Distribution of Candida sp. in the Genitourinary Tract in Diabetic Mellitus Patients Attending Ladoke Akintola University of Technology Teaching Hospital, Osun State, Nigeria

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ABSTRACT

Introduction: The incidence of opportunistic infections due to *Candida albicans* and other *Candida spp*. has been increasing. Rapid identification of *Candida spp* is important for the clinical management among these individuals. **Aim:** The purpose of this study was to identify *Candida spp*. isolated from the genitourinary tract of diabetic mellitus patients attending Ladoke Akintola University of Technology Teaching Hospital, Osogbo Osun State Nigeria. **Materials and Methods:** Clean-catch midstream urine and genital swabs (high vaginal swabs, endocervical swabs and urethral swabs) were collected from 70 asymptomatic and symptomatic patients attending genitourinary clinic of Ladoke Akintola University of Technology Teaching Hospital, Osogbo Osun State Nigeria. Fungal culture and identification were done using standard mycologic procedure. This study was carried out for twelve (12) months, between May 2013 and April 2014 **Results:** Thirty- four Candida isolates were obtained from 70 diabetic mellitus patients. The prevalence rate of genitourinary candidiasis in diabetic patients was 48.6%. The most common species were *C glabrata* (58.8%), *C albicans* (23.5%), *C dublinensis* (11.8%) and *C lusitaniae* (5.9%). Genitourinary candidiasis was highly prevalent in female 28/45(62.2%) than male 6/25(24.0%). *Candida* species were isolated in 6(17.6%) urine, 1(2.9%) urethral swab and 27(79.4%) vulvovaginal swabs samples. Significant genitourinary candidiasis was detected in 23.5% and 76.5% of asymptomatic and symptomatic diabetic patients, respectively and 18 out of 36 (50.0%) were positive for Type 1 while 16 out of 34 (47.1%) were positive for Type 2 (p=0.022)

Conclusions: It shows from this study that *C glabrata* had the highest proportion in the genitourinary tract of diabetic patients attending Ladoke Akintola University of Technology Teaching Hospital, Osogbo Osun State

Keywords: Prevalence, Genitourinary Candidiasis, Diabetes, CHROMagar Candida and API.

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INTRODUCTION

Genitourinary Candidiasis has been shown to be more prevalent in patients with diabetes than other immunocompromised patients (1) based on its re-occurrence, severity and requiring hospitalization (2). Diabetes is a "chronic" metabolic disorder that occurs when the body cannot produce enough or effectively use insulin (3). Type 1 Diabetes Mellitus (DM), previously known as insulin-dependent diabetes or childhood-onset diabetes, develops when the body cannot produce any insulin, and usually begins in childhood (4). Type 2 DM develops when the body cannot produce enough insulin or when the insulin that is produced does not work properly. Ninety percent of people with diabetes around the world have this type of diabetes, and it is largely the result of excess body weight and physical inactivity; it is usually diagnosed at age 40 or older, but it can occur at a younger age (4). This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger), weight loss, vision changes, and fatigue. All of these symptoms may occur suddenly (5). It is often associated with a high disease burden in developing countries such as Nigeria (3) and the disease frequency is on the increase among top executives, politicians, academicians, civil servants, farmers, traditional rulers, traders, businessmen, teachers, students, pupils, pre-school children and pregnant women (3). Therefore, it is obvious that diabetes mellitus touches almost every family and constitutes a drain on the economy and social life of sufferers and their families (3). Current estimates of DM prevalence are 1-2% in rural adults and 6-10% in urban dwelling adult in Nigeria (6). This translates to at least 5 million people living with DM, a large proportion of which are undiagnosed (7).

