

RESEARCH ARTICLE

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# Asymptomatic Candiduria among Type 1 and 2 Diabetes Mellitus Patients: Risk and Sociodemographic Factors, Prevalence, Virulence Markers and Antifungal Susceptibility

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## Abstract

Diabetes mellitus (DM) has been considered as one of the predisposing factors for candiduria and *Candida* urinary tract infections. The study determined the socio-demographic characteristics, risk factors of DM patients with asymptomatic candiduria and ascertained the prevalence, virulence factors and antifungal susceptibility of *Candida* isolated. Socio-demographic and risk factors were obtained via questionnaires. Microscopic, macroscopic and chemical analysis of mid-stream urine (MSU) samples were determined by microbiological method and dipsticks. The characterization, virulence factors, antibiotic susceptibility of *Candida* isolates were determined by conventional, mycological media and disc diffusion techniques, respectively. Of the 51 MSU samples,  $\geq 31.4\%$  were amber and clear in colour, contained yeast cells and leukocytes; between 5.9 to 25.5% had hyaline casts, urobilinogen, epithelial cells, red blood cells, pus cells and nitrite, while the specific gravity was  $\geq 1.015$ . The prevalence of candiduria among subjects with respect to age, types and duration of diabetes, gender, tobacco and alcohol consumption were not significant ( $p \geq 0.005$ ). *Candida dubliniensis* and *C. parapsilosis* prevalence was highest in subjects with random blood sugar (mg/dL) of  $\geq 400$  and 300-399, respectively. Of the 39 isolates, 64.1% were Fluconazole sensitive, 10.3% were dose dependent susceptible to Ketoconazole, 74.4% exhibited Voriconazole sensitivity, 100% *C. dubliniensis* were Clotrimazole sensitive,  $\leq 28.6\%$  *C. tropicalis* and *C. glabrata* were resistant to Amphotericin B and Itraconazole, while between 23.1% and 71.8% isolates produced hydrolytic enzymes and biofilm. This study revealed the socio-demographic characteristics and risk factors among subjects and the necessity to continuously investigate pathogenic *Candida* against antifungal agents for effective treatments of asymptomatic candiduria in diabetes mellitus patients.

**Keywords:** Candiduria, Diabetes, Risk Factors, Antifungal, Susceptibility, Virulence Factors

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## INTRODUCTION

Diabetes mellitus, chronic metabolic and degenerative disorder characterized by hyperglycemia, is an increasing global health problem with numerous complications (Lotfi-Kamran *et al.*, 2009; Zahra *et al.*, 2016). Human behavioural changes, lifestyle, genetics and environment factors such as high intake of refined carbohydrates, smoking and heavy consumption of alcohol have been implicated in the burgeoning prevalence of diabetes mellitus (Hu, 2011). The two types of diabetes mellitus are type 1 and type 2 (Mukhtar *et al.*, 2019). The type 1 diabetes mellitus is a heterogeneous disorder characterized by the destruction of pancreatic beta cells, resulting in absolute insulin deficiency (Maahs *et al.*, 2010; Mukhtar *et al.*, 2019), while type 2 consists of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action and an inadequate compensatory insulin secretory response (Mukhtar *et al.*, 2019). Diabetes mellitus, immunosuppression, gender, age, exposure to antimicrobial agents and organ transplantation have long been considered as a predisposing factor for *Candida* urinary tract infections (Kauffman, 2005; Sobel *et al.*, 2011).

The urinary tract consists of organs of the body involved in the production, storage and excretion of urine (Beer *et al.*, 2006). The urinary tract is sterile, devoid of microorganisms but may be invaded by pathogenic organisms (Akinjogunla *et al.*, 2010). Several studies have revealed increase in urinary tract infections (UTIs), attributable to *Candida* spp (Yashavanth *et al.*, 2013). *Candida* species are thin-walled, dimorphic fungi (8 to 12  $\mu\text{m}$  in diameter), that reproduce by budding (Talaro and Talaro, 1996). Although, >150 *Candida* spp have been reported, only few cause human diseases (Kwon-Chung *et al.*, 1992). Candiduria, the presence of *Candida* spp such as *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. parapsilosis* in urine, is classified into asymptomatic and symptomatic forms. Candiduria can lead to morbidity and mortality in immunocompromised and ill-patients (Sanglard and Odds, 2002). *Candida* spp promptly react to environmental changes and take advantage of compromised immunity for establishment of diseases (Akinjogunla *et al.*, 2017). The emergence of drug-resistant *Candida* spp is attributable to

the use of prolonged and inappropriate empirical therapy which has further complicated the management of patients (Chakrabarti *et al.*, 1996). Thus, it's important to appropriately identify the causal agents of the infection and administer proper treatment so as to prevent the disease from becoming chronic.

