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Identification and Antifungal Resistance among *Candida* species from the Genitourinary Tract

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Abstract

Background: *Candida* species are reportedly the most common human fungal pathogens. The incidence of urinary tract infections (UTIs) caused by *Candida* pathogens has increased in recent decades. However, such infections rarely occur in the absence of any predisposing factors.

Aim: The aim of the present study was to identify the *Candida* species causing UTIs and to determine the antifungal resistance among *Candida* species from the genitourinary tracts.

Methods: Five hundred (500) midstream urine samples were collected between January 2021 to January, 2022 from male and female patients clinically diagnosed of genitourinary tract infection and inoculated onto Sabouraud dextrose agar (SDA). Isolates from SDA were plated on CHROMagar to ensure detection of mixed cultures. Germ tube and carbohydrate assimilation tests performed were necessary for isolate identification. Susceptibility testing was carried on the isolates using broth dilution method.

Results: Distribution of *Candida* species among different age groups showed the highest incidence in age brackets 30-45, followed by 45-60, while the ages of 0-15 had the least. The occurrence rate of *Candida* species were as follows: *Candida* albicans 173(65.5%), *Candida* glabrata 61(23.1%), *Candida* krusei 19(7.2%) and *Candida* tropicalis 11(4.2%). High rate of susceptibility was observed for each isolate against fluconazole (92.0%) and ketoconazole (93.9%). The resistance rate was low for fluconazole (8.0%) and ketoconazole (1.2%).

Conclusions: These results incriminated C. albicans as the most common *Candida* species causing genitourinary tract infection in women. This surveillance study has established fluconazole and ketoconazole as very effective antifungal agents for the treatment of genitourinanry tract infections caused by *Candida* species.

Keywords: Urinary Tract Infections; *Candida albicans*; *Candida glabrata*; Fluconazole; Ketoconazole

Abbreviations: RPMI: Roswell Park Memorial Institute; GTT: Germ Tube Test; SDA: Sabourand Dextrose Agar; ICU: Intensive Care Unit; UTI: Urinary Tract Infections.

Introduction

In recent decades, *Candida* species, which are known as opportunistic pathogens, have been reported as the fourth leading cause of bloodstream infections in hospitalized patients [1]. Candiduria is defined as the presence of yeast in urine samples that indicates sample contamination, colonization of *Candida*, or urinary tract infections (UTI), such as disseminated candidiasis [2]. Candiduria is confirmed when 104-105 CFU/ml (colony forming unit/ml of urine) of *Candida* is detected in urine; however, *Candida*-associated UTI is mostly determined by >105 CFU/ml and generally related to the symptoms of the patient [3]. Among *Candida* species, *Candida albicans* has been reported as the most common cause of candiduria. Nevertheless, an increase in the rate of non-albicans species such as *Candida glabrata, Candida parapsilosis, Candida tropicalis*, *Candida kefir*, *Candida lusitanae*, *Candida guilhermondi*, and *Candida dubliniensis* has been reported during the last decades [4-6].

There is some evidence indicating that *Candida auris*, emerging multidrug-resistant yeast was recently isolated from the urine of a hospitalized patient with candidemia [7]. Therefore, accurate identification of species is very important for proper treatment. For example, some *Candida* species including *Candida krusei* and *C. glabrata* show intrinsic resistance to fluconazole. Predisposing factors of candiduria and *Candida* UTI include old age, female sex, diabetes mellitus, long hospital stay, admission to intensive care unit (ICU), using broad-spectrum antibiotics, immunosuppressive therapy, radiation therapy, genitourinary tuberculosis, neutropenia, urinary tract instrumentation, renal defect, transplantation, abnormalities of the urinary tract, and catheterization [8,9]. The incidence of candiduria caused by *Candida* spp. has increased in recent years, particularly in hospitalized patients. Depending on the clinical conditions and underlying diseases, the infection should be treated with effective antifungal agents [2,10]. More than 20% of hospitalized patients admitted to the ICU may develop candiduria following invasive therapeutic and diagnostic procedures [11,12]. Many studies demonstrated that candiduria in critically-ill ICU patients is a sign of severe colonization in the patients [13]. Recently, *Candida* has been reported as the most common nosocomial pathogen isolated from the urogenital tract of ICU patients [14]. The prevalence of candiduria in ICU patients was reported to be 19-44% [15]. In a study in Spain, 22% of patients who stayed more than seven days in ICU developed candiduria. Approximately one third of ICU patients with a positive *Candida* culture had a urinary catheter. It has been also reported that ICU patients who receive four different antibiotics have 35% increased risk of developing candidiasis. If *Candida* is isolated from clinical specimens such as urine, the risk increases to 80% [16].

