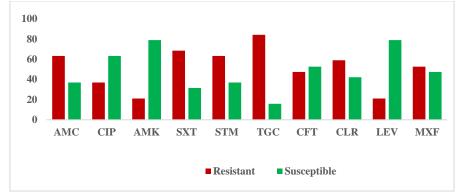


Figure.4. Percent frequency distribution of resistant and susceptible *E. coli* towards a panel of antibiotics

Antibacterial susceptibility profile of Proteus spp

Antibacterial activity of a panel of antibiotics against *Proteus spp* isolates have been demonstrated in the figure 5. The percent frequency of resistant and susceptible *Proteus spp* have been shown diagrammatically in figure whereas the antibacterial potential of antibiotics in term of zone of inhibition along with standard deviation (SD) against all *Proteus spp* isolates.

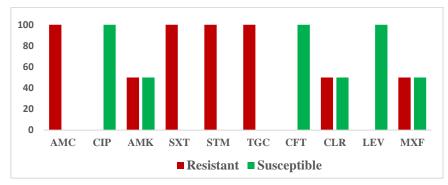


Figure.5. Percent frequency distribution of resistant and susceptible *Proteus spp* towards a panel of antibiotics

DISCUSSION

Diabetes is a disorder of pancreas, characterized by abnormally high sugar level in the blood. Abnormality in insulin production result diabetes. There are two types of diabetes, type I (insulin-dependent) and type II (non-insulin-dependent). In diabetic patients bacterial infection is very common and major source of UTI and also UTI is higher in diabetic patients as compare to healthy individuals. Therefore, the current research work focus on the comparative analysis of UTI in type II diabetic patients and healthy individuals as UTI which is one of the most common complications of diabetes. For this purpose, isolation of pathogens involved in UTI and screened with various antibiotics was carried out. Diabetic samples were also processed for the detection of HBs Ag and anti HCV viral profile. In the current study the overall frequency of pathogenic bacterial isolates/strain was 34% and 07% respectively for the diabetic and healthy

Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

individuals. The diabetic patients were also screened for HBs Ag and HCV through ICT method, Out of 100 diabetic samples 2% HBs Ag positive and 3% HCV were found positive. Bacterial isolates in urine samples of diabetic included E. coli15 (44.11%), S. aureus 12 (35.29%), P. aeruginosa 02 (5.88%), Klebsiella spp. 03 (8.82%), and Proteus spp were 02 (5.88%) whereas is E. coli 4 (57.15%) and S. aureus 3 (42.85%) were identified in the urine of healthy individuals. Similarly also evaluated the incidence of urinary tract infection (UTI) in patients with diabetes mellitus. Total of 100 diabetic and non-diabetic urine samples were collected. The study report the incidence of UTI is higher in people of high socioeconomic status. The common bacterial isolates responsible for the UTI according to them were E.coli, S. aureus ,P. aeruginosa, and Klebsiella spp [12]. Diabetic patients of poor socioeconomic status were suffering more from UTI as compared to patients of high socioeconomic status [13]. In type II diabetic patients E.coli (44%) was the most prevalent cause of UTI. Another study as reported that UTI in diabetic patents caused by E. coli (47%), K. pneumoniae (5%) and Proteus mirabelus (7%). Frequency of bacterial infection was relatively low in controlled glycaemic group14 (41.17%)as compared to uncontrolled diabetic patients 20 (58.82%) [12]. Earlier study also highlight this fact that UTI infection is relatively more common in hyperglycaemic patients as compare to control glycaemic patients [14]. Similarly, the glycaemia has great effect on UTI, associated with bacterial isolates and also reduce therapeutic response towards various bacterial isolates [15]. In this earlier report, the incidence of UTI was 35% with higher frequency in uncontrolled glycaemic patients (n=197) followed by glycaemic controlled patients (n=55) [15]. Age group 30-40 were more prone to UTI in case of healthy control whereas comparable finding was observed on both age groups among the diabetic patients in the current study. However, patients above 40 years have been reported who are more susceptible to UTI [15]. In the present study, E. coli were the predominant uropathogens followed by S.aureus. The involvement of E.coli as major contributor in the UTI has also been demonstrated in previous studies [16]. These results highlight the importance of controlling glycemia in diabetic patients to reduce the UTI regardless of age and gender. In the present work samples of diabetic patients were also screened for the Anti HCVantibody and HBV Agand it was found that 2% and 3% diabetic patients were infected with HBV and HCV respectively. Similar finding had also been reported i.e frequency of 5% HBV and 3% HCV in diabetes using immune chromatographic technique [17]. Moreover, other researcher had also described the infection of viral hepatitis in diabetic patients. For antibiotics sensitivity pattern of isolated bacterial pathogens, a panel of selected antibiotics were used. These included Amoxicillin, Ciprofloxacin, Levofloxacin, Amikacin, Sulfamethoxazole, Clarithromycin, Ceftriaxone, Moxifloxacin, Streptomycin and Tigecycline. The Antibiogram analysis of S. aureus isolates from UTI revealed that Amikacin (93.33%), Ciprofloxacin (80%) had relatively good results as compared to the rest of antibiotics in the panel against the S. aureus [18]. The Antibiogram analysis of Klebsiella spp isolates from UTI revealed that Ciprofloxacin, Amikacin, Levofloxacin and Moxifloxacin are 100% susceptible had relatively good results as compared to the rest of antibiotics in the panel against the Klebsiella spp. In previous studies of Klebsiella

Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group

patterns of resistance to Trimethoprim-Sulfamethoxazole (47%), pneumoniae showed Ampicillin/Sulbactam (42%), Cephalothin (42%), Ciprofloxacin (34%), Cefotaxime (25%), Amoxicillin/Clavulanate (24%), Ceftazidime (22%), Nitrofurantoin (11%), and Amikacin (2%), respectively [19]. In the current Antibiotic analysis revealed that E. coli washighly resistant to Tigycyclin (84.21%) and Sulfamethoxazole (68.42%) while Amikacin and Levofloxacin are 78.94% susceptible as compared to the previous findings E. coli relatively displayed high antimicrobial resistance rates against Cephalothin(58%), Trimethoprim-Sulfamethoxazole (48%), Ciprofloxacin and Ampicillin (34%), Cefotaxime (28%), Ceftazidime (26%), Amoxicillin (20%) and Amikacin (2%) [20]. In the present Antibiogram analysis Sulfamethoxazole, Streptomycin and Tigecycline were 100% resistant to Ciprofloxacin, Ceftriaxone, levofloxacin were 100% susceptible to Proteus spp and Ciprofloxacin are 100% susceptible and Amoxicillin, Amikacin, Clarithromycin, Ceftriaxone, Streptomycin, Tigecycline are 100% resistant against P. aeruginosa while according to showed that Gram-negative (i.e. E.coli, Proteus and P. aeruginosa) bacterial isolates were mostly sensitive to Nitrofurantoin (97.41%) followed by Gentamicin (88.57%), Norfloxacin (80.00%) and Was Resistance to Nalidixic Acid (71.42%) and Amoxycillin (80.00%) [19].

CONCLUSIONS

Diabetic patients (34%) proved relatively more prone towards UTI infections as compared to healthy control (07%). Bacterial isolates associated with UTI included were *E. coli* (44.11%), *S. aureus* 12 (35.29%), *P. aeruginosa* 02 (5.88%), *Klebsiella spp.* 03 (8.82%), and *Proteus spp* were (5.88%) in diabetic patients whereas only *E. coli* 04(57.15%) and *S. aureus* 03 (42.85%) were identified in the urine of some healthy individuals. Age and gender factor had either little or no effect on the susceptibility of diabetic as well as healthy individuals toward the UTI. Some of the diabetic patients were also infected with HBV and HCV but their prevalence was very low as compared to bacterial infections. Uncontrolled glycemic patients seem to be more prone to infection than the controlled glycemic patients.

