Middle-East Journal of Scientific Research 26 (5): 508-513, 2018
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2018.508.513

# Susceptibility of Clinical Isolates of *Candida albicans* and Non Albican Candida Species from Federal Teaching Hospital Abakaliki, South Eastern Nigeria to Three Commonly Used Antifungal Agents

*[1]Onwa Ndubuisi Collins, [1]Onuchukwu Ikenna Edwin, [1]Okonkwo Eucharia, [2]Nworie Okoro and [3]O. Ikeanumba Mikchael*

[1]Applied Microbiology Department, Ebonyi State University, Abakaliki, Nigeria
[2]Department of Biological Sciences, Alex Ekwueme-Federal University Ndufu-Alike Ikwo, Nigeria
[3]Department of Biology, Alvan Ikokwu Federal College of Education Owerri Imo State, Nigeria

**Abstract:** The susceptibility of clinical isolates of *Candida albicans* and Non albican Candida species from Federal Teaching Hospital Abakaliki, South Eastern Nigeria to three commonly used antifungal agents were carried out. A total of 245 clinical samples made of 70 Urine samples, 70 Endocervical swab samples, 65 High vaginal swab samples and 40 Throat swab samples were tested. The samples were collected using standard microbiological methods. Isolation of Candida species from the samples was done using cultural and microscopic methods. Differentiation of *Candida albicans* from Non albican Candida was carried out using the germ tube test. The susceptibility of the *Candida albicans* and Non albican Candida species were done using the Kirby-Bauer disc diffusion test. Results showed that out of the 245 clinical samples tested, *Candida species* were recovered from 83 (33.9%) of them. Endocervical swab gave the highest recovery of 47% (33/70), followed by High vaginal swab 35% (23/65), Throat swab 28% (11/40) and Urine 23% (16/70). Results of Differentiation of the 83 *Candida species* into *Candida albicans* and Non albican Candida revealed that 46 (55.4%) were *Candida albicans* while 37 (44.6%) were Non albican Candida. Also results of the susceptibility tests showed that Voriconazole had the greatest *in vitro* activity against both *Candida albicans* `(93%) and Non albican Candida (76%) followed by Fluconazole with 85% activity against *Candida albicans* and 70% activity against Non albican Candida. It was found that all the isolates of *Candida albicans* and Non albican Candida were (100%) resistant to the action of Nystatin. Also Non albican Candida resisted the action of the antifungals tested more than *Candida albicans*. It is therefore recommended that the use of Nystatin in the treatment of Candida infections be reviewed within the study area, while periodic monitoring of the antifungals employed in the treatment of Candida infections be carried out.

**Key words:** *Candida albicans* · Non Albican Candida · Susceptibility · Antifungal Agents

## INTRODUCTION

Candida species can cause human infection and the disease caused by the genus Candida could be referred to as candidiasis or candidoma. The genus *Candida* belongs to the phylum *Ascomycetes,* class *Blastomycetes* and order *Cryptococcales* and family Cryptococcaceae. Candidiasis ranges from mild infection such as Onychomycosis or perlish to potentially fatal systemic candidiasis. Among the causative agents of bloodstream infections, *Candida* ranks fourth in the United States and seventh in Europe [1]. Until recently, *Candida albican* was, by far, the predominant species in most of the countries, causing up to two-third of all cases of invasive candidiasis [2]. Candida organism has been implicated as one of the major causes of hospital acquired infection or nosocomial infection [3]. Any microorganism is capable of causing nosocomial infection, but those that are able to survive and persist in hospital environment for longer period and develop resistance to antimicrobial agents and

**Corresponding Author:** Onwa Ndubuisi Collins, Applied Microbiology Department, Ebonyi State University Abakaliki, Nigeria.

disinfectants are particularly important [3]. Advances in medical practice have increased the frequency of fungal infections in hospitalized patients. Fungal pathogens, now account for almost 10% of all nosocomial blood stream infections (BSIs) [4]. Among various fungal pathogens, Candida spp. is the important cause of substantial morbidity and mortality in hospitalized patients, it is fast becoming a very important pathogen among critically ill hospitalized patients [5, 6].