Candiduria can sometimes lead to systemic infection and candidiasis. Candidemia following candiduria that is associated with high morbidity and mortality [17]. Most UTIs are caused by bacterial agents and *Candida* is often ignored, while increasing evidence suggest the increased rate of UTI cases caused by *Candida* species, especially in critically-ill patients [18,19]. The present study aimed at molecular identification of *Candida* species isolated from hospitalized patients with candiduria.

Materials and Methods

Study Design

This cross-sectional study was conducted at Abia State University Teaching Hospital Aba from January 2021 to January 2022.

Collection of Samples

The sample comprised of 500 midstream urine specimens collected from men and women clinically diagnosed of genitourinary tract infections. Midstream urine specimens were obtained after instructing patients on how to collect the sample to eliminate contamination. The specimens were immediately transferred to laboratory for analysis.

Ethical Clearance

Ethical permission was obtained from the hospital authorities and the consent of the patients was also obtained before specimen collection.

Eligibility Criteria

Inclusion Criteria

- Indoor and outdoor patients with signs and symptoms of urinary tract infection like bladder discomfort, frequency, painful or difficulty in micturition and fever were included in the study [20].
- Pure growth of yeast isolates having significant colony count >10^3CFU/ml
- Willingness to participate in the study.

Exclusion Criteria

- Sample showing mixed growth of microorganisms on blood agar and MacConkey agar was excluded from the study [20].
- A colony count less than 10^3 CFU/ml was excluded.

- Unwillingness to participate in the study.

Culture Procedure

Samples were cultured on Sabourand dextrose agar (SDA), (Lab.M) at 37°C. Inoculated plates were examined after 48 h incubation. Isolates from SDA were plated on CHROMagar (France) to ensure detection of mixed cultures. Cultures were incubated at 37°C for 72 h. Identification of *Candida* species were based on colony morphology and pigmentations on the CHROMagar.

Germ Tube Test (GTT)

This was done according to the method of Odabasi Z, et al. [8]. Yeast isolates suspected to be *C. albicans* were inoculated into human serum, incubated for about 30 min at 37°C and examined microscopically for the production of germ tubes.

Sugar Assimilation Test

All isolates which could not be identified using CHROMagar and Germ tube test were subjected to sugar assimilation test as described by Odabasi Z, et al. [8]. Yeast was grown on a basal carbohydrate free medium supplemented with the test sugar. These were incubated at 30°C for 18 h. Opacity in the medium indicates the ability of the isolate to assimilate a sugar.

Antifungal Susceptibility Test

Susceptibility testing was carried on the banked isolates using broth microdilution method of Odabasi Z, et al. [8] and based on the approved National Committee for Clinical Laboratory Standards guidelines for a broth microdilution reference method, [21].

Seven different concentrations of each drug were tested as follows; Fluconazole (0.10, 0.50, 1.0, 5.0, 10.0, 50.0, 100) ug/ml and ketoconazole (0.01, 0.05, 0.10, 0.50, 1.0, 5.0, 10) ug/ml. 0.1 ml yeast inoculum from Roswell Park Memorial Institute (RPMI) 1640 medium visually matched to 0.5 McFarland and incubated at 35°C for 48 h were added to each microdilution well. The trays were incubated at 35°C for 48 h.

A numerical score from 0 to 4 were assigned to each set of well using the following scale: 0 = optically clear, 1 = slightly hazy, 2 = Prominent reduction in turbidity, 3 = Slight reduction in turbidity, 4 = No reduction in turbidity. Scores 0-2 was regarded as sensitive while scores 3 and 4 were said to be resistant.

The MIC was regarded as the lowest antifungal concentration with substantially lower turbidity compared to growth in the antifungal free growth control well. A susceptible interpretation was given to any strain for which the MIC of fluconazole was ≤10 ug/ml, and ketoconazole ≤ 5ug/ml [21].

Statistical Analysis

The Chi-square Test was used to test the occurrence of *Candida* species as well as the significance of antifungal resistance among the yeast isolates.

Table 1 shows the Sociodemographic Characteristics of patients. Out of a total of 500 urine samples, 264 yielded growth of fungi isolates There was no statistical significance of fungi infection in relation to gender (p = 0.183) and educational status (p = 0.067). Age and Occupation were significantly associated with fungi infection (p = 0.031) and (p = 0.014) respectively.

