CANDIDA SPECIES ASSOCIATED WITH GENITOURINARY TRACT INFECTIONS AT TWO HEALTH FACILITIES IN LAGOS, NIGERIA

***Temitope O. Samuel[1], Solayide A. Adesida[2], N. B. Ahmed[1], Deborah O. Akinyeye[2] and Rebecca F. Peters[3]**

[1]Mycology Laboratory, Department of Botany, University of Lagos, Nigeria.
[2]Department of Microbiology, University of Lagos, Nigeria.
[3]Department of Microbiology, Lagos University Teaching Hospital, Lagos, Nigeria.
Correspondence to: tosamuel@unilag.edu.ng; +2348036410449
DOI: https//orcid.org/0000-0003-2033-5206

ABSTRACT

Background: Genitourinary tract infections due to *Candida* species is one of the most frequently diagnosed infections in both community and hospital settings and poses a diagnostic and therapeutic challenge with the emergence of antifungal resistance and new pathogens.

Aim: This research aims to investigate the different *Candida* species associated with genitourinary tract candidiasis and examine their susceptibility to three commonly used antifungal agents.

Methods: Candida isolates from cases of genitourinary tract infections were collected using standard mycological methods. Differentiation of the species was achieved with Chromogenic agar. Susceptibility of the isolates to three antifungal agents was tested using disk diffusion method.

Results: Out of the 107 *Candida* isolates evaluated, Forty-one percent was *C. albicans* (44/107; 41%) followed by *C. tropicalis* (34/107; 31.8%). The frequency of genitourinary candidiasis due to non-albicans *Candida* species (63.3%) was higher than those assigned to *Candida albicans* (36.7%). Although, *C. parapsilosis* was limited to the tertiary-care center, a strain of *C. dubliniens*is was found in the private medical diagnostic facility. About 14% (15/107) of the isolates could not be discriminated against by Chromatic Candida agar.

Conclusion: The findings demonstrated heterogeneity in the distribution of species in the two facilities investigated. Although, nystatin was the most effective antifungal agent in this study, antifungal resistance to fluconazole and voriconazole seems to be a serious concern.

Keywords: Antifungal resistance, *Candida* species, Genito-urinary tract Infections, Phenotypic methods

INTRODUCTION

The genus *Candida* consists of typical yeast cells that are the normal microbiota of human oral mucosal cavity, digestive tract, and urinary tract. They are considered important pathogens due to their versatility and ability to survive in various anatomical sites (Sardi *et al.,* 2013). They are increasingly involved in hospital-acquired infections, sometimes causing outbreaks in healthcare settings (Bougnoux *et al.,* 2018). Invasive *Candida* infections are one of major causes of morbidity and mortality in immunocompromised as well as critically ill immunocompetent patients. *Candida albicans* is by far the most relevant species in human infections (Ksiezopolska and Gabaldón, 2018) and well documented as one of the leading causes of urinary tract infections in both men and women. But more recently, other species such as *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*, are becoming frequently associated with human infections (Kabir and Ahmad, 2012).

Citation: Temitope O. Samuel, Solayide A. Adesida, N. B. Ahmed, Deborah O. Akinyeye and Rebecca F. Peters (2021): *Candida* Species Associated With Genitourinary Tract Infections At Two Health Facilities In Lagos, Nigeria *BJMLS. 6(1): 1 -8*

Genitourinary tract infections due to *Candida* species may present as candiduria in males and females, balanoprosthitis and balanitis in males, and vulvovaginal candidiasis in females (Obisesan *et al*., 2015). Although, its incidence differs in relation to sex, host resistance, and healthcare facilities, the occurrence of genitourinary candidiasis is believed to be greater in women. Under, certain circumstances, *Candida* are capable of developing virulence factors that could make them pathogenic. The most severe candidiasis is usually acquired in health care settings (Aldardeer *et al*., 2020) and the source of the infection may be either endogenous or exogenous.

More critically, the emergence of resistance to several antifungal has become a prominent feature of the behaviour of *Candida* and a growing medical and economic problems in both developed and developing countries. Recent developments in antifungal resistance comprise the demonstration of increased azole resistance among non - albicans isolates and resistance to multiple classes of antifungal agents among emerging species such as *C. auris*. Consequently, information about the distribution and drug resistance patterns among *Candida* isolates is very essential for epidemiological surveillance, implementation of appropriate antifungal therapies and patient recovery. Africa and Abrante (2019) analyzed regional differences in the distribution of Candida species and trends of antifungal resistance in Africa. They concluded that high levels of *Candida* drug resistance are emerging in sub-Saharan Africa. In Nigeria, a number of studies have been done on the incidence and antimicrobial resistance patterns of *Candida* from a variety of clinical sources (Obisesan *et al*., 2015; Emeribe *et al*., 2015), but antifungal resistance and species distribution among genitourinary cases in Lagos remains poorly reported. Therefore, the objective of this study was to determine the occurrence of *Candida* species in individuals with symptomatic genito-urinary infections and to evaluate the antifungal susceptibility profile of the isolated species.

