

**ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA SPECIES CAUSING
VULVOVAGINAL CANDIDIASIS IN JOS, NIGERIA**Essien Unyime Cosmas¹, Kim Esther Garos², Okolo Mark Ojogba² and Idoko Emmanuel³¹Department of Medical Laboratory Science, Faculty of Medical Sciences, University of Jos.²Department of Medical Microbiology, Faculty of Medical Sciences, University of Jos.³National Hospital Abuja P.M.B 425 Garki-Abuja.***Corresponding Author: Essien Unyime Cosmas**

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ABSTRACT

This study was designed to identify, characterized and determine antifungal sensitivity pattern of *Candida* species from vaginal specimens. A total of 139 *Candida* isolates were obtained from specimens submitted to microbiology laboratory of two tertiary hospitals in Jos metropolis. Samples were processed using standard methods for *Candida* isolation. *Candida* speciations were performed by germ tube test and *Candida* CHROMagar medium. Antifungal sensitivity pattern was performed on Mueller Hinton Agar (MHA) supplemented with 2% glucose and 0.5 µg/ml methylene blue dye by disc diffusion method. Out of 139 *Candida* isolated 69 (49.6%) were *Candida albicans*, 61 (43.9%) were *Candida krusei*, 3 (2.2%) were *Candida tropicalis*, 2(1.4%) *Candida dubliniensis* and 4 (2.9%) were unidentified *Candida* species. Antifungal sensitivity pattern reveals Ketoconazole as the most active agent 111/139 (79.9%) against *Candida* isolates. Conversely, the highest resistance was recorded in Griseofulvin 0 (0.0%). The present study has shown that specie level identification of *Candida* isolates should be encouraged in view of the rising spate of antimicrobial resistance to fungal agents.

KEYWORDS: *Candida* species, Ketoconazole, Antifungal sensitivity.**INTRODUCTION**

Candidiasis is a term used to describe the overgrowth of *Candida* species. It is responsible for 90% of the cases of vaginitis (Adad *et al.*, 2001). Candidiasis is caused by yeast of the genus *Candida*. The genus *Candida* is composed of a group of heterogeneous organisms with more than 68 different *Candida species* (Soll, 2002). Although *Candida albicans* is the most prevalent species involve in infections, the trend towards non-*albicans* *Candida* (NAC) species has been documented in recent studies (Enoch *et al.*, 2006; Deorukhkar and Saini 2012b).

Surprisingly, the clinical manifestation caused by NAC species are indistinguishable from those caused by *Candida albicans* but they differ in their susceptibility to antifungal agents and often show high resistance to commonly used antifungal drugs (Enoch *et al.*, 2006). The most common antifungal agents in use are the polyenes and the azoles. The polyenes exert their activity by binding to ergosterol (fungal membrane sterol) resulting in an increased permeability to the cell wall and eventual cell death (Farooqi *et al.*, 2013). The azoles function by inhibition of the cytochrome P-450-mediated

removal of the C-14 methyl group from the ergosterol precursor, lanosterol (Farooqi *et al.*, 2013).

The polyenes include amphotericin B, nystatin and natamycin (Karaca and Koc, 2004). The azole derivatives include clotrimazole, econazole, ketoconazole, fluconazole and itraconazole of which fluconazole and itraconazole are members of the triazoles with 3 nitrogen molecules, whereas clotrimazole, miconazole and ketoconazole are imidazoles with 2 nitrogen molecules (Karaca and Koc, 2004).

Griseofulvin was reported to be active against dermatophytes (Karaca and Koc, 2004).

Prolonged usage of antifungals in treating infections caused by *Candida species* has led to the emergence of azole resistance. *Candida* species have evolved a multitude of mechanisms to survive exposure to antifungal drugs and some of which includes mutations. (White *et al.*, 2002).

However, accurate speciation of *Candida* with antifungal susceptibility testing has becomes very essential for

treatment and reduction in the spate of antifungal resistance.

The present study was designed to determine the sensitivity pattern of *Candida species* isolated from vaginal specimens in Jos, Plateau State Nigeria.

