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Characterization of Candida Species Isolated from Clinical Samples in a Tertiary Health Facility in Bida, North Central Nigeria

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ABSTRACT: *Candida* characterization is important for the management of candidiasis, helpful in understanding the epidemiology of infection particularly the source and mode of transmission and facilitates the development of effective measures to prevent and control transmission of resistant strains**.** This study was carried out to identify the most common *Candida* species and distribution of *Candida albicans* and non albicans *Candida* species in clinical specimen in Bida. Samples were processed according to standard mycological techniques. Isolation of the fungus was done using Sabouraud dextrose agar. Microscopy was done on wet preparation using Lacto phenol cotton blue. *Candida* isolates were sub-cultured for speciation using Chrom agar *Candida***.** A total of 204 *Candida* isolates were studied, *Candida* was mainly isolated from high vaginal swab (HVS) 50.5%, urine 25.0%, endocervical swab 15.7% and ear swab 8.8%. *Candida albicans* was the most common species isolated (53.9%), followed by *Candida tropicalis* 31.4% and 7.4% for *Candida krusei* and *Candida glabata* respectively. There was statistically significant difference ($P < 0.001$) between age, gender and *Candida* species distribution. *Candida albican* had the highest frequency of 53 (51.5%) in HVS, our study showed higher prevalence in HVS which confirms higher prevalence of vulvo-vaginal candidiasis in female of reproductive age group of 20-39 years in Bida. There was a statistical significant difference ($P < 0.001$) between *Candida* species distribution among out patients and in patients with high out patients preponderance of candidiasis of 159 out of 204. Speciation of *Candida* is important to identify the incidence and trends of *Candida* infections in a given population, our study reaffirms the prevalence of *Candida albicans* in Bida.

Key words: **Characterization, Chromo-agar, *Candida albicans*, Bida, Nigeria**

Introduction

The genus *Candida* has diverse species that are common residents of soil and of the mucosal surfaces of human gastrointestinal tract, genito-urinary tract and the mouth, which are capable of causing oral thrush or vaginal thrush. *Candida* species have been associated with differences in the morphotype and virulence factors such as germ tube and mycelia formation, proteinase secretion, the changes in vaginal pH, phenotypic switching and ability to cause vaginal candidiasis (Samaranayake *et al.*, 2003; Namkinga *et al.*, 2005).

The most common vaginal isolate include *C. albicans* with a prevalence of 70-90% and less frequently non-albicans *Candida* species such as *C. tropicalis*, *C. glabrata (Torulopsis glabrata)*, *C. kefyr (C. pseudotropicalis)*, *C. krusei*, *C. famata*, *C. parapsilosis*, and *C. lusitaniae* (Chong *et al*., 2003; Namkinga *et al.*, 2005). However, a significant increase in non-albicans *Candida* species and formation of virulence

factors have been reported to be associated with recurrent *Candida vaginitis* (Chong, *et al*., 2003; Namkinga, *et al.,* 2005).

The incidence of *Candida* infections has dramatically increased in recent years as a result of a large increase in HIV/AIDS cases, thus an ever-expanding population with immuno-compromise due to mucosal or cutaneous barrier disruption, defects in the number and function of neutrophils or in cell-mediated immunity, metabolic dysfunction, organ transplantation and extremes of age <1 year and > 70 years (Pfaller and Diekema, 2007). The expanded use of immunosuppressive chemotherapies, and transplantation further increases the risk for both common and uncommon *Candida* species. In addition, as our aging population becomes increasingly mobile, environmental exposures to a variety of endemic fungal pathogens become more common and sometimes, may further increase the risk of fungal diseases (Pfaller and Diekema, 2007).

Candidiasis is caused by specie of the genus *Candida*, mainly *Candida albicans. Candida* species are ubiquitous fungi which are commonly implicated in human infection. The rising incidence of mucosal and systemic candidiasis reflects the exceeding increase in the number of persons at risk, coupled with other opportunities that exist for *Candida* species to invade tissues normally resistant to invasion. (Pappas *et al.,* 2009).