Candida species are yeast fungi that are normally present on the skin and mucous membranes such as oral cavity, vagina and rectum. *Candida. albicans* is the major cause of infection in human [7]. It is also an important part of the normal flora in the oral cavity, gastrointestinal tract and vagina in healthy humans. Candida species mediate adhesion, biofilm formation, invasion into host cells, yeast-to hypha transition (Phenotypic switching), secretion of hydrolases, contact sensing and thigmotropism are the pathogenic potentials of *C. albicans* [8].

Several factors increase the incidence rate of candidiasis in colonized patients such as weakened immune system, mucosal & cutaneous barrier disruption, neutrophil dysfunction (Quantitative or qualitative) metabolic disorders and advanced age [9].

Over the past decade, the species associated with candidiasis has progressively shifted from *Candida albicans* to Non *albican Candida* (NAC) spp. a term used to describe other species in the genus Candida aside *Candida albicans* that can cause candidiasis. The most common Non albican Candida species include; Candida parapsilosis, Candida tropicalis, Candida krusei, Candida glabrata, Candida lusitaniae, Candida guilliermondii, Candida rugosa [10].

To manage the patients with candidiasis, antifungal susceptibility testing has become an important step in guiding physicians in the selection of proper antifungal therapy [11]. Amphotericin B, a polyene fungicidal agent has been the standard for *candida* infections for decades, but the toxicity of its conventional form and cost of its lipid forms limits its use [12]. More recently, azole antifungal compounds, with lower cytotoxicity and perfect efficacies, have emerged as the principal drugs used in treatment of *Candida* infection. However, prolonged use of azole has led to the development of drug resistance in *Candida albicans* and other (NAC) species [13]. Among the factors contributing to development of resistance to azole is the selection of intrinsically less susceptible organisms such as *Candida. glabrata* and *Candida krusei* and the acquisition of resistance by previously susceptible strain of *C. albicans* following long term azoles exposure have been documented [14]. This research work was aimed at isolating *Candida albicans and Non albican Candida* from different clinical specimens and to determine their antifungal susceptibility patterns against three commonly used antifungal agents namely; fluconazole (25µg), Nystatin (100units) and voriconazole (1µg) all produced by Oxoid UK.

## MATERIALS AND METHODS

**Study Area:** This study was conducted at the Federal Teaching Hospital Abakaliki, Ebonyi State, South Eastern Nigeria.

**Ethical Clearance:** The consent and permission of the Hospital management were inquired in order to carry out this research work. Subsequently, the confidentiality of the information obtained was kept.

**Sample Collection and Preparation:** A total of 245 Clinical specimens were collected as follows 70; urine, 65 high vaginal swab (HVS), 70 endocervical swab (ECS) and 40 throat swab (TS) samples. Urine samples were collected in sterile universal containers while HVS, ECS and TS samples were collected using swab sticks.

**Isolation of Candida Species:** Candida species were isolated from the clinical specimens using standard microbiological methods of microscopic, macroscopic and cultural characteristics [15].

**Differentiation of *Candida Albican* from non *Albican Candida*:** The differentiation of *Candida albicans* from Non-*albican Candida* was done using germ tube formation as described by Kothari and Sagar [16].

**Standardization of Test Organisms:** The Candida albicans and Non albican Candida isolates used for sensitivity tests were standardized using the 0.5 McFarland equivalent standard as described by Lockhart [17].

**Susceptibility Testing:** The susceptibility testing of the commonly used Antifungal agents were ascertained using Kirby Bauer agar well diffusion as described by Makhodo *et al*. [18].