MATERIALS AND METHODS

Study Design

Candida isolates were collected from a private medical diagnostic centre and tertiary-care hospital within Lagos metropolis. Ethical approval was not required as isolates collected were stored as part of patient clinical care were analyzed. Isolates were from defined conditions including urinary catheterization. We selected the two health facilities because the centers are well equipped for mycological cultures and identification.

Collection of *Candida* Isolates

Unduplicated clinical isolates of *Candida* recovered from urine and high vaginal samples of patients with suspected genito-urinary infections were randomly collected. Preliminary analyses of the samples collected from patients were based on direct microscopy with 10% Potassium Hydroxide and growth on Saboraud Dextrose Agar (SDA) (Hi-Media, Indian) supplemented with chloramphenicol and incubated at 37^{O}C for 24–48 h. All isolates with budding morphology were subjected to Gram-staining and subcultured on SDA for 24h at 37^{0}C for purity purposes.

Germ tube test

The germ tube-producing capability of the isolates was carried out suspending about 2-3 colonies in 0.5ml of fresh human serum using an inoculating loop in a test tube. The isolates were incubated for 2-3 hours. A drop was transferred to a glass slide, covered with a cover glass and observed under a light microscope (X40 magnification). Growth of a small tube with straight walls, without septum and without constriction at a junction between cells suggests positive results (Marinho *et al*., 2010). *Candida albicans* ATCC 10231 and *C. dubliniensis* ATCC 44508 were used as reference strains.

Candida Species Differentiation

Chrom ID Candida agar

Chromogenic agar (Chromatic™ Candida, Liofilchem®) was used to speciate the organisms according to the manufacturer's recommendations. Chromogenic agar differentiates *Candida* spp. by colour and colony morphology. The high specificity and sensitivity for the identification of the most commonly encountered *Candida* spp. has been well documented (Ghaddar *et al*., 2020). Green colony was interpreted as *C. albicans*, blue colonies defined as *C. tropicalis*, pale pink to white colonies were *C. parapsilosis*, pink-coloured colonies with pale edges were identified as *C. krusei,* and beige colonies described *Candida glabrata* while the rest of species produced white colonies.

Antifungal Susceptibility Testing

The susceptibility of the identified *Candida* strains to three commonly used antifungal agents was determined by the disk diffusion method using Mueller–Hinton agar supplemented with 2% glucose and 5 µg/ml methylene blue as recommended by (Khadka *et al*., 2017). The surface of the agar was inoculated using a swab dipped in a turbidity-adjusted cell suspension of 0.5 McFarland standard. The antifungal disks were purchased from Oxoid (Oxoids, UK) and included nystatin (NS; 100 IU), voriconazole (VOR; 1 µg)) and fluconazole (FCA; 25 µg). Zone of inhibition around the disc was measured after incubating the media at 37 °C for 24 h. Resistance to the antifungal agents was determined according to breakpoints as described by other authors (Adesiji *et al*., 2011). Quality control was ensured using *Candida albicans* ATCC 10231 and *C. dubliniensis* ATCC 44508.