Characteristics	Total Tested (%)	Number Positive (%)	χ2	df	P- Value
Gender					
Male	262(52.4)	145(55.3)	4.612	1	0.183
Female	238(47.6)	119(50.0)			
Total	**500(100)**	**264(52.8)**			
Age in Years					
0 -15	71(14.2)	25(35.2)	15.142	6	0.031
15-30	99(19.8)	36(36.4)			
30-45	122(24.4)	94(77.0)			
45-60	118(23.6)	65(55.1)			
>60	90(18.0)	44(48.9)			
Total	**500**	**264(52.8)**			

Occupation					
Schooling	68(13.6)	35(51.5)	11.732	5	0.014
Farming	90(18.0)	56(62.2)			
Civil Servants	80(16.0)	24(30.0)			
Trading	76(15.2)	31(40.8)			
Artisans	59(11.8)	26(44.1)			
Metal mining	62(12.4)	44(71.0)			
Stone Quarrying	65(13.0)	48(73.8)			
Total	**500(100)**	**264(52.8)**			
Educational Status					
Tertiary Education	121(24.2)	61(50.4)	6.101	2	0.067
Secondary Education	128(25.6)	77(60.2)			
Primary Education	117(23.4)	44(37.6)			
None/Illiterate	134(26.8)	82(61.2)			
Total	**500(100)**	264(52.8)			

Table 1: Sociodemographic Characteristics of patients.

Table 2 shows the Prevalence rate of *Candida* species. *C. albicans* had the highest prevalence of 173(65.5%) while c. tropicalis had the lowest prevalence of 11(4.2%).

Candida species	No (%)	Colony color	Germ Tubes	Beta-glucosidase
Candida tropicalis	11(4.2)	Blue purple with a halo around	-	-
Candida glabrata	61(23.1)	Dark pink	-	-
Candida albicans	173(65.5)	Green or light green	+	+
Candida krusei	19(7.2)	Cream	-	-

Table 2: Prevalence rate of *Candida* species.

Table 3 shows the summary of MIC of isolates against different concentrations (0.1-100g/ml) of fluconazole. With reference to the NCCLS standards, isolates giving clarity of growth (optical clarity) at concentrations ≤10 µg/ml were regarded as susceptible while those giving such clarity at concentrations >10 g/ml were regarded as resistant. 163 (94.2%) *Candida albicans* isolates gave optical clarity at lower concentrations ≤ 10 g/ml and were regarded as susceptible. The remaining 10(5.8%) *C. albicans* isolates which gave optical clarity at higher concentrations >10 g/ml were regarded as resistant. *Candida tropicalis*, was 100% susceptible to fluconazole since all the isolates showed optical clarity at concentrations ≤ 10 g/ml. High resistant rate (100%) was recorded for *Candida krusei* since all the19 isolates had their optical clarity at concentrations >10 g/ml.

Fluconazole _g/ml	*C. albicans* No (%)	*C. glabrata* No (%)	*C. krusei* No (%)	*C. tropicalis* No (%)
0.1	47(27.2)	28(46.0)	Nil	Nil
0.5	65(37.6)	10(1.6)	Nil	Nil
1	28(16.2)	18(30.0)	Nil	11(100)
5	14(8.1)	5(8.2)	Nil	Nil
10	9(5.3)	Nil	Nil	Nil
50	7(4.0)	Nil	6(31.6)	Nil
100	3(1.7)	Nil	13(68.4)	Nil
Total	173(100)	61(100)	19(100)	11(100)

Nil = No organism was tested at that concentration.

Table 3: Susceptibility of *Candida* species to fluconazole (%).

Table 4 shows the summary of the MIC of isolates against different concentrations of ketoconazole. According to NCCLS standards, optical clarity at concentrations ≤5µg/ml was regarded as susceptible while optical clarity at concentrations >5µg/ml was regarded as resistant. High rate of susceptibility was recorded for *C. albicans* (83.3%), *C. tropicalis* (100%) and *C. glabrata* (95.1%); since all their isolates gave optical clarity at lower concentrations (≤5 µg/ml). *C. krusei* showed moderate resistance since only 47.3% of its isolates gave optical clarity at higher concentrations (>5µg/ml).

Fluconazole _g/ml	*C. albicans* No (%)	*C. glabrata* No (%)	*C. krusei* No (%)	*C. tropicalis* No (%)
0.01	61(35.3)	8(13.1)	Nil	11(100)
0.05	31(17.9)	3 (4.9)	Nil	Nil
0.1	42(24.3)	15 (24.6)	3 (15.8)	Nil
0.5	10 (5.8)	32 (52.5)	7 (36.8)	Nil
1	15 (8.7)	Nil	Nil	Nil
5	7 (4.0)	Nil	7 (36.8)	Nil
10	7 (4.0)	3(4.9)	2 (10.5)	Nil
Total	173(100)	61 (100)	19 (100)	11(100)

Nil = No organism was tested at that concentration

Table 4: Susceptibility of *Candida* species to Ketoconazole (%).