Hyperglycemia or raised blood sugar, is a common effect of uncontrolled diabetes (2), the major cause of increased susceptibility of diabetic mellitus patients to urogenital candidiasis and may impair several aspects of humoral host defense, especially the nerves and blood vessels, resulting in decreased random motion of neutrophils, chemotaxis, phagocytosis, and microbial killing thereby enhancing yeast adhesion and growth in the genital tissues (4). Although, the distribution of *Candida spp* exist as normal flora in mouth, skin, gastrointestinal and urogenital tracts of healthy individuals with estimated overall carriage rate of 80% (8, 9), yet it flourishes and causes havoc in individuals with high blood sugar, HIV/AIDS, drug addiction, immunological deficiencies, pregnancy, broad-spectrum antibiotics therapy, administration of corticosteroids or immunosuppressive drugs, malnutrition, malignancy and anaemia (10 11, 12, 13, 14, 15). Some researchers also reported that systematic conditions such as vitamin B deficiency, hypothyroidism and lymphoblastoma favor *Candida* infection (16, 17, 18).

Genitourinary candidiasis manifests in different parts of the body. In the vagina are found, itching, redness and a thick white vaginal discharge with occasional white patches on the skin of the vaginal area (19). The irritation from vaginal candidiasis is responsible for the physical discomfort experienced by some patients (19). The offensive vaginal discharge is a product of organic decomposition of the proteins component on the vaginal mucosa (19). In the lower urinary tract, bladder irritation, including dysuria, hematuria, frequency, urgency, and supra-pubic tenderness are common (20) while in the upper urinary

tract patients present with fever, leukocytosis and costo-vertebral angle tenderness (21). Renal candidiasis may present with high fever, hemodynamic instability, and variable renal insufficiency (22). Patients infected with candida balanitis commonly complain of local burning and pruritus, and the clinical features include mild glazed erythema and papules with or without satellite-eroded pustules (23).

Epidemiologic data from the past decade reveal a paradigm shift in *Candida* infections with non-albicans Candida species such as *C. glabrata, C. tropicalis,* and *C. krusei* as emerging important pathogens (24, 25, 26, 27, 28).This transition has had a significant clinical impact due to decreased susceptibility of these non-albicans yeasts to antifungal agents (27, 28). Genitourinary candidiasis is common in diabetes mellitus patients and mostly in women (29, 30) *Candida albicans* and non-*albicans* species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and fungal susceptibility. Therefore, Candida species identification is important for successful management.

The risk of asymptomatic, complicated genitourinary candidiasis is higher in diabetes (36) which lead to serious side effects that includes carcinoma of the bladder, extensive renal parenchymal damage, increased prematurity, low birth weight and fetal death and the diagnosis of almost any form of *Candida* disease requires an integration of clinical, epidemiological, and laboratory findings. However, there is understudied of *Candida* in the genitourinary tract of diabetes patients in this locality. Therefore, this study was carried out for speciation of *Candida* associated with genitourinary tract of diabetes patients attending LAUTECH Teaching Hospital, Osogbo, Osun State for proper management.

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Materials and Methods

Study Site

This study was carried out within University Health services, Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo Osun State, Nigeria. It is located in the South-West geopolitical zone of Nigeria, serves as a referral hospital.

Study design

This research is a descriptive cross-sectional study carried out over a period of 12 months (May, 2013 - April, 2014). Random sampling method was used.

Subject selection

This study was carried out among the population suffering from diabetes, across all ages group and in both men and women.

Ethical approval

Approval of the study protocol was obtained from the Ethics Committee of LAUTECH Teaching Hospital, Osogbo, Nigeria.

Data collection

Questionnaire was prepared and distributed for this purpose after obtaining the approval of Ethics Committee of LAUTECH Teaching Hospital, Osun State and after seeking the consent of the patients.

Specimen collection

All these specimens; Blood, Endocervical, High vaginal, Urethra swabs and Urine were collected following aseptic precautions using fluoride oxalate bottle, sterile swab sticks and universal container respectively from patients suffering from diabetes attending genitourinary clinic. Firstly, 5ml of blood specimens were collected into fluoride oxalate bottles from genitourinary

clinic and immediately sent to Chemical pathology for the analysis of glucose level. Each diabetic mellitus patient was instructed to collect clean-catch midstream urine into a sterile screw-capped wide-mouth container. Genital swabs were collected by qualified medical officer, following aseptic precautions. The samples were collected from both asymptomatic and symptomatic patients and immediately delivered to the genital bench of Medical Microbiology laboratory where they were processed according to standard mycological procedures (31).

