IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 1 Ser. I (Jan. –Feb. 2021), PP 01-04
www.Iosrjournals.Org

Antifungal Susceptibility Profile Of Genitourinary Candida species AMONG Women Attending a Tertiary Hospital In Abakaliki, Nigeria.

[**1.] Nwankwo, C. E., [1.] Orji, J. O., [2.] Opara, J. K., [1.] Oru C. M., [3.] Ugadu, I. O., [1.]Inyogu, J. C., [3.]Ayomoh, E., *[1.]Agumah N. B.

[1]*Department of Applied Microbiology, Faculty of Science; Ebonyi state University, Abakaliki, Nigeria*
[2]*Microbiology unit, Department of Plant Science and Biotechnology, University of Jos. Nigeria.*
[3]*National veterinary research institute (Microbiology unit), Vom; Plateau State, Nigeria.*
***Lead Author*
***Corresponding Author:** agumahbuife@ebsu.edu.ng*

Abstract
This study was aimed at investigating the Antifungal susceptibility profile of Genitourinary Candida species among women attending a tertiary hospital in Abakaliki, Nigeria. One hundred and five swab specimens were collected. Fifty one were endocervical swab specimens (ECS) while Fifty four were vaginal swab specimens (VS). The specimens were analysed using standard microbiological methods. Wet preparations were examined for the presence of yeast cells. Antibiogram of Candida isolates obtained from this study was carried out using commercially available azole drugs. Fluconazole, voriconazole and nystatin were the drugs used for this study. The Kirby Bauer disc diffusion method was employed. Out of 105 specimens collected, 68.6% (n=72) were positive for the presence of Candida. Non Candida albicans Candida was predominant (65.3%) as against C. albicans (34.7%). Fluconazole was sensitive in 34.7% of the isolates and resistant in 65.3%. Voriconazole was sensitive in 81.9% of the isolates and resistant in 18.9%. Nystatin was sensitive in 23.6% of the isolates and resistant in 76.4%. Generally the highest rate of sensitivity was recorded with Voriconazole and the highest rate of resistance was recorded with Nystatin. Prompt diagnosis and employment of very standard techniques in assessing the antifungal susceptibility profile of Candida species is highly recommended.

Date of Submission: 26-12-2020 Date of Acceptance: 08-01-2021

I. Introduction

Candida infections are responsible for increased morbidity and mortality rates in at-risk patients, especially in developing countries where there is limited access to antifungal drugs (Charlene and Pedro, 2017). Candida spp. cause systemic diseases which are the fourth leading cause of nosocomial bloodstream infections in modern hospitals. Recent estimated burden of candida in Nigeria is 9,284 (6/100,000) (Oladele and Denning, 2014). Vulvovaginal candidiasis has a global burden of approximately 134,000,000. Tens of millions are estimated to have mucosal candidiasis. Studies have shown that mortality associated with fungal diseases is greater than 1.6 million and similar to that of tuberculosis. This estimate is 3 fold greater than that of malaria. Socioeconomic, geo-ecological characteristics and increasing number of at-risk populations are the main determinants of variations on incidence and prevalence of candidiasis.

The most challenging clinical problem is the increased rate of non-Candida albicans isolation and the rapidly growing resistance of Candida species (Li et al., 2015). The susceptibility degrees of Candida species towards the used antifungal drugs vary and due to the growing use of these antifungals, resistance to these agents has increased during the last decades. Candida albicans is the most prevalent among Candida spp., which causes both superficial and systemic infections. Other pathogenic Candida species include C. tropicalis, C. glabrata, C. parapsilosis, and C. krusei accounting for 25%, 8%, 7%, and 4% of candidiasis, respectively (Mukherjee and Chandra, 2015). Moreover the environmental stress with exposure to antifungal drugs can mediate resistance (Zaidi et al., 2016). Candidiasis can reoccur repeatedly. Some health-care providers prescribe antifungal drugs on a long-term basis, but this can lead to drug-resistant candidiasis that is more difficult to treat. Therefore, early identification of Candida spp. and monitoring their antifungal susceptibility help in treatment. This study was aimed at studying antifungal susceptibility pattern of genitourinary isolates of Candida species among women attending a tertiary health care centre in Abakaliki.