The virulence factors that enhance the pathogenicity of *Candida* spp are the: phenotypic switching (Soll, 1992); adhesins and invasins on the cell surface (Akinjogunla *et al.*, 2019); yeast hyphal morphogenetic transformation and hydrolytic enzymes e.g. proteases, phospholipases, haemolysins and lipase (Francois *et al.*, 2013). These hydrolytic enzymes aid in adherence, tissue penetration, invasion and destruction of host tissues (Silva *et al.*, 2011; Akinjogunla *et al.*, 2019). Biofilms, communities of microorganisms embedded in an extracellular matrix, similarly contribute to the pathogenicity of *Candida* spp and confer substantial resistance to antifungal therapy (Donlan and Costerton, 2002). The study determined the socio-demographic characteristics and risk factors of diabetic patients with asymptomatic candiduria and as well ascertained the prevalence, virulence markers / factors (amylase, lipase, phospholipase, caseinase, gelatinase, coagulase, biofilm, haemolysin) and susceptibility profiles of *Candida* isolated to antifungal drugs (Ketoconazole, Clotrimazole, Voriconazole, Itraconazole, Fluconazole and Amphotericin) using disc diffusion technique.

## MATERIALS AND METHODS

### Collection of Samples

Mid-stream urine (MSU) samples were aseptically collected using sterile wide-necked, leak-proof containers from 51 diabetic patients (aged < 40 yrs and  $\geq$  41 yrs), with random blood sugar ranging between 200 and  $\geq$  400 mg/dL, attending some hospitals / clinics in Uyo, Akwa Ibom State. The Ethics Review Committee approval and verbal informed consent of diabetic patients were obtained prior to samples collection. The MSU samples were properly labelled and transported in a cooler box (4°C) to microbiology laboratory for analysis.

### Administration of Questionnaires

Structured questionnaires reflecting the socio-demographic characteristics and associated

risk factors were administered to the participants after their verbal informed consent.

#### **Inclusion Criteria**

The diabetic patients who agreed and gave verbal consent to participate in the study.

#### **Exclusion Criteria**

The non-diabetic patients and diabetic patients who were on antibiotics within one week prior to enrolment and/or those that declined to participate in the study.

#### **Microscopic and Macroscopic Examination of Mid-stream Urine Samples**

Two loopful of each uncentrifuged MSU sample was aseptically placed on a clean grease-free slide and covered with a cover slip. The presence of epithelial cell, pus cell, yeast cell, granular cast, crystals, hyaline cast and red blood cells were observed using 10 x and 40 x objectives with condenser iris sufficiently closed. Macroscopic examination was done by observing colour of the MSU samples as previously described by Akinjogunla and Divine-Anthony (2013).

#### **Chemical (Dipstick) Analysis of Mid-stream Urine Samples**

The chemical analysis of MSU samples was carried out using dipsticks (Medi-Test Combi 10 SGL, Macherey-Nagel, Germany). The parameters evaluated were: urobilinogen, leukocytes, protein, ketone, bilirubin, nitrites, specific gravity and blood. The dipstick was dipped into each fresh uncentrifuged MSU sample and removed after 5-10 secs. A colour change of the dipstick within 2 mins was observed and recorded according to the manufacturer's instructions.

#### **Mycological Analysis of Mid-stream Urine Samples**

A loopful of each MSU sample was aseptically inoculated onto each plate of Sabouraud Dextrose Agar (SDA, Oxoid UK) supplemented with chloramphenicol and aerobically incubated at 35°C for 48 hrs. After incubation, the plates were observed for yeast growth and were subcultured onto fresh plates of SDA and incubated at 35°C ± 2 for 48 hr. After incubation, the pure isolates were subcultured onto plates of CHROMagar Candida (Difco BBL., USA), aerobically incubated at 35°C ± 2 for 48 hr and their colonial morphology and pigmentation on CHROMagar Candida were used for identification. The *Candida* species were further identified using Gram staining,

temperature tolerance, chlamydospore and germ tube formation, sugar fermentation and assimilation tests. The *Candida* isolates were maintained on SDA slant at 4°C.

#### **In vitro Antifungal Susceptibility of Candida Isolates**

*In vitro* antifungal susceptibility of *Candida* isolates to Ketoconazole (KET, 10 µg), Clotrimazole (CLO, 10 µg), Voriconazole (VOR, 1µg), Itraconazole (ITR, 10 µg), Fluconazole (FLU, 25 µg), and Amphotericin B (AMP, 20 µg) was carried out using disc diffusion technique (CLSI, 2009). Ten microlitre of inoculum suspension, adjusted to turbidity of 0.5 McFarland standards, was inoculated onto the plate of Mueller Hilton Agar supplemented with 2 % glucose and 0.5 g/mL methylene blue (GM-MHA). The GM-MHA plates were allowed to dry for 15 mins before antifungal discs were aseptically placed on the surfaces of the culture plates using sterile forceps. The plates were aerobically incubated at 35°C ± 2 for 24 hr, inhibition zones after incubation were observed, measured in millimeters (mm) using a ruler and interpreted as sensitive (S), dose dependent susceptible (DDS) and resistant (R) was made as follows: ITR, AMP and VOR (S: ≥ 16, DDS: 10-15, R ≤ 9), FLU (S: ≥19, DDS: 15-18, R ≤ 14), KET (S: ≥ 30, DDS: 23-29, R ≤ 22) and CLO (S: ≥ 20, DDS: 12-19, R ≤ 11).