Laboratory Analyses

Glucose estimation

For the diagnosis of DM, Glucose- Oxidase method was used. It was carried out by using spectrophotometric analysis of plasma using Agappe reagent kit (Switzerland GmbH). The working reagent was prepared by dissolving reagent 2 (R2) with the volume of reagent 1(R1) as indicated in the label and kept at 2-8^{o}C and laboratory procedure was followed according to the manufacturer instruction. All necessary precautions were taken into consideration.

Procedure for Glucose estimation

1000µl of working reagent was prepared for blank, 1000µl of working reagent and 10µl of standard was prepared for standard and 1000µl of working reagent and 10µl of plasma was prepared for sample. They are all mixed and incubated for 10mins at 37^{o}C. The change in absorbance of standard and sample were measured against reagent blank at wavelength of 505nm. Diabetics status was further confirmed using IDF standards; diabetes (FBS > 126mg/dl)

Wet Preparation

A small volume of normal saline (0.9% NaCl) was placed inside the tube containing the swab stick. The bottom of the tube was tapped gently for few seconds for homogeneous mixture. A drop of saline mixture was placed onto a glass slide and covered with a cover slip. The preparation was examined under the microscope using X10 and X40 objective lenses. Upon examination, oval budding cells presumed to be Candida species were seen and noted.

A deposit of centrifuged urine was placed onto a glass slide covered with a cover slip and examined microscopically at ×400 magnifications. The wet preparation was to detect yeast cells only, presence of trichomonads and clue cells were excluded (20).

Culture

Antibiotic-treated selective (Chloramphenicol and Gentamycin containing 50mg chloramphenicol and 5mg gentamycin) Sabouraud Dextrose Agar (SDA) was used as a growth medium for the isolation of *Candida* species. After inoculation of the clinical specimen by streaking, plates were incubated at 37°C for 2 days aerobically. Colonial morphology of Candida isolates was noted. Candida has a smooth, large, white to cream colored colonies with glabrous to waxy appearance.

Gram Staining

After inoculation on Sabouraud Dextrose Agar, a smear was made from the colonies formed, on a clean glass slide by placing a drop of sterile water in the middle of the clean glass slide. Sterilized inoculating wire loop (flamed and then cooled) was used to touch the colony and rubbed in the drop of water on the slide and spread into a thin smear. The smear was air dried and heat fixed by passing over a Bunsen burner flame for three times to fix the colony. The smear was flooded with crystal violet for one minute and then washed with clean water

and again flooded with lugol's iodine for 30 seconds. The smear was decolorized with acetone-alcohol and washed immediately with water and counterstained with neutral red for 3 seconds. The film was then washed with clean water and allowed to air dry, and then examined under the microscope at X100 magnification with oil immersion. Upon examination of stained preparation, the yeast cells were seen as violet oval cells (20, 31).

Germ tube test

A simple test for the identification of *Candida species* is the germ tube test. A colony was emulsified in 0.5 - 1 ml of sterile human serum and animal serum (rabbit), incubated at 35 - 37°C for 4 h. A drop of the suspension was placed on a microscope slide and it was examined under high power (x40) for the formation of germ tubes (20).

Species Identification

CHROM agar Candida

Identification of *Candida* species was carried out by sub culturing the isolated *Candida* from Saboraud Dextrose Agar onto CHROMagar incubated at 37°C for 48- 72 hours aerobically. The production of color and morphology as described by the manufacturer were recorded.

Biochemical test

A complete identification of *Candida* species was done by subjecting colonies from saboraud dextrose agar to biochemical test, using API 20C AUX (Biomuriex, France) following the manufacturer's instruction

Statistical Analysis

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 16.0). Percentage for proportions was reported, chi-square test was used for comparisons between groups. A *P* value less than 0.05 were considered significant.

RESULTS

Study Population

The demographic profiles of study subjects are presented in Table 1. Of the 70 diabetic mellitus patients investigated, 27 (38.6%) had no symptoms of genitourinary candidiasis (asymptomatic) and the remaining 43 (61.4%) presented with symptoms of genitourinary candidiasis (symptomatic). Diabetic patients living in urban and rural areas were 28(40.0%) and 42(60.0%) respectively. Types 1 and 2 diabetes mellitus were observed in 36(51.4%) and 34(48.6%) of the patients, respectively. The age range of the study participants was 16-70years. Of the 70 diabetic patients, 44(62.9%) were women and 26(37.1%) were

Table 1: Demographic profiles of diabetic mellitus patients at Sexually Transmitted Disease Clinic, in LAUTECH Teaching Hospital, Osun State Nigeria.