## RESULTS AND DISCUSSION

Table 1: Isolation of Candida species from the different clinical specimens.

| Clinical specimen | No. of sample collected | No. of Candida species isolated | Percentage of candida species isolated (%) |
|---|---|---|---|
| High vaginal swab | 65 | 23 | 35.4 |
| Endocervical swab | 70 | 33 | 47 |
| Throat swab | 40 | 11 | 28.5 |
| Urine | 70 | 16 | 23 |

Table 2: Differentiation of Candida species into *Candida albicans* and Non *albican Candida* from the different clinical specimens.

| Clinical specimen | No. of isolated Candida species | No. of *Candida albican* | Percentage of *Candida. Albican* (%) | No. of non *albican Candida* | Percentage of non *albican Candida* (%) |
|---|---|---|---|---|---|
| High vaginal swab | 23 | 14 | 61 | 9 | 39 |
| Endocervical swab | 33 | 15 | 45.5 | 18 | 54.5 |
| Throat swab | 11 | 7 | 64 | 4 | 36 |
| Urine | 16 | 10 | 62.5 | 6 | 37.5 |

Table 3: Susceptibility pattern of the *Candida albicans* to fluconazole

| Clinical specimen | Number tested | Number susceptible | Percentage susceptibility (%) |
|---|---|---|---|
| High vaginal swab | 14 | 14 | 100 |
| Endocervical swab | 15 | 14 | 93 |
| Throat swab | 7 | 7 | 100 |
| Urine | 10 | 9 | 90 |

Total sensitive 44/46=95.7%

Table 4: Susceptibility pattern of the Non albican Candida to Fluconazole

| Clinical specimen | Number tested | Number susceptible | Percentage susceptibility (%) |
|---|---|---|---|
| High vaginal swab | 9 | 6 | 67 |
| Endocervical swab | 18 | 12 | 67 |
| Throat swab | 4 | 4 | 100 |
| Urine | 6 | 4 | 67 |

Total sensitive 26/37= 70.3%

Table 5: Susceptibility pattern of the *Candida albicans* to Voriconazole

| Clinical specimen | Number tested | Number susceptible | Percentage susceptibility (%) |
|---|---|---|---|
| High vaginal swab | 14 | 14 | 100 |
| Endocervical swab | 15 | 14 | 93 |
| Throat swab | 7 | 7 | 100 |
| Urine | 10 | 10 | 100 |

Total sensitive 45/46= 97.8%

Table 6: Susceptibility pattern of the Non albican Candida to Voriconazole

| Clinical specimen | Number tested | Number susceptible | Percentage susceptibility (%) |
|---|---|---|---|
| High vaginal swab | 9 | 8 | 89 |
| Endocervical swab | 18 | 12 | 67 |
| Throat swab | 4 | 4 | 100 |
| Urine | 6 | 4 | 67 |

Total sensitive 28/37= 75.7%

Table 7: Susceptibility pattern of the *Candida albicans* to Nystatin

| Clinical specimen | Number tested | Number susceptible | Percentage susceptibility (%) |
|---|---|---|---|
| High vaginal swab | 14 | 0 | 0 |
| Endocervical swab | 15 | 0 | 0 |
| Throat swab | 7 | 0 | 0 |
| Urine | 10 | 0 | 0 |

Table 8: Susceptibility pattern of the Non albican Candida from the clinical specimen to Nystatin

| Clinical specimen | Number tested | Number susceptible | Percentage susceptibility (%) |
|---|---|---|---|
| High vaginal swab | 9 | 0 | 0 |
| Endocervical swab | 18 | 0 | 0 |
| Throat swab | 4 | 0 | 0 |
| Urine | 6 | 0 | 0 |

Table 9: Comparative summary of the Susceptibility and Resistance pattern of *Candida albicans* and Non *albican Candida* to the antifungal agents used

| Antifungal agent | No. of *Candida albicans* sensitive | No. of *candida albicans* resistant | No. of Non *albican candida* sensitive | No. of Non *candida albican* resistant |
|---|---|---|---|---|
| Voriconazole | 43(93) | 3(7) | 28(76) | 9(24) |
| Fluconazole | 39(85) | 7(15) | 26(70) | 11(30) |
| Nystatin | 0(0) | 46(100) | 0(0) | 37(100) |