MATERIALS AND METHODS

Study area/population

The study was conducted in Jos, the capital city of Plateau State, Nigeria. The city of Jos is the largest settlement in Plateau State with a population of over one million people. Women within the age group of 15 to 45 years formed the population of this study.

Ethical clearance

Ethical clearances for the study were sought and obtained from the following health institutions: Plateau specialist hospital and Jos University Teaching Hospital all located in Jos.

Sample collection

A total of 139 *Candida* isolates were obtained from vaginal swab specimens submitted to Microbiology Laboratory of Jos University Teaching Hospital and Plateau Specialist Hospital.

Isolation and characterization of *Candida*

Swab specimens were streak-inoculated on Sabouraud dextrose agar (SDA) and the plates were incubated for 24-48 hours at 37°C. Plates having no yeast growth were further incubated for 72 hours. Colonies of *Candida species* were presumptively identified by the creamy, smooth, pasty and convex appearance. Wet smears preparation and direct gram were also performed on swab specimens after inoculation. Presence of pseudohyphae, budding cells and gram positive budding cells also further confirm the presence of *Candida species*.

Germ tube test

A suspension of pure *Candida* isolate was made by inoculating a test tube containing 0.5 ml of human serum with a loopful of the organism. It was incubated in a water bath for 2-4 hours at 37°C. After incubation, a wet preparation was made by transferring an aliquot of the suspension onto a clean glass slide and cover with coverslip. This was examined using a x10 and x40 objectives respectively. The presence of elongated daughter cells from the mother cells without constriction at their origin is referred to as a germ tube, while those cells with constriction at the origin of mother cells were noted as pseudohyphae (Kim *et al.*, 2002). Germ tube and pseudohyphae were positive indication for *Candida albicans* (R. Rajendran, 2014).

Identification of *Candida species* using CHROM agar medium

Further identification was performed by streak-inoculating pure *Candida* isolates from SDA onto a

Candida CHROMagar. This was incubated at 37°C for 48 hours, identification of *Candida species* was based on the different colour changes observed on the medium. Briefly, this method is based on the enzymatic activity of *Candida* isolates on chromogenic substrate present in the medium (Devi and Maheshwari, 2014).

Preparation of standardized *Candida* inoculum

The Clinical and Laboratory Standards Institute guidelines (CLSI, 2004) were used to prepare BaSO₄ turbidity standard (0.5 McFarland standard). Briefly, 99.5 mL of solution A (1% v/v H₂SO₄) was added to 0.5 mL of solution B (1.17% w/v BaCl₂ · 2H₂O) with constant stirring. Using matched cuvette with a 1.0 cm light path, the OD_(625nm) was measured on the spectrophotometer. The 0.5 McFarland standard was distributed into disposable screw-capped universal bottle. From SDA plate, a discrete colony of test organisms were suspended in sterile distilled water and was agitated briefly to homogenize. The yeast density which gave an OD_(625nm) equivalent to that of 0.5 McFarland standard is referred to as the standardized inoculum.

Antifungal sensitivity testing (Disc diffusion method)

Mueller Hinton Agar (MHA) supplemented with 2% glucose and 0.5 µg/ml methylene blue dye was used to perform antifungal sensitivity testing. The agar surface was inoculated by using a sterile swab dipped in a standardized *Candida* cell suspension. Antifungal discs were dispensed on dried surface of Mueller Hinton glucose methylene blue agar and the plates were incubated aerobically at 35°C and read after 24 hours. Zone diameter were read manually using ruler and interpreted according to CLSI interpretative break point (CLSI, 2004). Antifungal susceptibility testing was performed for the following antifungal: Amphotericin B, Econazole, Fluconazole, Griseofulvin, Itraconazole, Ketoconazole and Miconazole.

Data analysis

Data obtained from the study were analyzed using SPSS version 21 computer package. P-value less than 0.05 were considered statistically significant.

RESULT

Of the 139 *Candida* isolates studied, *Candida albicans* was found to be the most predominant species (69 isolates, 49.6%), followed by *Candida krusei* (61 isolates, 43.9%), *Candida tropicalis* (3 isolates, 2.2%), *Candida dubliniensis* (2 isolates, 1.4%) and unidentified *Candida species* was (4 isolates, 2.9%) as shown in figure 1.