Table 5 shows Susceptibility of isolates to fluconazole and ketoconazole. Both antifungals (fuconazole and ketoconazole) showed high susceptibility (94.2 and 96.5% respectively) against *C. albicans* isolates. Their resistance rates (5.8 and 3.5% respectively) were however quite low. Antifungal resistance in non-albicans species was observed in case of *C. krusei* where high (100%) and moderate (47.4%) resistance rates were recorded against both antifungals. There was no significant difference between the activities of both drugs at > 0.05.

Isolates	Fluconazole		Ketoconazole	
	No. R (%)	No. S (%)	No. R (%)	No. S (%)
Candida albicans	10 (5.8)	163 (94.2)	6 (3.5)	167 (96.5)
Candida glabrata	0	61 (100)	1 (1.6)	60 (98.4)
Candida krusei	19 (100)	0	9 (47.4)	10 (52.6)
Candida tropicalis	0	11 (100)	0	11 (100)
Total	29 (8.0)	235 (92.0)	16 (1.2)	248 (93.9)

R = resistance; S = susceptibility.

Table 5: Susceptibility of isolates to fluconazole and ketoconazole (%).

Discussion

The observation in this study that *C. albicans* had the highest incidence rate (65.5%) among the yeast isolates studied is in agreement with the reports of other workers [20,21]. Richter SS, et al. [20] reported a 76% incidence rate among his yeast isolates. Tatfeng YM, et al. [21] reported *C. albicans* to be the most incriminated yeast isolate in urinary tract infections. This finding however contradicted the earlier report of Okungbowa FI, et al. [22] who reported *C. glabrata* as the most common *Candida* species among symptomatic individuals in Nigerian cities. Also in this study, an incidence rate of 34.5% was observed for non-albicans species. Reports from other work showed similar observation [23]. This variation in reports may be attributed to the period of specimen collection and differences in population types.

In addition, candiduria showed a significant relationship with age and gender. Out of 264 subjects with *Candida* infection, 145(55.3%) of the cases were male. A higher frequency of *Candida* species (77%) within age bracket 30-45 years as observed in this study is in agreement with report of other work [22]. 35% incidence rate was reported within age group 26-36 years in Benin City [22]. These reports points to this age group as a vulnerable group probably due to sexual promiscuity, drug abuse and use of contraceptives. Also, aging is accompanied by the appearance of glucose in the urine. The elevation of urine glucose to more than 150

mg/dl sets the ground for the growth of *Candida* strains [19].

The high fluconazole susceptibility rate (94.4%) in *C. albicans* found in this study is consistent with other reports. No fluconazole resistance was reported among yeast isolates in earlier works on vulvovaginitis conducted in the U.S and Brazil [24,25].

The low fluconazole-resistance rate (5.6%) in *C. albicans* found in this study is consistent with other research findings. A U.S. Study reported fluconazole resistance in 3.6% *C. albicans* isolates [24]. A 2.1% *C. albicans* resistance rate was reported in New York [26]. Azole resistant candidiasis appears to be on the increase, and the reasons for resistance may include incomplete therapy, overgrowth of resistant strains, and induction of drug resistance in the particular species, colonization and subsequent infection with a resistant organism [27]. *C. krusei* is naturally resistant to fluconazole even at high doses [28]. In this study, a 100% resistance rate was observed for *C. krusei,* which is consistent with research reports. The second azole antifungal studied in this work was ketoconazole. Similar susceptibility pattern was observed in this drug as in fluconazole. The 93.9% susceptibility and 1.2% resistance observed for ketoconazole were also consistent with previous research works. The similarity in the activity of these two anti-fungals shows that they both belong to same azole antifungal, the imidazoles

The research findings of this study, support previous observations that clinical *Candida* species and related yeast infections are increasing and that the widespread use of imidazoles (such as fluconazole and ketoconazole) appears to be associated with emerging resistance to these important antifungal agents in yeasts. As a consequence, *in vitro* testing of the susceptibility of yeasts to antifungal agents will likely play an ever-increasing role in the appropriate selection of antifungal agents for the treatment of fungal infections. Nonetheless, the high susceptibility rate of *Candida* species to azole drugs as observed in this work supports the continued use of azole antifungals for the treatment of genitourinary tract infections among women.

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