II. Methodology

IDENTIFICATION OF ISOLATES

Vaginal swab and endocervical swab specimens were collected from women who attended Federal teaching hospital Abakaliki. Methods as adopted from Okongbuwa (2003), Musa et al., 2017 and Khan et al., 2018 were employed. Microscopic examination of the swabs were done by the wet preparation method, 10% potassium hydroxide (KOH) preparation and Gram staining For phenotypic identification, the swab was streaked onto culture plates of SDA (Biofilchem) and Sabouraud dextrose agar with chloramphenicol (Biolilichem), which was then incubated for 48 hours at 27°C. The isolated pure colonies were confirmed on Gram staining for yeast cells. Candida spp. was isolated on CHROMagar Candida (Biofilchem) which is a differential and selective medium. Isolated Candida colonies on SDA were subcultured onto CHROMagar using an inoculating loop and incubated at 37°C for 24 hours. Presumptive identification was done based on colony color of the growing Candida strains. Identification of Candida was based on the color of individual colony. The cultural and morphological characteristics of the yeasts were studied. The yeast isolates were identified using the methods described by (Chijioke et al., 2016; Onianwah, 2014 and Uzoh et al., 2016). The methods used were germ tube test, carbohydrate fermentation, urea hydrolysis.

Antifungal Drug Susceptibility (Disc Diffusion Method)

Colonies of each isolate were picked up with a sterilized wire loop and emulsified in Nutirent broth and the turbidity adjusted to 0.5 McFarland standard. The Mcfarlands standard was prepared by adding 1ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) to 99ml of distilled water and dissolving 0.5g of dehydrated Barium Chloride ($Bacl_2 2H_2O$) in 50ml of distilled water in separate reaction flasks respectively.

Within 15 minutes of adjusting the turbidity, each isolate was plated onto a dried surface of a sterile Mueller-Hinton agar plate respectively using a sterile cotton swab. Muellar Hinton agar (Oxoid Ltd) were prepared according to the manufacturers instruction. The Antimicrobial discs used for this study were commercially prepared (Oxoid Ltd) Antimicrobial disks containing 25 µg of fluconazole, 1ug of Voriconazole and 100ug of Nystatin were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure its complete contact with the agar surface. The plates were incubated at 37ºC and examined after 24 hours of incubation. The zones of inhibition were measured in millimeter and the results were interpreted`using validated CLSI interpretive breakpoints for in vitro susceptibility testing of fluconazole as done by Iroha et al., (2012); Pfaller et al., (2006) and Pam et al., (2012).

Table showing interpretative breakpoints for different drugs used

Drug (Conc)	Susceptible (mm)	Resistant (mm)
Fluconazole (25µg)	≥19	≤14
Voriconazole (1µg)	≥17	≤13
Nystatin (100 µg)	≥19	≤14

III. Results And Discussion

TABLE 1: SHOWING GENERAL FREQUENCY OF ISOLATION OF Candida species FROM VAGINAL AND ENDOCERVICAL SWABS

SPECIMEN	NO COLLECTED	NO POSITIVE (%)	NO NEGATIVE (%)
VS	51	34 (66.7)	17 (33.3)
ECS	54	38 (70.4)	16 (29.6)
TOTAL	105	72 (68.6)	33 (31.4)

TABLE 2: SHOWING DISTRIBUTION VARIOUS Candida spp ISOLATED

Candida species ISOLATED	NO ISOLATED	PERCENTAGE FREQUENCY (%)
C. albicans	25	34.7
C. tropicalis	12	16.6
C. dubliniensis	5	6.9
C. glabrata	18	25.0
C. krusei	12	16.7
	72	100.0

Table 3: General Antifungal Susceptibility Profile of 72 Candida Species Isolated from Clinical Specimens; ECS (Endo-Cervical Swabs) and HVS (High Vaginal Swabs).

ANTIFUNGAL AGENTS	PERCENTAGE RESISTANCE OF CANDIDA SPECIES	PERCENTAGE SUSCEPTIBITY OF CANDIDA SPECIES
FLUCONAZOLE	47 (65.3)	25 (34.7)
VORICONAZOLE	13 (18.1)	59 (81.9)
NYSTATIN	55 (76. 4)	17 (23.6)

Table 4: Antifungal Susceptibility Profile of Candida albicans Isolated from Clinical Specimens; ECS (Endo-Cervical Swabs) and HVS (High Vaginal Swabs).

ANTIFUNGAL AGENTS	PERCENTAGE OF RESISTANCE	PERCENTAGE OF SUSCEPTIBITY
FLUCONAZOLE	16 (64)	9 (36)
VORICONAZOLE	4 (16)	21 (84)
NYSTATIN	18 (72)	7 (28)

Table 5: Antifungal Susceptibility Profile of Candidatropicalis Isolated from Clinical Specimens; ECS (Endo-Cervical Swabs) and HVS (High Vaginal Swabs).