#### **Detection of Phospholipase, Lipase, Gelatinase, Caseinase and Hemolysin Producing Candida isolates**

Phospholipase, lipase, gelatinase, caseinase and hemolysin producing *Candida* isolates were detected using egg yolk agar (SDA, 2% egg yolk), tributyrin agar (SDA, 1% tributyrin), gelatin agar (SDA, 1% gelatin), skim milk agar (SDA, 1% skim milk) and human blood - Sabouraud Dextrose Agar (SDA, 3 % glucose, 5% human blood) respectively (Deorukhkar *et al.*, 2014; Akinjogunla *et al.*, 2107). Ten microliters of *Candida* isolate suspension, adjusted to turbidity of 0.5 McFarland standards, was inoculated onto each plate of egg yolk agar, tributyrin agar, gelatin agar, skim milk agar and human blood-SDA. All the plates were aerobically incubated at 35°C ± 2 for 72 hrs. After incubation, transparent zones around the colonies were observed and considered positive for production of phospholipase, lipase, gelatinase, caseinase and hemolysin, respectively.

**Detection of Amylase producing *Candida* isolates**

Amylase producing *Candida* isolates was detected using starch agar (SDA, 2% starch). The *Candida* isolates were streaked onto starch agar plates and aerobically incubated for 48-72 hr at 35°C ± 2. After incubation, 3 drops of 10 % Lugol iodine was put on the culture plates and allowed to react for 10 min. Clear zone around the colony indicated the production of amylase (Akinjogunla et al., 2107).

**Detection of Biofilm Producing *Candida* Isolates**

A loopful of each *Candida* isolate from overnight agar plate was aseptically inoculated onto each tube of Glucose-Sabouraud Dextrose Broth (10 ml SDB, 2% glucose). Each tube was incubated aerobically for 48 hrs at 35°C ± 2. After incubation, the contents of each tube were poured out, each tube washed with phosphate buffer saline (PBS, pH 7.2), dried and stained with safranin (1%). Excess stain in each tube was rinsed with PBS and tubes dried in an inverted position. A biofilm formation was indicated when a visible film lined the wall and bottom of the tube (Deorukhkar et al., 2014; Akinjogunla et al., 2107).

**Detection of Coagulase Producing *Candida* Isolates**

The production of coagulase by *Candida* isolates was determined using EDTA-human plasma. Ten microlitre of each *Candida* isolate from overnight agar plate was aseptically inoculated onto each tube of EDTA-human plasma. Each tube was incubated at 35°C ± 2 and observed for clot formation after 2, 4, 6 and 24 hr. The presence of clot that could not be resuspended

by gentle shaking indicated positive for coagulase production (Rodrigues et al., 2003).

**Statistical Analysis**

The Statistical Package for Social Sciences (IBM SPSS Version 22.0. Armonk, NY: IBM Corp.) was used for data analysis. The significant difference in the socio-demographic characteristics and associated risk factors among the diabetic patients at p ≤ 0.05 were determined using chi-square ( $\chi^2$ ) test. Descriptive data were presented as charts and percentages.

**RESULTS**

The microscopic analysis of MSU samples based on age and gender of subjects are shown on Table 1. Of the 51 samples collected, 13.7%, 25.5%, 35.3%, 9.8%, 13.7%, 5.9% and 11.8% had epithelial cells, pus cells, yeast cells, granular cast, crystals, hyaline casts and red blood cells (RBCs), respectively. The occurrence of epithelial cells, pus cells, yeast cells, crystals, hyaline casts and RBCs was higher in female subjects and those aged ≥ 40 yrs than male subjects and those aged < 40 yrs. Only 5.9% MSU samples of subjects aged ≥ 40 yrs contained hyaline casts, while none of the samples of subjects within age group < 40 yrs had hyaline casts (Table 1).

The results of macroscopic and dipstick analysis of MSU samples are shown in Table 2. The colour of MSU samples was: pale yellow and clear 11(21.6%), pale yellow and turbid 6(11.8%), yellow and turbid 3(5.9%), colourless and clear 7(13.7%); amber and clear 10 (35.3%); amber and turbid 6(11.8%). Of the 51 MSU samples collected,

**Table 1.** Microscopic Analysis of Urine Samples of Diabetic Patients Based on Age and Gender

Parameters		No of Samples Collected	No (%) of Occurrence						
			Epithelial Cell	Pus Cell	Yeast Cell	Granular Cast	Crystals	Hyaline Cast	RBC
Age (yrs)	< 40	13	1 (7.7)	3 (23.1)	4 (30.8)	2 (15.4)	1 (7.7)	0 (0.0)	1 (7.7)
	≥ 40	38	6 (15.8)	10 (26.3)	14 (36.8)	3 (7.9)	6 (15.8)	3 (7.9)	5 (13.2)
	Total	51	7 (13.7)	13 (25.5)	18 (35.3)	5 (9.8)	7 (13.7)	3 (5.9)	6 (11.8)
Gender	Male	29	3 (10.3)	5 (17.2)	8 (27.6)	4 (13.8)	3 (10.3)	1 (3.4)	1 (3.4)
	Female	22	4 (18.2)	8 (36.4)	10 (45.5)	1 (4.5)	4 (18.2)	2 (9.1)	5 (22.7)
	Total	51	7 (13.7)	13 (25.5)	18 (35.3)	5 (9.8)	7 (13.7)	3 (5.9)	6 (11.8)

RBC: Red Blood Cell. values in parentheses expressed the percentages.