The increased prevalence of topical and invasive disease caused by *Candida* has led to new clinical syndromes, the expression of which depends solely on the immune status of the host. *Candida* species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to systemic illnesses. The clinical manifestations may be acute, sub-acute or chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditis and meningitis. In healthy individuals, *Candida* infections are usually due to damaged epithelial barrier functions and affects all age groups, but are commonly seen in the newborn and the elderly. They usually remain superficial and respond readily to treatment. Systemic candidiasis is frequently seen in patients with cell-mediated immune deficiency, and those receiving aggressive cancer treatment, immunosuppression, or transplantation therapy. The management of life threatening invasive candidiasis remains highly hindered by lack of reliable diagnostic methods that allow detection of both fungemia and tissue invasion by *Candida* species (Marodi and Johnson 2007).

The first step in the development of *Candida* infection is colonization of the mucocutaneous surfaces (Pappas 2006). All of the factors outlined above are associated with increased colonization rates. The routes of *Candida* invasion include disruption of a colonized surface (skin or mucosa), allowing the organisms access to the bloodstream, and (Romani 2008) persorption via the gastrointestinal wall, which may occur following massive colonization with large numbers of organisms that pass directly into the bloodstream (Guary *et al*., 2009).

Candida species characterization is important for the management of candidiasis .Speciation helps in understanding of epidemiology of infection particularly the source and mode of transmission which facilitates the development of effective measures to prevent and control transmission of resistant strains (Shaheen and Taha, 2006) .

This study was carried out to identify the most common *Candida* species and distribution of *Candida albicans* and non-albicans *Candida* species in clinical specimen in a tertiary health facility in Bida, North Central Nigeria.

Materials and Methods

The study was carried out in Medical Microbiology Laboratory of Federal Medical Centre Bida, located in Bida local government area of Niger State, North Central Nigeria.

Samples were processed according to standard mycological techniques (Milne, 1996). Each specimen was examined for the presence of fungi by microscopy using wet mounts (KOH and Nigrosin mounts), and stained preparation using (lacto phenol cotton blue stain).

Isolation of the fungus was done using Sabouraud dextrose agar, the plates were incubated at 37^0c for 24 – 72 hours. Growth rate, colony characteristics and microscopy were used to identify *Candida* by their

characteristic shape and hyphe on wet preparation using Lacto phenol cotton blue. *Candida* isolates were sub -cultured into agar slope for speciation using Chrom agar *Candida*.

Chrom agar Candida

Chrom agar Candida is a special medium for isolation and differentiation of major clinical species of *Candida*. It displays high specificity and sensitivity for three of the major *Candida* species at a time namely *Candida albicans, Candida tropicalis and Candida krusei.*
Candida isolates were identified to species level by culturing on Chrom agar *Candida*. Incubated aerobically at 37ºC for 36- 48 hrs. Identification was based on colour production.

Interpretation:

- *Candida albicans* Green
- *Candida tropicalis* Metallic blue
- *Candida krusei* Pink, Fuzzy
- *Candida glabrata* Mauve

The data generated from this study were analysed by the statistical software SPSS version 16 (SPSS Inc. Chicago. Illinois) for windows.

Results

Two hundred and four (204) samples were collected from patients attending various clinics of the Federal Medical Centre Bida. Table 1 shows distribution of specimen with the highest frequency of 103 (50.5%) High Vaginal Swab, Urine 51 (25.0%), Endocervical Swab 32 (15.7%), Ear Swab 18 (8.8%).

