World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY PROFILE OF VULVOVAGINAL CANDIDIASIS AMONGST WOMEN OF REPRODUCTIVE AGE IN JOS METROPOLIS, NIGERIA

[1]*James Garba Damen, [1]Essien Unyime Cosmas and [2]Victoria M. Daminabo

[1]Department of Medical Laboratory Science Faculty of Medical Sciences University of Jos.
[2] Department of Science Laboratory Technology, School of Science and Technology, Port Harcourt Polytechnic, Rumuola, P.M.B. 5936, Port Harcourt, Rivers State

***Corresponding Author: James Garba Damen**
Department of Medical Laboratory Science Faculty of Medical Sciences University of Jos.

Article Received on 19/03/2017 **Article Revised on 08/04/2017** **Article Accepted on 29/04/2017**

ABSTRACT

Background: Vaginal candidiasis is the most common opportunistic mucosal infection that affects large numbers of healthy women of childbearing age. Candida is capable of causing various clinical manifestations ranging from mucocutaneous overgrowth to disseminated infection. This was a cross-sectional study conducted between September to December 2015. **Aim of the study:** To determine the prevalence and antifungal susceptibility of Candida isolates of vaginal swab among reproductive age women. **Materials and Methods:** Women within the age group of 15 to 45 years formed the population of this study. Socio-demographics information, were gotten through a standard questionnaire. Vaginal swabs were collected from each participant and cultured on Sabouraud dextrose agar supplemented with chloramphenicol. Identification of the isolates were based on morphological appearance, germ tube and chlamydospore formation. Antifungal susceptibility testing was performed by using the CLSI guidelines (CLSI, 2012). **Results:** Of the 246 participants who submitted vaginal swabs, yeasts was isolated in 47 (19.1%). Out of 47 yeasts isolates, 28 (11.4%) were *Candida albicans* while 19 (7.7%) were non- albicans Candida species (NAC). Antifungal susceptibility testing performed on *C. albicans* showed a high susceptibility to fluconazole 23/28 (82.1%) and nystatin 15/28 (53.6%) while NAC exhibited high resistance 4/19(21.1) and 2/19(10.5) to fluconazole and nystatin respectively. **Conclusion:** Hence, there is need to understand the pattern of antifungal susceptibility in our community in order to adequately check the spread of resistant species in this population.

KEYWORDS: Vaginal candidiasis, Antifungal Susceptibility, *C. albicans.*

INTRODUCTION

Vulvovaginal candidiasis (VVC) is an opportunistic fungal infection of the female lower genital tract caused by *Candida species.*[1] Mucosal candidiasis, especially vulvovaginal candidiasis, is the most common fungal disease in normal healthy women[2,3] *Candida* is the most common cause of fungal infections and also an important cause of community and health care associated infections.[4] Although considered to be part of normal microbial flora, Candida is capable of causing various clinical manifestations ranging from mucocutaneous overgrowth to disseminated infection like candidemia.[5] However, factors like HIV/AIDS, treatment with broad spectrum antibiotics and immunosuppressive drugs increase the vulnerability to Candida infections.[6]

The role Candida in establishment and progression of infection was considered to be passive, whereas the immune status of host was considered as a vital mechanism responsible for candidiasis.[7] Therefore candidiasis was considered "disease of diseased''. Lately this concept has changed and it is established that Candida can actively participate in the pathogenesis of the disease progression by using mechanism of aggression like tissue adhesion, phenotypic switching, biofilm formation, and production of extracellular hydrolytic enzyme which play an important role in colonization and invasion of host tissue.[8,9,10]

The genus Candida consists of a group of heterogeneous organisms with more than 17 different *Candida species.*[11] Although Candida albicans is the most prevalent species involve in infections, the trend towards non-albicans Candida (NAC) species is documented in recent studies.[12,13] Surprisingly, the clinical manifestation caused by NAC species. is 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.[14]

In routine clinical practice very low attention has been laid on the antifungal sensitivity of Candida isolates in Jos, Plateau State. Furthermore, there is inadequate data on the pattern of sensitivity of yeast isolates to commonly used antifungal drugs in our community.