**Table 2.** Macroscopic and Chemical Analysis of Urine Samples of Diabetic Patients (n=51)

Variables	Categories	No (%) of Occurrence		
		Male (29)	Female (22)	Total No (%)
Appearance	PYC	7 (24.1)	4 (18.2)	11 (21.6)
	PYT	3 (10.3)	3 (13.6)	6 (11.8)
	YT	2 (6.9)	1 (4.5)	3 (5.9)
	CC	4 (13.8)	3 (13.6)	7 (13.7)
	AC	11 (37.9)	7 (31.8)	18 (35.3)
Protein	AT	2 (6.9)	4 (18.2)	6 (11.8)
	++	2 (6.9)	1 (4.5)	3 (5.9)
	+	4 (13.8)	3 (13.6)	7 (13.7)
Ketone	-	23 (79.3)	18 (81.8)	41(80.4)
	++	5 (17.2)	3 (13.6)	8 (15.7)
	+	4 (13.8)	4 (18.2)	8 (15.7)
Bilirubin	-	20 (69.0)	15 (68.2)	35 (68.6)
	++	0 (0.0)	0 (0.0)	0 (0.0)
	+	1 (3.4)	3 (13.6)	4 (7.8)
Nitrite	-	28 (96.6)	19 (86.4)	47 (92.2)
	+	5 (17.2)	1 (4.5)	6 (11.8)
Urobilinogen	-	24 (82.8)	21 (95.5)	45 (88.2)
	++	1 (3.4)	0 (0.0)	1 (2.0)
	+	1 (3.4)	1 (4.5)	2 (4.0)
Leukocytes	-	27 (93.1)	21 (95.5)	48 (94.1)
	++	3 (10.3)	3 (13.6)	6 (11.8)
	+	5 (17.2)	7 (31.8)	12 (23.5)
Blood	-	21 (72.4)	12 (54.5)	33 (64.7)
	++	0 (0.0)	1 (4.5)	1 (2.0)
	+	1 (3.4)	4 (18.2)	5 (9.8)
Specific Gravity	-	28 (96.6)	17 (77.3)	45 (88.2)
	1.000	2 (6.9)	1 (4.5)	3 (5.9)
	1.005	8 (27.6)	6 (27.3)	14 (27.5)
	1.010	4 (13.8)	3 (13.6)	7 (13.7)
	1.015	15 (51.7)	12 (54.5)	27 (52.9)

-: Negative; +: Present in low amount; ++: Present in high amount; PYC: Pale yellow and clear; PYT: Pale yellow and turbid; YT: Yellow and turbid; CC: Colourless and clear; AC: Amber and clear; AC: Amber and turbid

6 samples had leukocytes (+), 12 samples had leukocytes (++); 19.6% were positive for proteins; 15.7% contained ketone (+), 15.7% had ketone (++), 4.0% contained urobilinogen (+), 2.0% had urobilinogen (++) , 11.8% contained blood; 5.9% had specific gravity (SG) of 1.000; 27.5% had SG of 1.005; 13.7% had SG of 1.010, SG of 52.9% was 1.015, while nitrites and bilirubin were present in 11.8% and 7.8%, respectively.

The socio-demographic characteristics and risk factors of subjects are presented in Table 3. Of the 51 MSU samples from subjects, 35.3 % had *Candida* isolates growth, indicating

candiduria, while 64.7 % had no growth of *Candida* isolates. Of 18 subjects with candiduria, 9(50.0%) were males and 9(50.0 %) were females, giving a gender ratio of 1:1. The hypertensive subjects had higher prevalence of candiduria (8/20; 40.0%) than non-hypertensive subjects (10/31; 32.3%). The prevalence of candiduria was lower among subjects with type I diabetes, family history of diabetes and duration of diabetes < 5 yrs than among subjects with type 2 diabetes, duration of diabetes ≥ 5 yrs and without family history of diabetes. There was no statistically significant relationship between the prevalence of candiduria