In this study, a total of 83 Candida species were recovered from a total of 245 clinical samples. Endocervical swab had the highest recovery with 47%, followed by High vagina swab 35%, Throat swab 28% and Urine 23%. Candida species were isolated more from genital organs; endocervical and vaginal than from the other samples from the other parts of the body. This might be attributed to the fact that Candida species are normal flora of the genital organs especially vagina. This agrees with the work of Marchetti *et al*. [19] who found Candida as the major etiological agent in genital infections? However, *Candida albican* at 55% remains the most isolated Candida species which is in line with the study by Mayer *et al*. [20] and Méan *et al*. [21], emphasizing the prevalence of *Candida albican* amongst other species. However, results of Michele *et al*. [22] study disagree with the present study, with 54.3% of non *albican Candida* in preference to *Candida albican.* Giving each sample prevalence of *Candida albican*, High vagina swab 61%, Ecs 45%, throat swab 64% and Urine 63% while for Non *albican Candida*, Hvs 39%, Ecs 55%, Ts 36% and urine 38%.

Antifungal agents are used basically for treatment and preventive purposes of various fungal infections, though they differ in their mode of actions. For the purposes of this study, two groups of antifungal were selected based on routine use within the locality. Nystatin which is a polyene, which are broad spectrum antifungal drugs which binds to the ergosterol content of the cytoplasm, hence alters the cell permeability leading to leakage of cellular content and death [23]. Whereas fluconazole and voriconazole from Azole group of antifungals which block the synthesis of ergosterol (Major component of fungal cytoplasmic membrane) by inhibiting P450 dependent enzyme sterol 14-α-demethylase leading to cessation of cell growth, reproduction and increase permeability [24].

*Candida albicans* in this study recorded 85% susceptibility to fluconazole and 15% resistance (Table 9). Ostrosky-Zeichner and Pappas [25] in his work reported 83% susceptibility to fluconazole by *Candida albicans* with resistance of 20%. In this study also, Non *albican candida* had 70% susceptibility to fluconazole with 30% resistance. Rajkumari *et al.* [26] recorded 81% susceptibility of Non albican Candida species to fluconazole, while Sachin *et al*. [27] had 56% susceptibility to fluconazole by Non *albican Candida* with 44% resistance. Among the factors contributing to development of resistance to fluconazole include the selection of intrinsically less susceptible organisms such as *C. glabrata* and *Candida krusei* and the acquisition of resistance by previously susceptible strain of *Candida* species following long term azoles exposure have been documented [28].

*Candida albican* and non *albican candida* alike in this study presented 100% resistance to nystatin which is relatively in line with the work done by Warnock and Cambell [29]. In his work, resistance of 80% to nystatin by candida species was reported but contrasted with the work done by Wayne [30] which recorded 99.4 and 99.5% susceptibility of *Candida albicans* and non *albican Candida* respectively to nystatin and further buttressed by the work done by Yar zever and Ibrahim [31] who expresses high susceptibility of 94% to nystatin by Candida species. Suffice it to say at this juncture, that high level of resistance to nystatin by *Candida albicans* and Non *albican Candida* could be best attributed to increase use of antifungals as topical ointment, prolonged therapy or suppository as a result of its availability and low cost. Susceptibility of 93 and 76% to *Candida albicans* and Non *albican Candida* respectively to voriconazole in this study, has given high preference to the dual above, as a drug of choice in Candida infection treatment. According to Feng-Juan *et al*. [12] in his

study, this had 100% susceptibility of both *Candida albicans* and Non albican Candida to voriconazole, followed by Campbell *et al*. [4] who equally reported 100% susceptibility of all Candida species. Denning and Hope [9] in their work reported 76.6 and 100% susceptibility of *Candida albicans* and Non *albican candida* to voriconazole respectively. Nevertheless, studies have reported 56% resistance of Candida species to voriconazole, which is according to Feng-Juan *et al*. [12]. The results of antifungal susceptibility in this study showed that voriconazole has potent *in-vitro* activity against Candida species, including those that were fluconazole-susceptible or fluconazole-resistant. This finding suggests that voriconazole might be effective in the treatment of refractory candidiasis caused by fluconazole-resistant strains.