RESULTS

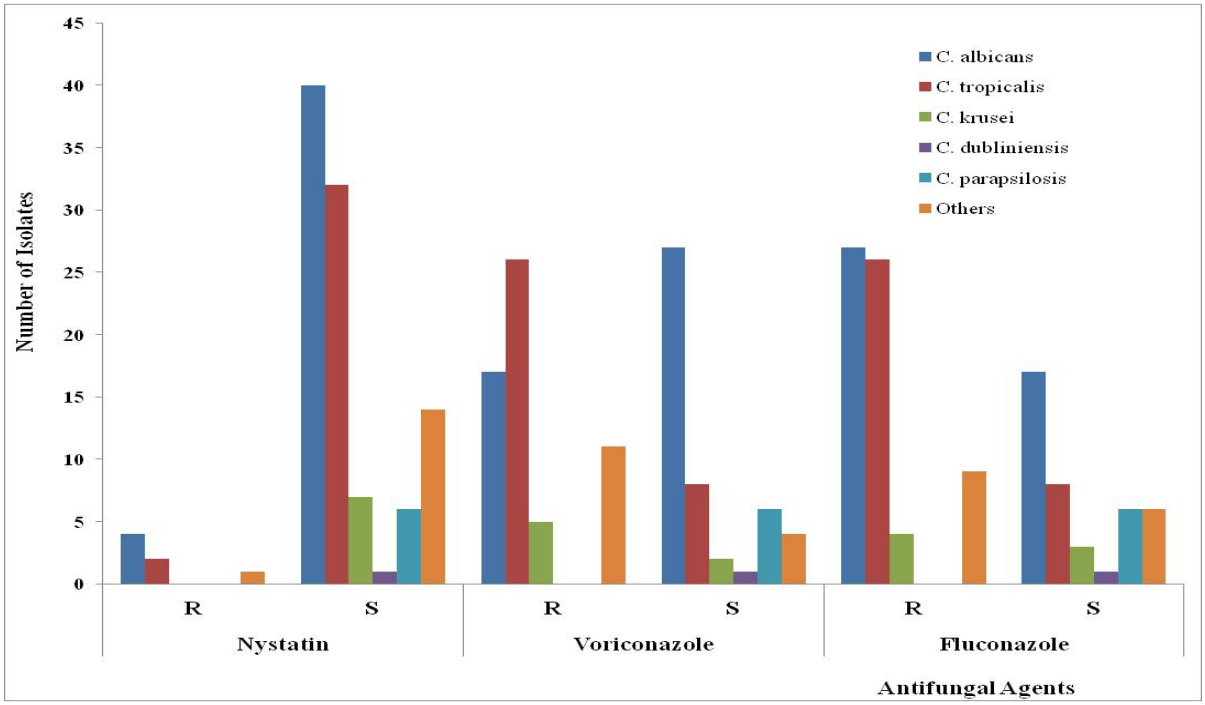
A total of 107 *Candida* isolates were evaluated, 44 from the private medical diagnostic centre and 63 from the tertiary-care hospital which include nineteen (17.8%; 19/107) urine, and 85.9% (92/107) HVS. Subsequently, most of the analyzed isolates, 96.3% (103/107), were from female patients. Majority of the isolates were *C. albicans* (44/107; 41%) followed by *C. tropicalis* (34/107; 31.8%). The rate of occurrence of the species in HVS and urine in relation to the centres where they were collected is depicted in Table 1.

Predominance of *C. albicans* (37/63; 58.7%) was seen in the private medical diagnostic centre while *C. tropicalis (*24/44; 50%) was common among the isolates obtained from tertiary-care hospital. By and large, the occurrence of genitourinary candidiasis due to non-*albicans Candida* species (63.3%) was higher than those attributed to *Candida albicans* (36.7%). *C. dubliniensis* was uncommon among the isolates analysed but formed yellow-green colonies on chromogenic medium thus distinguishing it from *C. albicans*. All the isolates classified as *C. albicans* and *C. dubliniensis* exhibited the ability to produce germ-tube. The main non-*Candida albicans* species identified include *C. tropicalis* (31.8%), *C. krusei* (6.5%) and *C. parapsilosis* (5.6%). Whilst *C. parapsilosis* was resisted to the tertiary-care centre, *C. dubliniensis* (1 isolate) was observed only in the private medical diagnostic facility. Approximately 14% (15/107) of the isolates could not be discriminated by the Chromatic *Candida* agar.

Antifungal susceptibility test indicated that 93.5% (100/107) of the isolates was susceptible to nystastin but 55.1% and 61.7% were resistant to voriconazole and fluconazole respectively. Figure 1 shows the antifungal susceptibility pattern of the *Candida* species. The observed resistance of C. albicans isolates to Nystastin, VOR, FLU were 9.1, 38.6 and 61.4%, respectively. Conversely, the majority of non-*albicans Candida* species were resistant to voriconazole (58.8%) and fluconazole (61.9%). Resistance to triazoles (fluconazole and voriconazole) was significantly associated with *C. tropicalis.*

Table 1: Distribution of *Candida* species in relation to the samples sites

Species	Tertiary-Care Centre (n=44)		Private Medical Diagnostic Centre (n=63)		Total (n=107)
	Urine (n=4)	HVS (n=40)	Urine (n=15)	HVS (n=48)	
Candida albicans	1	6	7	30	44
Candida tropicalis	2	22	3	7	34
Candida krusei	0	2	0	5	7
Candida dubliniensis	0	0	0	1	1
Candida parapsilosis	1	5	0	0	6
Others	0	5	5	5	15
Total	4	40	15	48	107



R = Resistant, S= Sensitive

Figure 1: Antifungal Susceptibility Patterns of the *Candida* Species

DISCUSSION

In this survey, more isolates were obtained from high vaginal samples than urine, which indicates that *Candida* spp. as part of the genital microbiota of up to 30% of healthy people (Kalia *et al.* 2020) possess the potential to trigger lower urinary tract infection, particularly in women.