**Table 3.** Socio-Demographic Characteristics and Risk Factors of Diabetic Patients

Socio-Demographic Characteristics /Risk Factors	No of Samples Collected	No (%) of Subjects		$\chi^2$	p-value
		with Candiduria	without Candiduria		
Age (yrs)					
< 40	13	3 (23.1)	10 (76.9)	1.14	0.286
≥ 40	38	15 (39.5)	23 (60.5)		
Gender				0.534	0.465
Male	29	9 (31.0)	20 (69.0)		
Female	22	9 (40.9)	13 (59.1)		
Marital Status				0.164	0.921
Single	7	2 (28.6)	5 (71.4)		
Married	30	11 (36.7)	19 (63.3)		
Widowed / Divorced	14	5 (35.7)	9 (62.3)		
Occupation				0.717	0.869
Teaching	8	2 (25.0)	6 (75.0)		
Civil / Public Service	21	7 (33.3)	14 (66.7)		
Self -Employed	12	5 (41.7)	7 (58.3)		
Unemployed	10	4 (40.0)	6 (60.0)		
Educational Status				1.45	0.693
Primary	10	4 (40.0)	6 (60.0)		
Secondary	9	3 (33.3)	6 (66.7)		
Tertiary	19	5 (26.3)	14 (73.7)		
None	13	6 (46.2)	7 (53.8)		
Type of Diabetes				0.386	0.534
Type 1	17	5 (29.4)	12 (70.6)		
Type 2	34	13 (38.2)	21 (61.8)		
Duration of Diabetes				0.433	0.510
< 5 yrs	23	7 (30.4)	16 (69.6)		
≥ 5 yrs	28	11 (39.3)	17 (60.7)		
Family History of DM				0.395	0.530
Yes	11	3 (27.3)	8 (72.7)		
No	40	15 (37.5)	25 (62.5)		
Alcohol Consumption				0.386	0.534
Yes	17	7 (41.2)	10 (58.8)		
No	34	11 (32.4)	23 (67.6)		
Tobacco Intake				0.203	0.652
Yes	7	3 (42.9)	4 (57.1)		
No	44	15 (34.1)	29 (65.9)		
Blood Pressure				0.319	0.572
Hypertensive	20	8 (40.0)	12 (60.0)		
Non-Hypertensive	31	10 (32.3)	21 (67.7)		

DM: Diabetes Mellitus

among subjects with respect to age ( $\chi^2 = 1.14$ ;  $p = 0.286$ ), marital status ( $p = 0.921$ ), occupations ( $p = 0.869$ ), educational status ( $p = 0.693$ ), type of diabetes ( $p = 0.534$ ), duration of diabetes ( $p = 0.510$ ), family history of diabetes ( $p = 0.530$ ) and alcohol consumption ( $p = 0.534$ ). The prevalence

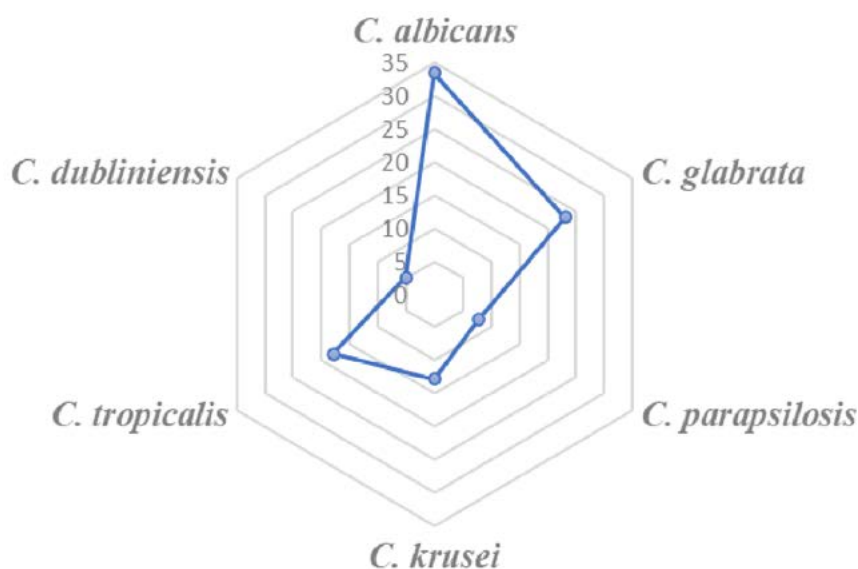
of candiduria was higher among subjects that smoked tobacco (42.9%) than non-tobacco smokers (34.1%), but there was no statistically significant difference ( $\chi^2 = 0.203$ ,  $p = 0.652$ ) (Table 3).



The percentage occurrence of *Candida* isolates in MSU samples of subjects in decreasing order was: *C. albicans* (33.3%) > *C. glabrata* (23.1%) > *C. tropicalis* (17.9%) > *C. krusei* (12.8%) > *C. parapsilosis* (7.7%) > *C. dubliniensis* (5.1 %) (Fig 1). With regards to total *Candida* isolates (n=39) obtained, subjects aged ≥ 40 yrs had 79.5 % isolates; subjects aged < 40 yrs had 20.5 % isolates; male subjects (46.2%) and female subjects (53.8%) isolates. The predominant candida isolate in female and male subjects was *C. albicans* and *C. parapsilosis*, respectively (Table 4). *C. dubliniensis* prevalence was found to be highest (100%) in subjects with random blood sugar (RBS) of ≥ 400 mg/dL, while *C. krusei* and *C. parapsilosis* was

predominant candida isolate in subjects with RBS (mg/dL) of 200-299 and 300-399, respectively (Table 4).