ANTIFUNGAL AGENTS	PERCENTAGE OF RESISTANCE	PERCENTAGE OF SUSCEPTIBITY
FLUCONAZOLE	0 (0)	12 (100)
VORICONAZOLE	0 (0)	12 (100)
NYSTATIN	6 (50)	6 (50)

Table 6: Antifungal Susceptibility Profile of Candida krusei Isolated from Clinical Specimens; ECS (Endo-Cervical Swabs) and HVS (High Vaginal Swabs).

ANTIFUNGAL AGENTS	PERCENTAGE OF RESISTANCE	PERCENTAGE OF SUSCEPTIBITY
FLUCONAZOLE	12 (100)	0 (0)
VORICONAZOLE	2 (16. 67)	10 (83. 33)
NYSTATIN	12 (100)	0 (0)

Table 7: Antifungal Susceptibility Profile of Candida dubliniensis Isolated from Clinical Specimens; ECS (Endo-Cervical Swabs) and HVS (High Vaginal Swabs).

ANTIFUNGAL AGENTS	PERCENTAGE OF RESISTANCE	PERCENTAGE OF SUSCEPTIBITY
FLUCONAZOLE	1 (20)	4 (80)
VORICONAZOLE	0 (0)	5 (100)
NYSTATIN	1 (20)	4 (80)

Table 8: Antifungal Susceptibility Profile of Candida glabrata Isolated from Clinical Specimens; ECS (Endo-Cervical Swabs) and HVS (High Vaginal Swabs).

No. of Candida glabrata=18

ANTIFUNGAL AGENTS	PERCENTAGE OF RESISTANCE	PERCENTAGE OF SUSCEPTIBITY
FLUCONAZOLE	18 (100)	0 (0)
VORICONAZOLE	7 (38. 89)	11 (61.11)
NYSTATIN	18 (100)	0 (0)

Out of 105 ECS and VS specimens, 72 Candida isolates were obtained. C. albicans had the highest frequency of isolation (34.7%) followed by C. glabrata (25.0%), C. krusei (16.7%), C. tropicalis (16.6%) and C. dubliniensis (6.9%). Hence Non Candida albicans Candida was predominant (65.3%) as against C. albicans (34.7%). This agrees with findings by Oli et al (2017). In their study Non Candida albicans Candida were the predominant etiological agents (66.67%) of the vulvovaginal candidiasis (VVC) among female undergraduates in a Nigerian University.

Fluconazole was sensitive in 34.7% of the isolates and resistant in 65.3%. Voriconazole was sensitive in 81.9% of the isolates and resistant in 18.9%. Nystatin was sensitive in 23.6% of the isolates and resistant in 76.4%. Generally the highest rate of sensitivity was recorded with Voriconazole and the highest rate of resistance was recorded with Nystatin. Our findings disagree with findings from a study by Efunshile et al (2016). They recorded 0% resistance for Fluconazole in a study at Ogbomosho. A study by Munmun and Dhanashree (2018) reported 18.8% resistance for voriconazole and 24.4% resistance to fluconazole

Our observation agrees with findings from various studies. Adesisji et al. (2011) recorded 73% resistance to Fluconazole and 61.5% resistance to Voriconazole. In their study they observed 42.3% susceptibility to Nystatin. Our findings agree with that of Khan et al (2018). Candida species isolated in their study were most susceptible to voriconazole (89.8%). Fluconazole and Nystatin recorded relatively low susceptibility rates (38% and 37% respectively).

Results from our study shows that in C. albicans isolated, Fluconazole was sensitive in 36% and resistant in 64%. Voriconazole was sensitive in 84% and resistant in 16%. Nystatin was sensitive in 28% and resistant in 72%. In C. tropicalis, Fluconazole and voriconazole was sensitive in all the isolates. Nystatin was sensitive in 50% and resistant in 50%. In C. krusei, Fluconazole and Nystatin were resistant in all the isolates. Voriconazole was sensitive in 83.33% and resistant in 16.67%. In C. dubliniensis, Fluconazole was sensitive in 80% and resistant in 20%. Voriconazole was sensitive in all the isolates. Nystatin was sensitive in 80% and resistant in 20%.