Characteristics	Total (n=70)	Asymptomatic (n=27)	Symptomatic (n=43)
Age (years)			
16-30	5	2	3
31-45	39	15	24
46-60	19	7	12
Above 60	7	3	4
Sex			
Female	44	18	26
Male	26	9	17
Living area			
Urban	28	9	19
Rural	42	18	24
Diabetes type			
Type 1	36	13	23
Type 2	34	14	20

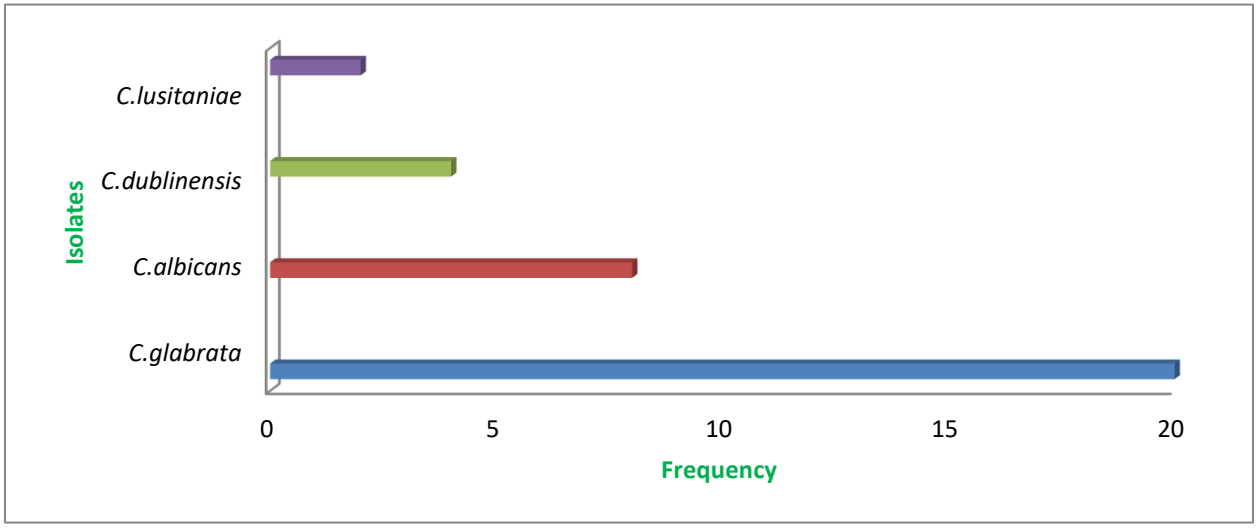

Figure 1: Distribution of *Candida Spp* recovered from genitourinary tract of diabetic mellitus patients.

A total of 34 *Candida* species were isolated from 70 diabetic mellitus patients investigated for genitourinary candidiasis. *C. glabrata* accounted for 20 (58.8%) of the cases, followed by, *C. albicans* (23.5%), *C. dublinensis* (11.8%), *C. lusitaniae* (5.9%). The overall prevalence of genitourinary candidiasis was 34 of 70(48.6%).