Therefore, the present study is aimed to determine the prevalence and susceptibility pattern of *Candida species* causing vulvovaginal candidiasis among women of reproductive age our local population.

MATERIALS AND METHODS

Study area
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.

Study population
Women within the age groups of 15 to 45 years formed the population of this study. The study period was between September and December 2015.

Ethical clearance
Ethical clearances were sought and obtain from the following hospitals where samples were collected for the study. Plateau state specialist hospital, Faith Alive Foundation and Our Lady of Apostle Hospital all located in Jos metropolis. Specimens were collected from individual who gave consent to be part of the study.

Data collection
Structured questionnaires were used as a source of data collection and were administered to study participants. Information on the age, occupation and marital status were captured in the questionnaire.

Sample collection
A total of 246 high vaginal swab specimens were collected from female with vaginal discharge in the aforementioned hospital using sterile swab sticks. The specimens were transported without any delay to hospital laboratory for analysis.

Isolation and characterization of yeast
Swab specimens were streak-inoculated on Sabouraud dextrose agar (SDA) media containing Chloramphenicol 10% (Plasmatec Laboratory Products LTD, UK), culture plates were incubated for 24-48 hours. Plates showing 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 Candida presence.

Germ tube test
A suspension of pure Candida isolate was made by inoculating a test tube containing 0.5ml of human serum with a loopful of the organism. It was incubated in a water bath for 2-4hours at 37^{o}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 a germ tube while those cells with constriction at the origin of mother cells were noted as pseudohyphae[15].Germ tube and pseudohyphae were positive indication for *Candida albicans*.[16]

Chlamydospore formation test
Test colonies were stab- inoculated on corn-meal agar plate by slide culture technique and was incubated for 72 hours at 25^{0}C. Chlamydospore formation was demonstrated by staining with Lactophenol cotton blue.[16,17] Yeast isolates found to be positive for Chlamydospore formation were further confirmed as *Candida albicans* whereas those showing negative results were regarded as non albicans Candida spp.

Preparation of standardized yeast inoculum
The Clinical and Laboratory Standards Institute guidelines[19] were used to prepare BaSO4 turbidity standard (0.5 McFarland standard). Briefly, 99.5 mL of solution A (1% v/v H_2SO4) 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
The antifungal susceptibility testing for nystatin and fluconazole was based on Clinical Laboratory Standards Institute[19] disc diffusion method. Mueller Hinton glucose methylene blue agar surface was inoculated by using a sterile swab dipped in a standardized Candida cell suspension, it was allowed to dry. The antifungal discs were dispensed on the inoculated SDA plates, sensitivity plates were incubated at 37^{o}C for 24hours. The zone size were measured and interpreted according to CLSI interpretative break point.

Data analysis
Data obtained from the study were analyzed using EPI info Version 3.5.1. $P< 0.05$ was considered statistically significant.

RESULTS

Of the 246 women studied, vaginal candidiasis was found in 47 (19.1%) patients. *Candida albicans* was the predominant specie with a prevalence of 28 (11.4%) while non *Candida albicans* was 19 (7.7%) as shown in figure 1.

The demographic characteristics of study participants were recorded in table1. Results from this table revealed that individuals within the age group 30-34 years recorded the highest occurrence of Candidiasis with *Candida albicans* prevalence 7 (15.6%) and non *Candida albicans* 5 (11.1%). In addition there was no statistically significant difference in the occurrence of candiasis among age groups p>0.05. Regarding occupation and marital status Candida isolates were predominant amongst housewives and married women with a prevalence of 5(31.3%) and 18(15.8%) respectively.

The results of antifungal sensitivity pattern of Candida isolates to fluconazole and Nystatin was shown in table 2. *Candida albicans* exhibit higher sensitivity 23/28 (82.1%) to fluconazole when compared with Nystatin 15/28 (53.6%). Conversely, non *Candida albicans* exhibited high resistant to fluconazole 4/19 4 (21.1%) and Nystatin 2/19 2 (10.5%).