The results showed that 64.1% *Candida* isolates were Fluconazole (FLU) sensitive, while 24.5% were FLU resistant (Table 5). Of the 39 *Candida* isolates, 71.8 %, 10.3 % and 17.9 % were sensitive, dose dependent susceptible (DDS) and resistant to Ketoconazole (KET) respectively; 74.4% isolates were sensitive to Voriconazole (VOR), 12.8% were DDS, while 12.8% isolates were resistant. All (100%) *C. dubliniensis* were sensitive to Clotrimazole (CLO), KET and FLU; between 22.2% and 28.6% *C. tropicalis* and *C. glabrata* were resistant to Amphotericin B and Itraconazole (ITR);



**Fig. 1.** Percentage Occurrences of *Candida* Isolates in Urine Samples of Diabetic Patients

**Table 4.** Distribution of *Candida* Isolates Based on Age, Gender and RBS Values of Diabetic (Candiduric) Patients

<i>Candida</i> Isolates	No of Isolates	No (%) of Occurrence Age (yrs)		Gender		Random Blood Sugar (mg/dL)		
		< 40	≥ 40	Male	Female	200-299	300-399	≥ 400
<i>C. albicans</i>	13	3 (23.1)	10 (76.9)	4 (30.8)	9 (69.2)	3 (23.1)	4 (30.8)	6 (46.2)
<i>C. glabrata</i>	9	2 (22.2)	7 (77.8)	5 (55.6)	4 (44.4)	2 (22.2)	5 (55.6)	2 (22.2)
<i>C. krusei</i>	3	0 (0.0)	3 (100)	1 (33.3)	2 (66.7)	3 (100)	0 (0.0)	0 (0.0)
<i>C. parapsilosis</i>	5	2 (40.0)	3 (60.0)	3 (60.0)	2 (40.0)	0 (0.0)	4 (80.0)	1 (20.0)
<i>C. tropicalis</i>	7	1 (14.3)	6 (85.7)	4 (57.1)	3 (42.9)	1 (14.3)	2 (28.6)	4 (57.1)
<i>C. dubliniensis</i>	2	0 (0.0)	2 (100)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100)
Total	39	8 (20.5)	31 (79.5)	18 (46.2)	21 (53.8)	9 (23.1)	15 (38.5)	15 (38.5)

RBS: Random Blood Sugar; Values in parentheses expressed the percentage of *Candida* isolates

**Table 5.** Antifungal Susceptibility of *Candida* Isolates from Diabetic (Candiduric) Patients (n = 39)

Antifungal Drug		<i>Candida</i> Isolates						Total No(%)
		<i>C. albicans</i> No(%)	<i>C. glabrata</i> No(%)	<i>C. krusei</i> No(%)	<i>C. parapsilosis</i> No(%)	<i>C. tropicalis</i> No(%)	<i>C. dubliniensis</i> No(%)	
Fluconazole	S	7 (53.8)	6 (66.7)	2 (66.7)	4 (80.0)	4 (57.1)	2 (100)	25 (64.1)
	DDS	3 (23.1)	1 (11.1)	0 (0.0)	1 (20.0)	1 (14.3)	0 (0.0)	6 (15.4)
	R	3 (23.1)	2 (22.2)	1 (33.3)	0 (0.0)	2 (28.6)	0 (0.0)	8 (20.5)
Ketoconazole	S	10 (76.9)	6 (66.7)	3 (100)	3 (60.0)	5 (71.4)	1 (50.0)	28 (71.8)
	DDS	0 (0.0)	2 (22.2)	0 (0.0)	1 (20.0)	0 (0.0)	1 (50.0)	4 (10.3)
	R	3 (23.1)	1 (11.1)	0 (0.0)	1 (20.0)	2 (28.6)	0 (0.0)	7 (17.9)
Voriconazole	S	9 (69.2)	8 (88.9)	2 (66.7)	3 (60.0)	5 (71.4)	2 (100)	29 (74.4)
	DDS	1 (7.7)	0 (0.0)	1 (33.3)	2 (40.0)	1 (14.3)	0 (0.0)	5 (12.8)
	R	3 (23.1)	1 (11.1)	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	5 (12.8)
Amphotericin B	S	10 (76.9)	6 (66.7)	2 (66.7)	4 (80.0)	5 (71.4)	1 (50.0)	28 (71.8)
	DDS	0 (0.0)	1 (11.1)	0 (0.0)	1 (20.0)	0 (0.0)	1 (50.0)	3 (7.7)
	R	3 (23.1)	2 (22.2)	1 (33.3)	0 (0.0)	2 (28.6)	0 (0.0)	8 (20.5)
Itraconazole	S	11 (84.6)	5 (55.6)	2 (66.7)	3 (60.0)	4 (57.1)	0 (0.0)	25 (64.1)
	DDS	1 (7.7)	2 (22.2)	1 (33.3)	0 (0.0)	1 (14.3)	1 (50.0)	6 (15.4)
	R	1 (7.7)	2 (22.2)	0 (0.0)	2 (40.0)	2 (28.6)	1 (50.0)	8 (20.5)
Clotrimazole	S	9 (69.2)	8 (88.9)	3 (100)	3 (60.0)	5 (71.4)	2 (100)	30 (76.9)
	DDS	1 (7.7)	0 (0.0)	0 (0.0)	1 (20.0)	1 (14.3)	0 (0.0)	3 (7.7)
	R	3 (23.1)	1 (11.1)	0 (0.0)	1 (20.0)	1 (14.3)	0 (0.0)	6 (15.4)

S: Sensitive; DDS: Dose Dependent Susceptible; R: Resistant; Values in parentheses expressed the percentage of *Candida* isolates.