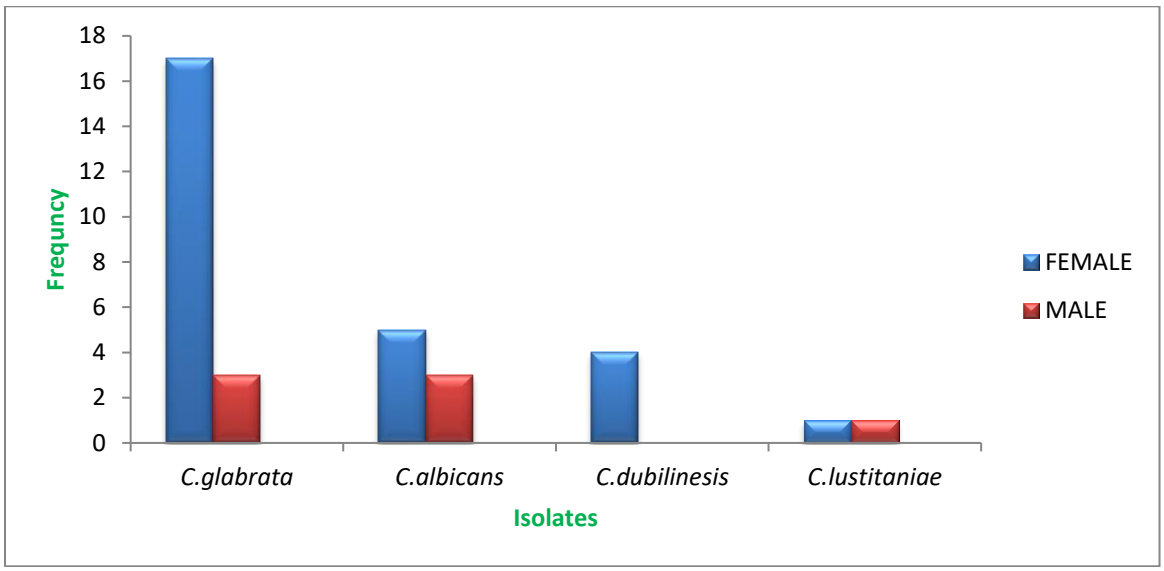

Figure 2: Occurrence of *Candida* isolates according to sex
Genitourinary candidiasis in diabetic patient was highly prevalent in females 27(79.4%) than males 7(20.6%) with p= 0.090

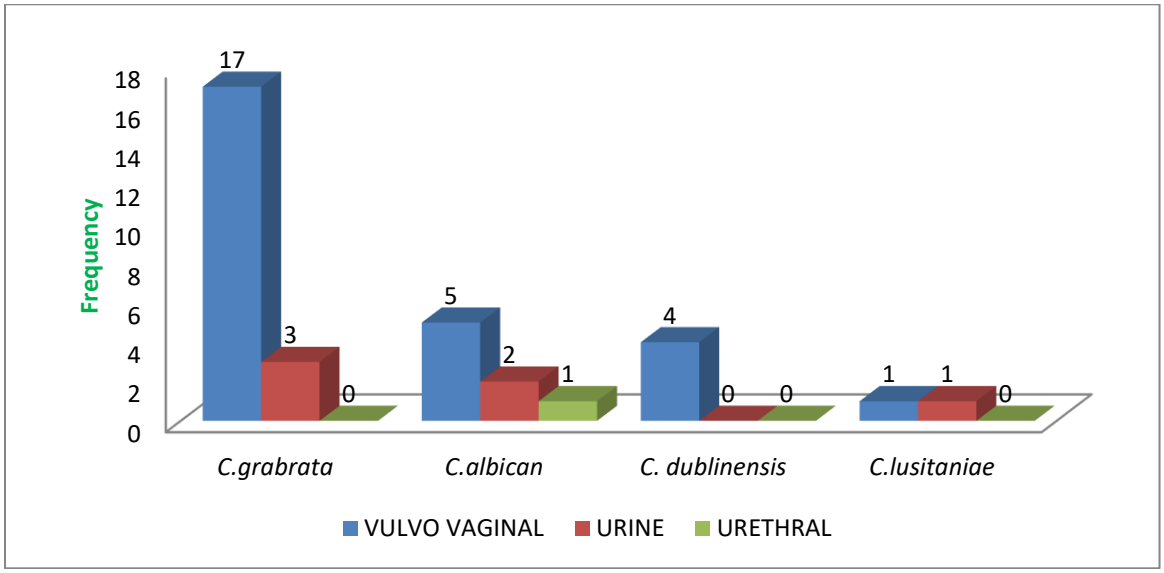

Figure 3: Species of *Candida* recovered from urogenital specimens of diabetic patients.
Genitourinary candidiasis was found in the urogenital system of diabetic patients but commonly found in the vulvovaginal swab.