REFERENCES

- 1. Dall, T.M., et al., The economic burden of elevated blood glucose levels in 2017: diagnosed and undiagnosed diabetes, gestational diabetes mellitus, and prediabetes. Diabetes care, 2019. 42(9): p. 1661-1668.
- 2. Cantley, J. and F.M. Ashcroft, Q&A: insulin secretion and type 2 diabetes: why do β-cells fail? BMC biology, 2015. 13(1): p. 1-7.
- 3. Koeslag, J.H., P.T. Saunders, and E. Terblanche, *A reappraisal of the blood glucose homeostat which comprehensively explains the type 2 diabetes mellitus–syndrome X complex.* The Journal of physiology, 2003. **549**(2): p. 333-346.
- 4. Buchanan, T.A., *Pancreatic beta-cell loss and preservation in type 2 diabetes.* Clinical Therapeutics, 2003. **25**: p. B32-B46.

- Muhammad Fayaz et. al Comparative Biodiversity and Antibiotics Sensitivity Analysis of Bacterial Pathogens in the Urine of Diabetic and Control Group
- 5. Kraegen, E., et al., *The role of lipids in the pathogenesis of muscle insulin resistance and beta cell failure in type II diabetes and obesity.* Experimental and Clinical Endocrinology & Diabetes, 2001. **109**(Suppl 2): p. S189-S201.
- 6. Beach, K.W., et al., The correlation of arteriosclerosis obliterans with lipoproteins in insulin-dependent and non-insulin-dependent diabetes. Diabetes, 1979. 28(9): p. 836-840.
- 7. Abdulaziz Al Dawish, M., et al., *Diabetes mellitus in Saudi Arabia: a review of the recent literature.* Current diabetes reviews, 2016. **12**(4): p. 359-368.
- 8. Boyle, J.P., et al., Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the US. Diabetes care, 2001. 24(11): p. 1936-1940.
- 9. Mohebi, R., et al., Cardiovascular disease projections in the United States based on the 2020 census estimates. Journal of the American College of Cardiology, 2022. **80**(6): p. 565-578.
- 10. Ronald, A., The etiology of urinary tract infection: traditional and emerging pathogens. The American journal of medicine, 2002. 113(1): p. 14-19.
- 11. Sorlozano, A., et al., Evolution of the resistance to antibiotics of bacteria involved in urinary tract infections: a 7-year surveillance study. American journal of infection control, 2014. 42(10): p. 1033-1038.
- 12. Linhares, I., et al., Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000–2009). BMC infectious diseases, 2013. 13(1): p. 1-14.
- 13. Beyene, G. and W. Tsegaye, *Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university specialized hospital, southwest ethiopia.* Ethiopian journal of health sciences, 2011. 21(2): p. 141-146.
- 14. Peleg, A.Y., et al., Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. Diabetes/metabolism research and reviews, 2007. 23(1): p. 3-13.
- 15. Stapleton, A., *Urinary tract infections in patients with diabetes.* The American journal of medicine, 2002. 113(1): p. 80-84.
- 16. Ahmad, S., et al., Diabetes mellitus and urinary tract infection: Causative uropathogens, their antibiotic susceptibility pattern and the effects of glycemic status. Pakistan Journal of Medical Sciences, 2020. 36(7): p. 1550.
- 17. Bailiff, N., et al., Frequency and risk factors for urinary tract infection in cats with diabetes mellitus. Journal of veterinary internal medicine, 2006. **20**(4): p. 850-855.
- 18. Worku, G.Y., Y.B. Alamneh, and W.E. Abegaz, *Prevalence of bacterial urinary tract infection and antimicrobial susceptibility patterns among diabetes mellitus patients attending Zewditu memorial hospital, Addis Ababa, Ethiopia.* Infection and Drug Resistance, 2021. 14: p. 1441.
- 19. Chandra, H., et al., Promising roles of alternative medicine and plant-based nanotechnology as remedies for urinary tract infections. Molecules, 2020. 25(23): p. 5593.
- 20. Claus, S.P., H. Guillou, and S. Ellero-Simatos, *The gut microbiota: a major player in the toxicity of environmental pollutants?* Npj biofilms and microbiomes, 2016. **2**(1): p. 1-11.