The characterization of *Candida species* by *Candida* Chrom agar, germ tube test and growth at 37°C is presented in Table 1. *Candida albicans* was identified as (green colonies, germ tube positive, and growth at 37°C), *Candida krusei* (Pink colonies, germ tube negative and no growth at 37°C), *Candida tropicalis* (Blue colonies, germ tube negative and no growth at 37°C) and *Candida*

dubliniensis (green colonies, germ tube positive and no growth at 37⁰C).

Table 2. Shows the sensitivity pattern of different antifungal agents used, of the 139 *Candida* isolates tested (111 isolates, 79.9%) were sensitive to ketoconazole, (91 isolates, 65.5%) were sensitive to Miconazole, (83 isolates, 59.7%) were sensitive to Econazole, (52 isolates, 37.4%) were sensitive to Fluconazole, (4 isolates, 2.9%) were sensitive to Itraconazole, (1 isolate, 0.7%) was sensitive to Amphotericin B while the highest resistance was recorded in griseofulvin (None, 0.0%).

Regarding *Candida albicans* (n=69) (54 isolates, 78.3%) were susceptible to Ketoconazole, (40 isolates, 57.9%) susceptible to Miconazole, (29 isolates, 42.0%) susceptible to Fluconazole, (39 isolates, 56.5%) susceptible to Econazole, (3 isolates, 4.4%) susceptible to Itraconazole and (1 isolate 1.5%) was susceptible to Amphotericin B.

Of the 61 isolates of *Candida krusei*, (49 isolates, 80.3%) were sensitive to Ketoconazole, (48 isolates, 78.7%) were sensitive to Miconazole, (42 isolates, 68.9%) were sensitive to Econazole, (15 isolates, 24.6%) were sensitive to Fluconazole and (None, 0.0%) was sensitive to Amphotericin B, Griseofulvin, and Itraconazole.

All the three (3) isolates of *Candida tropicalis* showed (100%) sensitivity to Fluconazole and Ketoconazole.

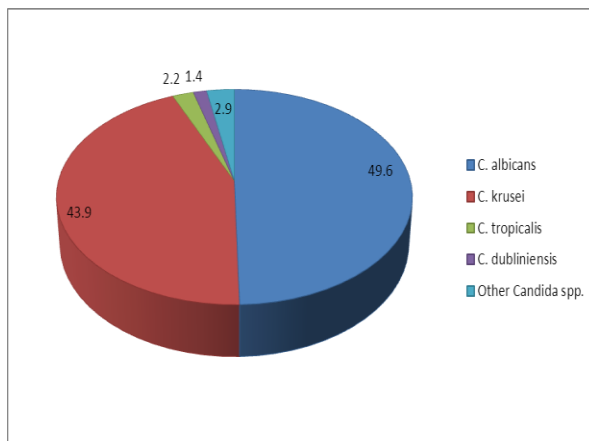


Figure 1: Prevalence of vaginal *Candida* isolates.

Table 1: Characterization of vaginal *Candida* isolates.

<i>Candida</i> species	Colony on Chrom agar	Germ tube	Growth at 45 ⁰ C
<i>Candida albicans</i>	Light green	+	+
<i>Candida krusei</i>	Pink	-	-
<i>Candida dubliniensis</i>	Green	+	-
<i>Candida tropicalis</i>	Dark blue	-	-

Table 2. Antifungal susceptibility pattern of Vaginal *Candida* isolates.