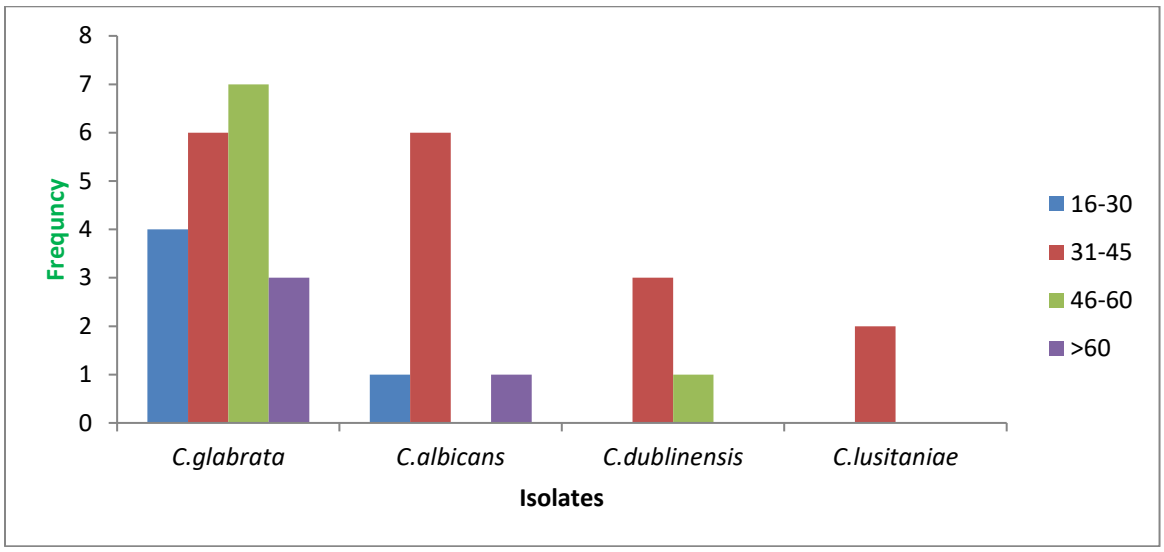

Figure 4: Distribution of *Candida spp* among age groups

In this study, genitourinary candidiasis in diabetic patient was found frequently in the age group 31-45 followed by 46-60 as presented below

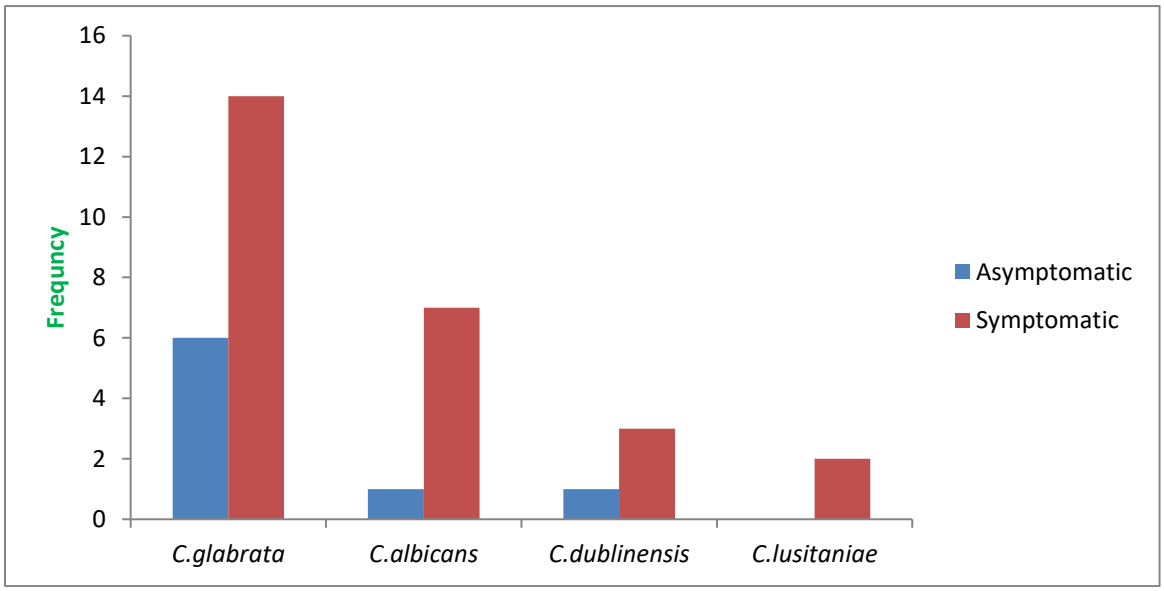

Figure 5: Distribution of *Candida* species of asymptomatic and symptomatic diabetic patients