Table 2 shows the distribution of *Candida* species isolated with the highest frequency of *Candida albican* 110 (53.9%) following *Candida tropicalis* 64 (31.4%), *Candida krusei* and *Candida glabrata* 15(7.4%) respectively.Table 3 shows the age distribution of *Candida* species among the participants in the study. *Candida tropicalis* had the highest frequency of 16 (51.6%) followed by *Candida albican* 15(48.4%) in age group 0-9 years while age group 10-19 years had 2 (100%) of *Candida albican* , the age distribution of *Candida* species among age 20-29 years shows a prevalence of 47 (50.5%) *Candida albicans,* 16 (17.3%), *Candida tropicalis* 15(16.1%) of *Candida krusei* and *Candida glabrata* respectively while age group 30-39 years had a highest frequency of 46(59.0%) *Candida albican* followed by 32 (41.0%) *Candida tropicalis.* There was a statistical significant difference ($P < 0.001$) between age and *Candida* species distribution among participants.

Table 4 shows gender distribution of *Candida* species in this study. *Candida tropicalis* had the highest frequency of 16 (88.9%) in male followed by *Candida albican* 2 (11.1%) while female had 108 (58.1%) *Candida albican* followed by 48 (25.8%) *Candida tropicalis* with 15 (8.1%) *Candida krusei* and *Candida glabrata* respectively. There was statistically significant difference ($P < 0.001$) between gender and *Candida* species distribution.

Table 5 shows the distribution of *Candida* species among various clinical specimens. *Candida albicans* had the highest frequency of 53 (51.5%) in High Vagina Swab followed by 32(31.1%) *Candida tropicalis* while *Candida krusei* and *Candida glabrata* had 9 (8.7%) respectively. In urine specimens *Candida albicans* had the highest frequency of 23(45.0%) followed by 16 (31.4%) *Candida tropicalis* and 6 (11.8%) *Candida krusei* and *Candida glabrata* respectively while in Endocervical Swab had 32 (100%) *Candida albicans.*

Table 6 shows the distribution of *Candida* species in clinical specimen based on patient's group. The highest frequency of 30(66.7%) *Candida albicans* was found among in- patients followed by 15 (33.3%) *Candida glabrata* while among out- patients *Candida albican* had the highest frequency of 80(50.3%) followed by 64 (40.3%) *Candida tropicalis* and 15 (9.4%) *Candida krusei*. There was a statistically significant difference ($P < 0.001$) between *Candida* species distribution among out-patients and in-patients.

Table 1: Sample Distribution of Specimens

	Frequency	**Percentage**
High Vaginal Swab	103	50.5
Urine	51	25.0
Endocervical Swab	32	15.7
Ear swab	18	8.8
Total	204	100

Table 2: Distribution of *Candida* Species in Clinical Specimens

Candida species	**Frequency**	**Percentage**
Candida albican	**110**	**53.9**
Candida tropicalis	**64**	**31.4**
Candida krusei	**15**	**7.4**
Candida glabata	**15**	**7.4**
Total	**204**	**100**

Table 3: Age Distribution of *Candida* Species in Participants

Age (Years)	***Candida albican***	***Candida Tropicalis***	***Candida Krusei***	***Candida glabata***	**Total**
0-9	15 (48.4%)	16(51.6%)	0(0%)	0(0%)	31(100%)
10-19	2(100%)	0(0%)	0(0%)	0(0%)	2(100%)
20-29	47(50.5%)	16(17.3%)	15(16.1%)	15(16.1%)	93 (100%)
30-39	46(59.0%)	32(41.0%)	0(0%)	0(0%)	78(100%)
Total	110(53.9%)	64(31.4%)	15(7.4%)	15(7.4%)	204(100%)

Table 4: Gender Distribution of *Candida* Species in Participants

Sex	***Candida albican***	***Candida Tropicalis***	***Candida Krusei***	***Candida glabata***	Total
Male	2(11.1%)	16(88.9%)	0(0%)	0(0%)	**18(100%)**
Female	108(58.1%)	48(25.8%)	15(8.1%)	15(8.1%)	**186(100%)**
Total	110(53.9%)	64(31.4%)	15(7.4%)	15(7.4%)	**204(100%)**