**Table 6.** Virulence Factors of *Candida* Isolates from Diabetic (Candiduric) Patients

<i>Candida</i> Isolates	No of Isolates	No (%) of Occurrence							
		AMY	LIP	PHO	CAS	GEL	COA	HAE	BIO
<i>C. albicans</i>	13	7 (53.8)	5 (38.5)	8 (61.5)	10 (76.9)	9 (69.2)	6 (46.2)	11 (84.6)	5 (38.5)
<i>C. glabrata</i>	9	4 (44.4)	3 (33.3)	5 (55.6)	6 (66.7)	5 (55.6)	2 (22.2)	5 (55.6)	2 (22.2)
<i>C. krusei</i>	3	0 (0.0)	1 (33.3)	2 (66.7)	2 (66.7)	3 (100)	0 (0.0)	2 (66.7)	0 (0.0)
<i>C. parapsilosis</i>	5	1 (20.0)	0 (0.0)	2 (40.0)	2 (40.0)	1 (20.0)	1 (20.0)	3 (60.0)	1 (20.0)
<i>C. tropicalis</i>	7	3 (42.9)	2 (28.6)	4 (57.1)	4 (57.1)	3 (42.9)	1 (14.3)	5 (71.4)	1 (14.3)
<i>C. dubliniensis</i>	2	0 (0.0)	1 (50.0)	2 (100)	1 (50.0)	2 (100)	0 (0.0)	2 (100)	0 (0.0)
Total	39	15 (38.5)	12 (30.8)	23 (59.0)	25 (64.1)	24 (61.5)	10 (25.6)	28 (71.8)	9 (23.1)

AMY: Amylase; LIP: Lipase; PHO: Phospholipase; CAS: Caseinase; GEL: Gelatinase; COA: Coagulase; BIO: Biofilm; HAE: Haemolysin; Values in parentheses expressed the percentage occurrences of *Candida* isolates.

≤ 23.1% *C. parapsilosis* and *C. albicans* were CLO resistant, while 33.3% *C. krusei* were DDS to VOR and ITR (Table 5).

Table 6 shows the distribution of virulence factors in *Candida* isolates from MSU samples of subjects. The percentage occurrence of each virulence factor was as follows: 38.5% amylase, 30.8% lipase, 25.6% coagulase and 23.1% biofilm. A higher prevalence, > 50%,

was observed for haemolysin, phospholipase, gelatinase and caseinase (71.8%, 59.0%, 61.5% and 64.1%, respectively). Among candida isolates showing biofilm formation were *C. albicans* (n=5), *C. glabrata* (n=2), *C. parapsilosis* (n=1) and *C. tropicalis* (n=1) while none of the *C. dubliniensis* and *C. krusei* were amylase, coagulase and biofilm producers (Table 6).



## DISCUSSION

*Candida* infections have increased vastly among immuno-compromised patients especially those with diabetic mellitus (DM). In this study, microscopic examinations of MSU of DM patients revealed the presence of epithelial cells, pus cells, yeast cells, crystals, hyaline casts and RBCs. The 13.7% MSU with epithelial cells and crystals obtained in this study was  $\leq$  27.5% epithelial cells and crystals obtained by Abebe *et al.* (2019) among DM patients at University of Gondar Hospital, Ethiopia. The presence of pus cells and RBCs in MSU had been reported by Akinjogunla and Divine-Anthony (2013) and Matulewicz and Meeks (2016), respectively. The presence of RBCs in urine may be attributed to lower UTI (cystitis), upper UTI (pyelonephritis) and cancer of the urinary tract, which are directly or indirectly related to DM (Matulewicz and Meeks, 2016). In our study, 25.5% DM patients had pus cells in their MSU and this value was  $<$  35.5% reported by Abdulla *et al.* (2015). The pus cells are white blood cells that have succumbed in defense of the body against pathogens that invade it, therefore their presence indicates microbial infections. The MSU of DM patients in this study had specific gravity of  $\leq$  1.015 and also contained leukocytes, proteins, ketone, urobilinogen, blood, nitrites and bilirubin. The percentage of MSU with protein (19.6%), ketone (31.4%) and leukocytes (35.3%) in our study was higher than 11.3%, 4.5% and 24.9% for protein, ketone and leukocytes, respectively obtained by Abebe *et al.* (2019) among DM patients at University of Gondar Hospital, Ethiopia. The presence of nitrite has a predictive value for UTIs (Akinjogunla and Divine-Anthony, 2013) and the value obtained for nitrite (11.8%) in this study was lower than 21.5 % MSU with nitrite reported by Fernandes *et al.* (2018).