## CONCLUSIONS

This work has shown that Voriconazole and fluconazole from the azole family of antifungals used within the locality of the research area remains effective for both *Candida albicans* and Non *albican Candida*. This work has also confirmed the increasing rate of Non albican Candida species in the cause of Candidiasis. The 100% resistance recoded by both *Candida albicans* and Non albican Candida species to Nystatin should warrant the review of the use of the drug in the treatment of Candidiasis in the locality. With various types of antifungals available in the market, it has become necessary to perform antifungal susceptibility testing and reporting for effective therapeutic outcome and continuous evaluation of newer antifungal agents

## REFERENCES


1. Adhikary, R. and S. Joshi, 2011. Species distribution and antifungal susceptibility of Candidaemia at a multi-specialty center southern India. J. Med. Microbial., (29): 309-3011.
2. Ane- Anyagwe, I., H.D. Meriki, S.P. Silum, F.R. Nsongommanyi and D. Zofou, 2015. Antifungal susceptibility profile and risk factors of vaginal Candidiasis among female University students in southwest region, Cameroon. Afr. J. Cln. Exper. Microbiol, 16(1): 67-72.
3. Bhaskar U.A. Yashavanth and R. Ronald, 2015. Identification of Candida Species from Clinical Samples and their Antifungal Susceptibility Patterns.Journal of Evolution of Medical and Dental, 4: 12998-13004.
4. Campbell, C.K., A.D. Holmes, K.G. Davey, A. Szekely and D.W. Warnock, 1998. Comparison of a new chromogenic agar with the germ tube method for presumptive identification of Candida albicans. Eur J. Clin Microbiol. Infect. Dis., (17): 367-368.
5. Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries. 2nd ed., Cambridge University Press, Cambridge, ISBN-10: 113944929X, pp: 440.
6. Choukri, F., M. Benderdouche and P. Sedonaoui, 2014. Invitro susceptibility profile of 200 recent clinical isolates of Candida spp. To topical antifungal treatment of Vulvovaginal Candidiasis, the imidazole's and Nystatin agents. J. Mycol. Med, (24): 303-307.
7. Chumei, Y., Z. Hongju, T. Zhenhua, C. Huifen, G. Jing and Y. Chaoyan, 2016. Antifungal susceptibility and molecular typing of 115 Candida albican isolates obtain from vulvovaginal candidiasis patient in 3 shanghai maternity hospital. J of international society for human and Animal Mycology, (54): 394-399.
8. Enwuru, C.A., A. Ogunledun, N. Idika, N.V. Enwuru, F. Ogbonna, M. Aniedobe and A. Adeiga, 2008. Fluconazole resistant opportunistic oro-pharyngeal Candida and non-Candida yeast-like isolates from HIV infected patients attending ARV clinics in Lagos, Nigeria. Afr. Health. Sci., 8(3): 142-148.
9. Denning, D. and W. Hope, 2010. Therapy for fungal disease; opportunities and priorities. Trends Microbiol, 18: 195-204.
10. Clinical Laboratory Standard Institute (CLSI), 2004. Method for Antifungal disk diffusion susceptibility testing of yeasts; approved guideline, 24(15): 2-6.
11. Deorukhkar, S., S. Saini and S. Mathew, 2014. Non-Candida albicans infection: An emerging threat. Interdiscip Perspect Infect Dis, 25: 131-36.
12. Feng-Juan, W., L. Dai zhang, zhao-Hui, W. Wen-Xiang Hui Bai and D. Han-Yu, 2014. Species Distribution and invitro Antifungal susceptibility of Vulvovaginal Candida isolates in China. Chin Med J., (129): 1161-1165.
13. Haddadi, P., S. Zareifar, P. Badiee, A. Alborzi, M. Mokhtari and K. Zomorodian, 2014. Yeast colonization and drug susceptibility pattern in the pediatric patients with neutropenia. Jundishapur J. Microbiol, 7(9): 118-158.
14. Kavitha, H., K. Anuradha and D. Venkatesha, 2014. Comparison of susceptibility of various Candida species isolated from Neonatal septicaemia to Voriconazole and fluconazole. J. of Pharmacy and Biological Science, (9): 78-81.