<i>Candida</i> isolates	No. of isolates	AMP	ECN	FLN	GF	ITN	KTN	MCN
<i>Candida albicans</i>	69	1(1.5)	39(56.5)	29(42.0)	0(0.0)	3(4.4)	54(78.3)	40(57.9)
<i>Candida krusei</i>	61	0(0.0)	42(68.9)	15(24.6)	0(0.0)	0(0.0)	49(80.3)	48(78.7)
<i>Candida tropicalis</i>	3	0(0.0)	0(0.0)	3(100.0)	0(0.0)	0(0.0)	3(100.0)	0(0.0)
<i>Candida dubliniensis</i>	2	0(0.0)	1(50.0)	2(100.0)	0(0.0)	1(50.0)	2(100.0)	1(50.0)
Other <i>Candida</i> species	4	0(0.0)	1(25.0)	3(75.0)	0(0.0)	0(0.0)	3(75.0)	2(50.0)
Total	139	1(0.7)	83(59.7)	52(37.4)	0(0.0)	4(2.9)	111(79.9)	91(65.5)



Figure 2: Mueller Hinton glucose methylene blue agar plate showing susceptibility to four antifungal agents.



Figure 3: Colonies of *C. albicans* (Green) and *C. krusei* (Pink) on *Candida* chrome agar.

DISCUSSION

Candida is the most common cause of vaginitis (Adad *et al.*, 2001). The genus *Candida* consists of over 68 different *Candida* species, with *Candida albicans* being the most prevalent (Deorukhkar and Saini 2012b).

In the present study we investigated the distribution of *Candida* species and determine invitro antifungal sensitivity of 139 isolates of *Candida* obtained from vaginal swabs. *C. albicans* was the predominant species isolated in our study, which is in tandem with some reports from studies conducted elsewhere (Hazen *et al.*, 2003; Akortha *et al.*, 2009 ; Nelson *et al.*, 2013, Taura *et al.*, 2013; Yang *et al.*, 2013). Moreover, *Candida krusei* was the most prevalent non albicans *Candida*(NAC), this result is contrary to the findings of Akortha *et al.*, (2009) South South Nigeria, Zhang *et al.*, (2015) from China, Nelson *et al.*, 2013 from Kenya who reported *Candida glabrata* as the predominant NAC isolates. However, similar study in Pakistan revealed *C. tropicalis* as the most prevalent NAC (Khan and Baqai, 2010). In this study, *Candida albicans* was differentiated from *Candida dubliniensis* by the ability of the former to grow at 45°C. The two *Candida dubliniensis* studied showed (100%) sensitivity to fluconazole. However, Ketoconazole was the most effective drugs against *Candida albicans* and non albicans species. This is in consonant with report of some studies elsewhere (Nawrat *et al.*, 2007; EL-sayed and Hamouda, 2007; Dias *et al.*, 2011). Although it contradicts the report of Suneel *et al.*, (2015) in India who found Amphotericin B and Voriconazole to be the most active antifungal drugs. The highest ketoconazole susceptibility rate 54 (78.3%) to *C. albicans* found in this study is consistent with other reports. This is result is similar to the report of Ungureanu *et al.*, 2016 who recorded (100%) sensitivity to Ketoconazole.

This finding however contradicted the earlier report of Akortha *et al.*, (2009) who reported highest sensitivity 132 (95.75) in Fluconazole, Al-mama *et al.*, (2014), observed the highest sensitivity (89 of 93) in Miconazole and Babin *et al.*, (2013) noted (100%) sensitivity in Amphotericin B and Flucytosine. All the 139 *Candida* isolates tested showed (100%) resistance to Griseofulvin. This is in agreement with earlier study which found that Griseofulvin have no inhibitory action on yeasts (Karaca and Koc, 2004). The reason for this could be attributed to the fact that Griseofulvin is used in treating dermatophytes (Karaca and Koc, 2004).

Regarding *Candida krusei* (n=61), 15(24.6%) of isolates were sensitive to Fluconazole. The reason for the high resistance to fluconazole is that *C. krusei* isolates are inherently resistant to fluconazole (Trick *et al.*, 2002; Pfaller and Diekema, 2012).

CONCLUSION

Prior knowledge of species distribution in clinical isolates of *Candida* and drug sensitivity pattern will also

enable clinicians to choose appropriate antifungal agents. In conclusion, the present study showed that *Candida albicans* was the most commonly isolated yeast from vaginal specimens, also the increase in the resistance especially to azoles is a major concern.

Therefore, the species level identification of *Candida* isolates and its sensitivity profile should be encouraged.

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