The prevalence of candiduria was more in subjects aged  $\geq$  40 than in subjects  $<$  40 yrs and this substantiated the earlier report of Hassaneen *et al.* (2014) and Abebe *et al.* (2019) that candiduria was more frequent in elderly patients. Several reports have showed frequency of candiduria to be more in diabetic women than in diabetic men (Janifer *et al.*, 2009; Abdulla *et al.*, 2015) and this study similarly confirmed these reports. The hypertensive subjects had higher prevalence of candiduria than non-hypertensive subjects

in our study and this was in conformity with a study conducted by Muller *et al.* (2007) in which diabetic patients were more at risk for candiduria / UTI than hypertensive patients without diabetes. Other predisposing factors such as duration of diabetes, family history of diabetes, alcohol in-take and tobacco consumption were observed to be associated with occurrence of candiduria in this study. The prevalence of candiduria was more in subjects with duration of diabetes  $\geq$  5 yrs than those subjects with duration of diabetes  $<$  5 yrs and this was similar to the findings of Janifer *et al.* (2009).

*Candida albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, *C. dubliniensis* and *C. parapsilosis* were isolated from MSU of DM patients in our study with random blood sugar ranging from 200 to  $\geq$  400 mg/dL. Isolation of non albicans *Candida* (NAC) from MSU of DM patients in our study corroborated the previous findings of Pandey and Pandey (2013) who reported the emergence of NAC in MSU of diabetic patients at Gwalior, India. The percentage occurrence of NAC (66.7%) in MSU of DM patients was higher than *C. albicans* (33.3%) and this agreed with the results of Ekpo *et al.* (2017) in Cameroon. Our observation also concurred with Kauffmann (2005) who reported that  $>$ 50% of urinary *Candida* isolates were NAC.

The susceptibility of *Candida* spp to antifungal agents is not often predictable, hence, investigating pathogenic *Candida* isolates against appropriate antifungal agents is required for effective treatments of patients. In our study, 20.5% Fluconazole and 20.5% Itraconazole resistant *Candida* spp were obtained and these values were  $>$  13% Fluconazole and 18.5% Itraconazole resistant *Candida* spp reported by Sojakova *et al.* (2004). The resistance of *Candida* spp to Ketoconazole in this study correlated with the reports of Hugo and Russell (2007). Over-expression of CtERG11 or CDR1/CDR2 gene (Sanglard and Odds, 2002), over-expression of efflux proteins which act by pumping the drug out of the cell at a rate faster rate than how the drug enters the cell (Akinjogunla and Eghafona, 2012) and modifications of target enzymes have been attributed to the resistance of *Candida* spp to azole group of antifungal agents (Silva *et al.*, 2011). A high percentage of Amphotericin B sensitive *C. albicans*, *C. parapsilosis* and *C. tropicalis* was

observed and this substantiated the findings of Metin *et al.* (2011).

The transition of *Candida* isolate from a harmless *commensal* to potent pathogen is determined by several host predisposing factors and virulence attributes of organisms. The pathogenicity of *Candida* isolates is attributable to secretion of extracellular hydrolytic enzymes that act synergistically under favourable conditions (Silva *et al.*, 2011). The occurrence of haemolysin, lipase and phospholipase producing *Candida* spp in this study corroborated the reports of Saha (2018) and Akinjogunla *et al.* (2019). Phospholipase facilitates *Candida* invasion by damaging host cellular contents and cleaves phospholipids of host cell membrane (Deorukhkar *et al.*, 2014). Haemolysin aids in lysis of host erythrocytes and strips iron from haemoglobin molecules (Manns *et al.* 1994). *Candida albicans* and NAC exhibited biofilm formation and the occurrence of biofilm producing *Candida* spp agreed with Seneviratne *et al.* (2008). In our study, 14.3% coagulase producing *C. tropicalis* obtained was similar to 14.6 % by Deorukhkar *et al.* (2014), but < 82.6% reported by Rodrigues *et al.* (2003). Coagulase binds plasma fibrinogen and activates a cascade of reactions that induce clotting of plasma (Rodrigues *et al.*, 2003).

## CONCLUSION

This study has shown the socio-demographic characteristics and risk factors in diabetic mellitus patients with or without candiduria; revealed the high sensitivity of *Candida* isolates to azole drugs and varied percentages of haemolysin, coagulase, biofilm, phospholipase and lipase producing *Candida* isolates in MSU samples and as well showed the necessity to continuously investigate pathogenic candida isolates against appropriate antifungal agents for effective treatments of asymptomatic candiduria in type 1 and 2 diabetes mellitus patients.

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## CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

## AUTHORS' CONTRIBUTIONS

Author AOJ designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors DO and AYO wrote part of the manuscript and managed the analyses of the study. Authors EIU and EIJ managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

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None.

## ETHICS STATEMENT

The study protocol was in accordance with ethical procedures and recommendations of State Hospitals Management Boards guidelines.

## DATA AVAILABILITY

The dataset used and/or analyzed during the current study are included in the manuscript.

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