Significant genitourinary candidiasis was detected in 8 of 27 (11.4%) and 26 of 43 (37.1%) asymptomatic and symptomatic diabetic patients, respectively (P =0.001).

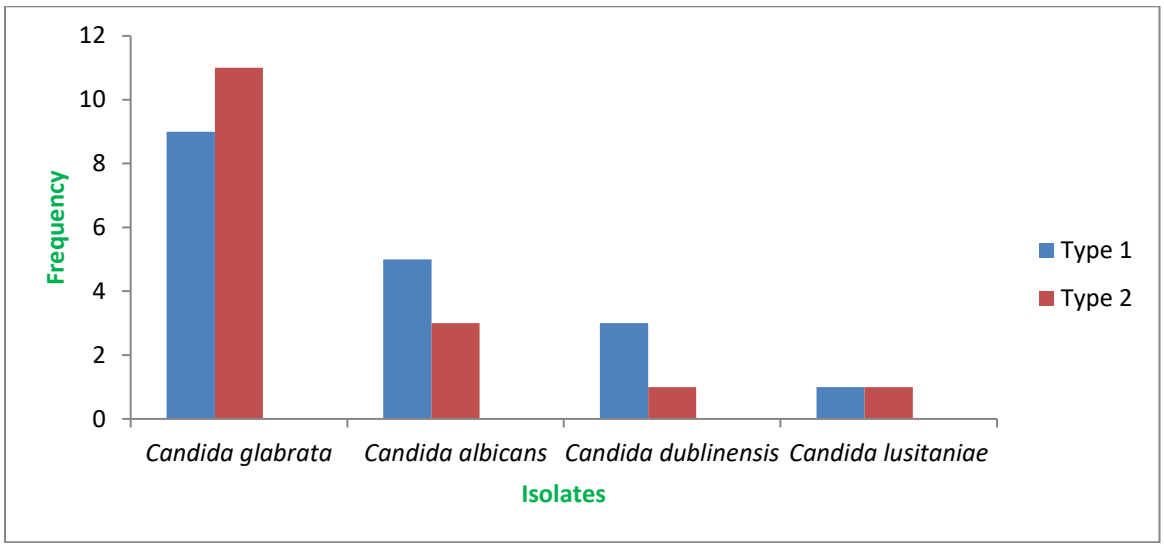

Figure 6: Occurrence of *Candida* species in Types of Diabetic mellitus patients

As presented above, this study showed that 18 out of 36 (50.0%) were positive for Type 1 while 16 out of 34 (47.1%) were positive for Type 2.

DISCUSSION

This study investigated the distribution of *Candida* isolates in the genitourinary tract of diabetic mellitus patients over the period of twelve (12) months in Medical Microbiology Department, LAUTECH Teaching Hospital, Osun State, Nigeria.

The prevalence of genitourinary candidiasis of diabetic patients in this study was significant (48.6%) in this locality In Nigeria, studies have shown higher prevalence of genitourinary candidiasis in diabetic patients; Ophori and his co- worker reported 36.2%, Richard et al. (2008) reported 37.7% in Benin city, 27% reported by Oni and his researcher in Ibadan (2012). Tobias revealed 30% in 2016 and 18.8% reported in Ibadan, Oyo State Nigeria by Adebiyi et al. (2015) (32, 33, 34, 35, 36). The variation in the prevalence may be due to geographical variability, period of specimen collection and management of diabetic patients.