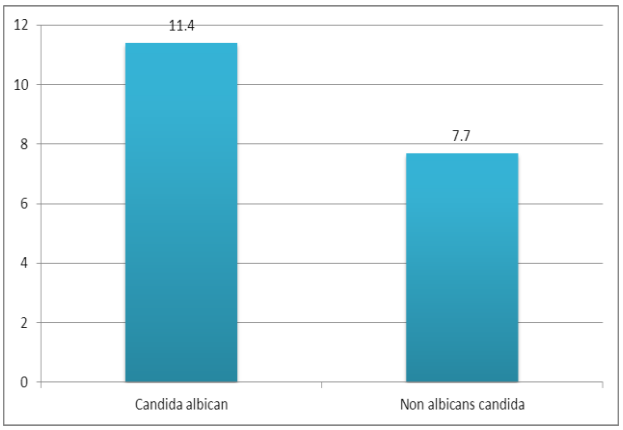

Figure 1: Percentage frequency of Candida species.

Table 1: Demographic characteristics of women with vaginal candidiasis in Jos.

Demographic variables	No. Examined	C. albicans No. Positive (%)	Non Candida albicans No. Positive (%)	P value
Age groups (years)				
15-19	11	1(9.1)	0(0.0)	
20-24	73	6(8.2)	5(1.8)	
25-29	73	8(11.0)	7(9.6)	
30-34	45	7(15.6)	5(11.0)	0.8417
35-39	27	4(14.8)	4(7.4)	
44-44	17	2(11.8)	0(0.0	
Occupation				
Civil servants	63	8(12.7)	3(4.8)	
Business	23	3(13.4)	2(8.7)	
House wife	16	5(31.3)	1(6.3)	0.1884
Student	144	12(8.3)	13(9.0)	
Marital status				
Married	114	18(15.8)	9(7.9)	0.1234
Single	132	10(7.6)	10(7.6)	
Total	**246**	**28(11.4)**	**19(7.7)**	

Table 2: Antifungal sensitivity pattern of Candida isolates to fluconazole and nystatin.

Candida Isolates	No. isolated	Fluconazole No. Sensitive (%)	Nystatin No. Sensitive (%)
Candida albicans	28	23 (82.1)	15(53.6)
Non albicans Candida	19	4(21.1)	2(10.5)
Total	**47**	**27(57.4)**	**17(36.2)**

DISCUSSION

Vulvovaginal candidiasis is a common female genital infection affecting mostly women of child bearing age. Candida colonizes mucous membranes such as the vagina, urinary tract and the oral cavity[20]. However, vaginal candidiasis occurs as a result of multiplication of Candida especially when the physiologic conditions of the vagina are altered.[21]

A prevalence of 19.1% of vaginal candidiasis was recorded in our study. However this value is lower than the 40.6% observed in Edo state, Nigeria[22] and other similar studies elsewhere.[33,34] The reason for this variation in prevalence could be due to the differences in methodology. Nevertheless, our study prevalence is similar to 20.6% reported in Costa Rica[23] and 20.15% reported in Brazil.[24]

Our study further revealed *Candida albicans* as the most frequent yeast isolates. This observation is consistent with reports obtain in Libya,[25] Edo state, Nigeria,[26] Egypt.[33] and Pakistan.[34] On the contrary, a study by Sandra *et al.*, from Iowa city has non albicans Candida as the preponderant yeast isolates.[35]

In relation to the sensitivity pattern of yeasts to antifungal drugs, *Candida albicans* showed more susceptibility to Fluconazole 23/28(82.1%) and Nystatin 15/28(53.6%) compared with the non albicans Candida with 4/19(21.1%) susceptibility to fluconazole and 2/19(10.5) susceptibility to nystatin. This sensitivity rates are comparable to the result of Mona from Egypt[33] who reported a higher resistant in the non albicans Candida isolates than in Candida albicans. In addition, other studies conducted elsewhere have further buttressed our finding.[34]

CONCLUSION

The present study has showed that *Candida albicans* was the predominant yeast isolated from the vagina of sexually active women in Jos. The efficacy of fluconazole against the species of Candida studies has justified its use as a drug of choice for the treatment of vaginitis caused by Candida in our study area.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of the hospitals who gave permission and ethical clearance for this study and appreciate the cooperation of the patients who willingly give consent to be part of the study.