Some studies have shown *Candida albicans* to be the most prevalent species of *Candida* (Khadka *et al*., 2017; Ghaddar *et al*., 2020) associated with genitourinary tract infections, an observation that was established in this study. In particular, we noticed that the predominance of *C. albicans*, accounting for about 40% of the total isolates, is linked to most of the isolates listed in the private medical diagnostic center and generally represents approximately one-sixth of the isolates found in the tertiary health care facility. This result was compatible with a previous study (Akorth *et al*., 2009). Elsewhere, ElFeky and co-workers also found that 60.3% of positive cases of vulvovaginal candidiasis among women in Cairo were due to *Candida albicans* (ElFeky *et al*., 2016). Our findings were nevertheless in contradiction with the result of Obisesan *et al*. (2015) who documented that *C. glabrata* (46.9%) was the most common among sexually transmitted disease clinic attendees in Ladoke Akintola University Teaching Hospital, Nigeria. Nevertheless, the variability in the distribution of *Candida* spp. depends on hygiene level of individuals, geographic location, socio-economic level as well as the study population.

Candida tropicalis in this study showed a higher predominance over other non-albicans and may be a prospective pathogen in cases of genitourinary candidiasis. This is consistent with the data presented by Yesudhason and Mohanram (2015). Other surveys, such as the one carried out by Rajendra *et al*. in 2010, also affirm the findings of this study. While the other non-albicans Candida (*C. krusei*, *C. dubliniensis* and *C. parapsilosis*) have been recovered at low frequency, our findings are consistent with the analysis conducted by Akotha *et al*. (2009) and Obisesan *et al*. (2015) but contradicts other works that have identified high occurrences of non-*albicans Candida* other than *C. tropicalis* (Ejike *et al.*, 2018; Seyoum *et al*., 2020). Likewise, *Candida krusei* was the most common non-albicans species isolated from specific clinical specimens in a study in Ethiopia (Seyoum *et al*., 2020). The difference between the results of these studies could be due to the study population and the health care settings. It was observed that *C. parapsilosis* was only associated with the tertiary-care centre while *Candida dubliniensis* was associated with the private medical diagnostic centre. In this study, 14 per cent of Candida isolates were not classified, a finding that was consistent with the work of Vecchione *et al*. (2017). While, this can be considered as a limitation of this study, it indicates that the identification of *Candida* species should not be focused on phenotypic methods alone, a priority for future research.

The antifungal susceptibility report in this study showed that Nystatin was the most active antifungal drug in which 93.5% of the isolates were susceptible irrespective of species. These data are consistent with those reported by Monroy-Pérez *et al*. (2016) but in disagreement with the results of Abdu *et al*. (2019). In this present study, fluconazole and voriconazole (the azoles) were less effective and this goes in tandem with the report of Pesewu *et al*. (2020) who also observed that *Candida* was highly resistant to voriconazole and fluconazole. The resistance rate of our isolates to fluconazole and voriconazole negates a previous report by Akotha *et al*. 2009 who established fluconazole and ketoconazole are very effective antifungal agents for the treatment of genitourinanry tract infections caused by *Candida* species.

The present outcome revealed that azole resistance in *Candida* may be on the increase in our setting. In Nigeria, fluconazole is routinely used in our hospitals for treating candidiasis and it is a common practice that antimicrobials can be purchased without a prescription thus development of resistance is inexorable. Thus, the importance of this result is that it reinforces the need to identify different *Candida* species as well as to assess their susceptibility profile to antifungal agents.

Worldwide, increased prevalence of azole resistance in Candida is also being observed with variations according to geographical regions. As the case in our study, a study in Philippines analysed isolates from clinical specimens and concluded that *s*ome Candida species have certain characteristics for antifungal resistance (Juayang *et al.,* 2019). Interestingly, in this current investigation, *C. parapsilosis* was susceptible to voriconazole and fluconazole while *C. krusei* was susceptible to both antifungal drugs. This contradicts the observation made by Pfaller *et al.,* in 2009 but agrees with the findings of Lima *et al*. (2017) where *C. parapsilosis* exhibited no resistance against voriconazole and fluconazole.

CONCLUSION

The findings demonstrated heterogeneity in the distribution of species and the susceptibility of *Candida* spp. to antifungal agents in the two facilities under investigation. *C. tropicalis* is growing substantially and may be a facade for non-albicans species in the two centers. Although, nystatin was the most effective antifungal agent in this study, antifungal resistance to fluconazole and voriconazole seems to be a serious concern. It is therefore important that plans for continuous surveillance to promote the management of the associated infections are put in place and antifungal susceptibility testing should be done before drugs administration.

Conflict of Interest

The authors declared no conflicts of interest.

Acknowledgments

The authors would like to acknowledge the management and staff of the private medical diagnostic centers that kindly allowed access into their facilities and provided some of the *Candida* isolates analyzed in this study.

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