It is noteworthy in this study that *C. glabrata* was the most prevalent aetiologic agent (58.8%), followed by *C. albicans* (23.5%). A similar finding was reported by Peer et al.. (37). Increase in the frequency of other *Candida spp* may be as a result of increased use of immunosuppressive agents, not identifying *Candida* to species level, not performing antifungal susceptibility testing and purchasing of drug over the counter without prescriptions.

A high proportion of genitourinary candidiasis was observed in women 27(79.4%) than in men 7(20.6%) in this present study and this is in agreement with studies conducted elsewhere (38, 39) as this may due to ovarian activity (40). During the period, of ovarian activity, the ovary produce adequate amount of estrogen which favors the *Candida* growth by maintains the acidic pH and enhancing the yeast adherence to vaginal epithelial cells (30). However, the high prevalence in women may indicate the presence of genitourinary candidiasis or just

colonization since *Candida* is a normal flora of the genitourinary tract in women.

Furthermore, from the urogenital samples collected, it was shown in this study that increased of fungal urinary tract and genital infection have been associated with diabetes and that patients with diabetes often have increased complication of urogenital infection including rare complication such as emphysematous, cystitis, pyelonephritis.

In this present study, genitourinary Candidiasis was found mostly in the age group 31- 45, followed by 46- 60.

Asymptomatic genitourinary carriages of *Candida* species, as well as incidence of symptomatic infection have been demonstrated in diabetic mellitus individuals. In this study, the prevalence of asymptomatic and symptomatic genitourinary candidiasis was 8(23.5%) and 26(76.5%) respectively. *Candida* isolates were more common from the genitourinary tract of symptomatic group. This finding may have important implications for treatment in that *Candida* species other than C. *albicans* are inherently less susceptible to conventional antifungal therapy (59). Regarding *Candida* infections, the mere presence of asymptomatic genitourinary candidiasis usually does not require specific treatment (38). Correcting risk factors, such as glycemic control and removal of urinary catheters, can result in remission of this infection and symptomatic genitourinary candidiasis is an indication for treatment and may signal diverse pathological states (42)

Finally, predisposition to fungal genital and urinary tract infection in type 1 diabetic mellitus could be tailored to environmental factors such as infection and diet interacting with a genetically susceptible person (2). Type 2 diabetic mellitus results from several factors such as glycosuria, adherence of *Candida* to the uro-epithelium and immune dysfunction. Suzanne and Vivian, (2014) reported that studies have shown that pharmacologically induced glycosuria with SGLT2 inhibitors raises the risk of developing genital infections and to a relatively lesser extent in urinary tract infections (43).

Conclusions

Both *Candida albicans* and non-*albicans* species were identified in the genitourinary tract of diabetes patients. The presence of genitourinary candidiasis in diabetic patients should not be neglected as non- albicans species of *Candida* predominated over *Candida albicans* as key opportunistic pathogens. These non- *C. albicans* species are responsible for re-occurrence, thus, early and specific diagnosis of genitourinary candidiasis is crucial for effective treatment and Phenotypic methods can be used as rapid identification of *Candida* species.

Recommendations

It is noteworthy that conventional methods which include macroscopic, microscopic characteristics, culture and biochemical profile by API system of identification (Gold standard) facilitate the diagnosis with efficiency and accuracy, should be possible simultaneously, so that identification will be corrected and accomplished thereby selection of appropriate antifungal agents or prophylaxis become ease. It will also make clinicians to have confidence in the accuracy and quality of laboratory test results thereby prescribing the exact (appropriate) antifungal agents which will reduce the economic burden of the society, which will also contributes to the reduction of emergence of resistant *Candida* species.

It is also crucial that health education should be held at different clinic because it will create more awareness and correct their perception towards the need for its prompt treatment.

School health care services should be encouraged so that health personnel can assess the health needs of students.
There should be proper control of blood glucose in diabetes patients, children to be well fed and screening of blood before transfusion, proper sanitation of the environment and hospital.
People should undergo proper hygiene and stop self – treatment as this will reduce re-occurrence of the disease.
To avoid resistant to antifungal treatment or clinical failure, speciation and antifungal resistance tests are needed to guide treatment options.
There should be training of health personnel on mycology, proper supply of reagents and equipment that can make detection and rapid presumptive identification of yeast species.

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