REFERNCES

1. Sobel J. (2007).Vulvovaginal candidiasis. *Lancet*, 369(9577): 1961-71
2. Fidel, P.J. (1999). Host defense against oropharyngeal and vaginal candidiasis: site-specific differences. *Rev Iberoam Micro*, 16(1): 8-15.
3. Mohanty, S., Xess, I., Hasan, F., Kapil, A., Mittal, S., and Tolosa, J.E. (2007). Prevalence & susceptibility to fluconazole of Candida species causing vulvovaginitis. *India J Med Res*, 126(3): 216-219.
4. Sullivan, D., Henman, M., and Moran, G. (1996). Molecular genetic approaches to identification, epidemiology and taxonomy of non-albicans *Candida species*. *J. Med. Microbiology*, 44: 399-408.
5. Eggimann, P., Garbinm, J. and Pittet, D, (2003). Epidemiology of Candida species infections in critically ill non immunosuppressed patients. *Lancet Infectious Disease*, 3: 685-702.
6. Deorukhkar, S., and Saini, S. (2012). Species distribution and antifungal susceptibility profile of Candida species isolated from blood stream infections. *J. Med. Dental. Sc*, 1: 241-249.
7. Sardi, J., Scorzoni, L., Bernardi, T., Fusco Almeida, A, and Mendes Giannini, M. (2013). Candida species: Current epidemiology, pathogenicity, biofilm formation, natural antifugal products and new therapeutic option. *Journal of Medical Microbiology*, 62: 10-24.
8. Sachin, C.D., Ruchi, K and Santosh. (2012). In-vitro evaluation of proteinase, phospholipase and haemolysin activities of Candida species isolated from clinical specimens. *International Journal of Medical and Biomedical Research*, 1: 153-157.
9. Tamura, N., Negri, M., Bonassoli, L., and Svidzinski, T. (2007). Virulence factors for Candida spp. recovered from intravascular catheters and hospital workers hands. *Rev. soc. Bras. Med. Trop*, 40: 91-93.
10. Sardi, J., Gullo, F.P., Pitangui, N.S., Fusco-Almeida, A.M and Mendes-Giannini, M.J.S (2013). In vitro Antifungal Susceptibility of Candida albicans Isolates from Patients with Chronic Periodontitis and Diabetes. *Clin Microbial* 2: 103.
11. Pfaller, M., and Diekema, D. (2007). Epidemiology of invasive Candidiasis: A persistent public health problem. *Clin. Microbiology Rev*, 20: 133-1163.
12. Enoch, D., Ludlam, H., and Brown, N. (2006). Invasive fungal infections: a review of epidemiology and management options. *J. Med. Microbiology*, 55: 809-818.
13. Deorukhkar, S., and Saini, S. (2013 a). Vulvovaginal candidiasis due to non albicans Candida: its species distribution and antifungal susceptibility profile. *Int J Curr Microbiol App Sci*, 2: 323-328.
14. Johnson, E., Warnock, D., Luker, J., Porter, S., and Scully, C. (1995). Emergence of azole resistance in Candida species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. *J. antimicrobial Chemotherapy*, 35: 103-114.
15. Kim, D., Shin, W., Lee, K., Park, J., Koh, C. (2002) Rapid differentiation of Candida albicans and Candida species using its unique germ tube formation at 39 ° C. Yeast, 19: 957-962.
16. R.Rajendran, K. F. (2014). A study of isolation and identification of non albicans Candida species from clinically suspected cases of vulvovaginitis. *Int. J. Curr. Microbiol. App. Sci*, 3(12): 147-159.
17. Sullivan, D and Coleman, D. (1998). Candida dubliniensis: Characteristic and identification J. Clin. *Microbiology* 36:329-334.
18. McCullough, M., Clemons, K., and Stevens D. (1999). Molecular and Phenotypic characterization of genotypic Candida albicans subgroups and comparison with Candida dubliniensis and Candida stellatoidea. *J. Clinc. Microbiology*, 37: 417-421.
19. Clinical and Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion susceptibility testing of yeasts: approved standard, M44-A. Wayne (PA): CLSI; 2004.
20. Moyes, D.L and Naglik, J.R. (2011). Mucosal Immunity and Candida albicans Infection. Clinical and Developmental Immunology.