15. Krcmery V.C. and A.J. Barress, 2002. Non albican Candida spp causing fungaemia:Pathogenicity and antifungal resistance. Journal of Hospital infection, 50 (4): 243-260
16. Kothari, A. and V. Sagar, 2009. Epidemiology of blood stream infection in a tertiary care institute in india. Indian J med Microbiol, (27): 171-172.
17. Lockhart, S., 2014. Current epidemiology of Candida infection. Clin. Microbiol. News, 36: 131-36.
18. Makhodo, N.A., F. Ismal, Y. Dango, T.Y.H. Chephe, A.A. Hoosen and M. Chabeleng, 2014. Susceptibility profile by yeast isolates from Sterile site at a public teaching Hospital in south Africa. South African J. Infect, (29): 97-100.
19. Marchetti, O., P. Moreillon, J.M. Entenza, J. Vouillamoz, M.P. Glauser and J. Bille, 2003. Fungicidal synergism of FLU and cyclosporine in Candida albicans is not dependent on multidrug efflux transporters encoded by the CDR1, CDR2, CaMDR1, and FLU1 genes. Antimicrob Agents Chemother, 47: 1565-1570.
20. Mayer, F.L., D. Wilson and B. Hube, 2013. Candida albicans pathogenicity mechanisms. Virulence, 4(2): 119-128.
21. Méan, M., O. Marchetti and T. Calandra, 2008. Bench-to-bedside review: Candida Infections in the Intensive Care Unit, 27: 1-6.
22. Michele, K., V. Siri, J. Shu, N. Mbekem and A. Olivia, 2017. Antifungal susceptibility pattern of Candida spp, isolated from female genital tract at the Yaounde Bethesda Hospital in Cameroon. Pan African Medical Journal, (28): 294-300.
23. Montravers, P. and K. Jabbour, 2006. Clinical consequences of resistant Candida infections in intensive care. Int J. Antimicrob Agents, 27: 1-6.
24. Naseema, S., Uma Penmetcha, Ramesh Babu Myneni, Padmaja Yarlagadda and Susmitha Singamsetty, 2016. A study of Identification and Antifungal Susceptibility Pattern of Candida species Isolated from Various Clinical Specimens in a Tertiary care Teaching Hospital, Chinakakani, Guntur, Andhra Pradesh, South India. Int. J. Curr. Microbiol. App. Sci., 5(7): 71-91.
25. Ostrosky-Zeichner, L. and P. G. Pappas, 2006. Invasive candidiasis in the intensive care unit. Crit Care Med., 34:857–63.
26. Rajkumari, N., P. Mathur, I. Xess and M. Misra, 2014. Distribution of different yeasts isolates among trauma patients and comparison of accuracy in identification of yeasts by automated method versus conventional methods for better use in low resource countries. Indian J. Med. Microbiol, 32: 391-394.
27. Sachin, C., Deorukhkar and S. Santosh, 2016. Why Candida Species have Emerged as Important Nosocomial Pathogens? Int. J. Curr. Microbiol. App. Sci., 5(1): 533-545.
28. Snydman, D.R., 2003. Shifting patterns in the epidemiology of nosocomial Candida infections. Chest, 123: 500-503.
29. Warnock, D. and C. Cambell, 1996. Medical Mycology. Mycol Res., 100: 1153-1162.
30. Wayne, P.A., 2006. Method for antifungal disk diffusion susceptibility testing of yeasts: approved standard. Clinical and Laboratory Standards Institute; Clinical and Laboratory Standards Institute. CLSI M44-A.
31. Yar zever, S. and A.A. Ibrahim, 2013. Genital tract infection ; Prevalence and causes in women attending Aminu Kano Teaching Hospital Kano, Nigeria. Journal of National Science Research, 3(2): 35-44.