Table 5: Distribution of *Candida* Species in Clinical specimens

Specimen	*Candida albican*	*Candida Tropicalis*	*Candida Krusei*	*Candida glabata*	Total
HVS	53(51.5%)	32(31.1%)	9(8.7%)	9(8.7%)	103(100%)
Urine	23(45.0%)	16(31.4%)	6(11.8%)	6(11.8%)	51(100%)
Ear swab	2(11.1%)	16(88.9%)	0(0%)	0(0%)	18(100%)
ECS	32(100%)	0(0%)	0(0%)	0(0%)	32(100%)
Total	110(53.4%)	64(31.4%)	15(7.4%)	15(7.4%)	204(100%)

Table 6: Distribution of *Candida* Species in Clinical specimens Based on Patient's Group

Patient group	*Candida albican*	*Candida Tropicalis*	*Candida Krusei*	*Candida Glabata*	Total
In - patients	30(66.7%)	0(0%)	0(0%)	15(33.3%)	45(100%)
Out patients	80(50.3%)	64(40.3%)	15(9.4%)	0(0%)	159(100%)
	110(53.9%)				
Total		64(31.4%)	15(7.4%)	15(7.4%)	204(100%)

P value = 0.000

Discussion

The frequency of Candidiasis has increased during the past years. This study investigated *Candida* species from different clinical specimens from all age groups and gender. A total of 204 *Candida* isolates from various clinical specimens were included in this study, *Candida* was mainly isolated from high vaginal swab 50.5% and urine 25.0%, Endocervical swab 15.7% and Ear swab 8.8%. *Candida albicans* was the most common species isolated from this study 53.9% followed by *Candida tropicalis* 31.4% and 7.4% for *Candida krusei* and *Candida glabata* respectively which agrees well with some studies (Mohandas and Ballal.2011; Dharwads and Bominie 2011,Tavleen *et al.*,2014) which reaffirms the high prevalence of albican *Candida* and a gradual shift towards non-albican *Candida* species, however Charkabati *et al.,* (1996) reported a higher prevalence of 75.0% non-albicans *Candida* which suggest that non-albican are emerging as important pathogens in human infection. The speciation of *Candida* is important to identify the incidence and trends of *Candida* infections in a given population as it is also essential for the choice of antifungal because of variation in the sensitivity of different species to different antifungal (Pfaller,1996).

Though candidiasis can occur at all ages, our study showed highest prevalence among age group 20-29 years followed by 30-39 years which is in agreement with the findings of Dalal and Kelkar, 1980 ; Tavleen *et al*., 2014 who reported high prevalence of candidiasis in age group 21-40 years. Our study found a significant difference ($P < 0.001$) between age and distribution of *Candida* species among participants.

A high female preponderance of candidiasis of 186 out of 204 was found in this study with a species distribution of 58.1% *Candida albican* which agrees well with Sjatha *et al.*, 2015.

Vulvoviginal candidiasis is the most common manifestation of genital candidiasis in female (Achkar, 2010) .Our study showed higher prevalence in high vaginal swab followed by urine sample which confirms higher prevalence of vulvo-viginal candidiasis in female of reproductive age group of 20-39 years in Bida, which agreed with Sujatha *et al.,* in Kanpur, Saldanha and Shivanand (2011) also found high vaginal swab having the highest number of *Candida* isolates in their study.

Our study found a statistically significant difference ($P < 0.001$) between *Candida* species distribution among out-patients and in-patients with high out-patients preponderance of candidiasis of 159 out of 204 in Bida.

In conclusion, continuous evaluation in the trends of species distribution is of high importance in the control and optimization of the management of *Candida* infection. This study recommends Chromogenic medium as a more rapid method for speciation of *Candida* isolates in clinical specimens as it facilitates the identification to species levels within 24 hours of incubation.

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