21. Kauffman, C.A., Fisher, J.F., Sobel, J.D., Newman, C.A. (2011). Candida Urinary Tract Infections-Diagnosis. *Clinical Infectious Diseases*, 52: S452-S456.
22. Esebelahie, N.O., Enweani, I.B., and Omoregie. R. (2013). *Candida* colonisation in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria *Libyan J Med*, 8(10).
23. Gross, N.T., Arias, M.L., Moraga, M., Baddasarow, Y and Jarstrand, C. (2007). Species distribution and susceptibility to azoles of vaginal yeasts isolated prostitutes. *Infect Dis Obstet Gynecol.*, 2007; 82412.
24. Passos, X.S., Sales, W.S., Maciel, P.J., Costa, C.R., Miranda, K.C., Lemos Jde, A., Batista Mde, A and Silva Mdo, R. (2005). Candida colonization in intensive care unit patients' urine. Mem Inst Oswaldo Cruz., 100: 925-928.
25. Ellabib, M.S and ElJariny, I.A. (2001). In vitro activity of 6 antifungal agents on candida species isolated as causative agents from vaginal and other clinical specimens. *Saudi Med J*., 22: 860-863.
26. Enweani, I.B., Gugnani, H.C., Okobia, R., Ojo, S.B. (2001). Effect of contraceptives on the prevalence of vaginal colonization with Candida species in Edo State, Nigeria. *Rev Iberoam Micol.*, 18: 171-173.
27. Citak, S., Ozcelik, B., Cesur, S and Abbasoglu, U. (2005). In Vitro Susceptibility of Candida Species Isolated from Blood Culture to Some Antifungal Agents. *J Infect Dis*, 58(1): 44-6.
28. Badiee, P and Alborzi, A. (2011). Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. *Iran J Microbiol*, 3(4): 183-8.
29. García, H.M., García, S.D., Copolillo, E.F., Cora, E.M., Barata, A.D., Vay, C.A, et al. (2006). Prevalence of vaginal candidiasis in pregnant women. Identification of yeasts and susceptibility to antifungal agents. *Revista Argentina de Microbiología*, 38(1): 9-12.
30. Mukasa, K.J., Herbert, I., Daniel, A., Sserunkuma, K L., Joel, B., and Frederick, B. (2015) Antifungal Susceptibility Patterns of Vulvovaginal Candida species among Women Attending Antenatal Clinic at Mbarara Regional Referral Hospital, South Western Uganda *British Microbiology Research Journal*, 5(4): 322-331.
31. Kalkanci, A, Güzel AB, Khalil IIJ, Aydin M, Ilkit M, Kustimur S. (2012). Yeast vaginitis during pregnancy: susceptibility testing of 13 antifungal drugs and boric acid and the detection of four virulence factors. *Med Mycol*, 50(6): 585-93.
32. Sabatelli, F., Patel, R., Mann, P.A., Mendrick, C.A., Norris, C.C., Hare, R., Loebenberg, D., Black, T.A and McNicholas, P.M. (2006).In vitro activities of Posaconazole, Fluconazole, Itraconazole, Voriconazole, and Amphotericin B against a large collection of clinically important moulds and yeasts. Antimicrob. *Agents Chemother*, 50(6): 2009-2015.
33. Mona, F., Asmaa, N., Ashgan, B., Mohamed, N. E., and Hydi, A.(2015). Antifungal Susceptibility Pattern and Species Distribution of *Candida* Isolates from Patients with Vulvovaginal Candidiasis. International Journal of Advanced Research, 3(5): 1376-1386.
34. Fouzia, K.,and Rakhshanda, B .(2010). In vitroantifungalsensitivity of fluconazole, clotrimazole and nystatin against vaginal candidiasis in females of childbearing age. J Ayub Med Coll Abbottabad, 22(4): 197-200.
35. Sandra, S.R.., Rudolph, P. G., Shawn A. M., Richard, J. H., Daniel, J. D., and Michael, A. P.(2005). Antifungal Susceptibilities of *Candida* Species Causing Vulvovaginitis and Epidemiology of Recurrent Cases. Journal of Clinical Microbiology, 43(5): 2155–2162.