Unlike in our study, a study by Khan et al., 2018 reported that in C. albicans, Fluconazole was sensitive in 46.7% and resistant in 53.3%. Nystatin was sensitive in 44.5% and resistant in 55.5% while voriconazole recorded a low resistance of 6.6%. In C. tropicalis, Fluconazole was sensitive in 61.2% and resistant in 38.8%. Nystatin was sensitive in 61.2% and resistant in 38.8% while voriconazole recorded a low resistance of 5.5%. In C. krusei, Fluconazole was resistant in all the isolates 100%. Nystatin was sensitive in 50.0% and resistant in 50.0% while voriconazole recorded resistance of 11.1% of the isolates. In C. glabrata, Fluconazole was sensitive in 37.5% and resistant in 62.5%. Nystatin was sensitive in 31.3 and resistant in 68.7% while voriconazole recorded a resistance of 18.7%.

Reduced susceptibility to some of the antifungal drugs especially Fluconazole and Nystatin shown in our study is a pointer to a trend reported by other scientists that Fluconazole resistance over the decades is gradually becoming a menace. Fluconazole has always been regarded as the most commonly available antifungal. Resistance to fluconazole could be attributed to abuse owing to non-compliance or over exposure on the side of the patient. Genetic flexibility of the Fungus could also be a contributory factor. Prompt diagnosis and employment of very standard techniques in assessing the antifungal susceptibility profile of Candida species is highly recommended as various strains may pose different reactions to different anitfungals.

References

[1]. Adesiji, Y. O., Ndukwe, N. and Okalawon, (2011). Isolation and antifungal sensitivity to candida isolates in young females central. European Journal of Medicine,**6:**172-176.

[2]. Charlene, W. J and Pedro, M. D. S (2017). Candida antifungal drug resistance in sub-Saharan African populations: A systematic review. *F1000Research*,**5**:2832.

[3]. Efunshile, A. M., Oduyebo, O., Osuagwu, C.S. and Koenig, B. (2016). Species distribution and antifungal susceptibility pattern of candida isolates from pregnant women in a tertiary hospital in Nigeria. *African Journal of Clinical and Experimental Microbiology*,**3:**183-189.

[4]. Oladele, R. O and Denning, D. W (2014). Burden of serious fungal infection in Nigeria. *West African Journal of Medicine* **33**(2):107-14.

[5]. Pam V. K., Akpan, J. U., Oduyebo, O. O., Nwaokorie, F. O., Fowora, M. A., Oladele, R. O., Ogunsola, F. T and Smith, S. I (2012). Fluconazole susceptibility and ERG11gene expression in vaginal candida species isolated from lagos Nigeria. *International Journal of Molecular Epidemiology and Genetics*. **3**(1): 84–90.

[6]. Li, X., Hou, Y., Yue, L., Liu, S., Du, J and Sun, S (2015). Potential targets for antifungal drug discovery based on growth and virulence in *Candida albicans*. Antimicrobial Agents and Chemotherapy;**59**(10)5885–5891.

[7]. Munmun, B. M and Biranthabail, D. (2018). Antifungal Susceptibility and Biofilm Production of Candida spp. Isolated from Clinical Samples. *International Journal of Microbiology*; **10**:1-5.

[8]. Zarei, M. A., Zarrin, M and Beheshti, F. M (2013) Antifungal Susceptibility of *Candida* Species Isolated From Candiduria. *Jundishapur J Microbiol*.;**6**(1):24-8.

[9]. Zaidi, K. U., Mani, A. , Thawani, V., Mehra, A (2016). Total protein profile and drug resistance in *Candida albicans* isolated from clinical samples. *Mol Biol Int* 2016: 4982131.

[10]. Musa F. M., Ado A., Yakasai M. A (2017). Prevalence of Candidiasis in Non-pregnant women attending Yusuf Dansoho Memorial hospital in Kaduna state Nigeria. *Bayero Journal of Pure and applied sciences* **10**(1): 180-183.

[11]. Okungbowa, F. I., Isikhuemhen, O. S and Dede, A. P. O (2003). The distribution frequency of *Candida* species in the genitourinary tract amongsymptomatic individuals in Nigerian cities. *Rev Iberoam Micol*; **20**: 60-63.

[12]. Oniawah, I. F (2014). The Incidence and Prevalence of Candida albicans infection of the urogenital tract of females between the ages of 18 and 45 years old: A Case study of Patients receiving treatment in Ashford and Patrice clinic in Port Harcourt. *International Journal of environmental sciences.* **3**(4): 101-104.

[13]. Uzoh, C. V., Iheakwumere, I. U., Unezurike, K. C. and Onyewenjo, S. C. (2016). Prevalence of Candida albicans among women attending Federal Micheal Centre Asaba, South South, Nigeria. *Advances in Life Sciences and Technology*,**41**:54-58

[14]. Oniawah, I. F (2014). The Incidence and Prevalence of Candida albicans infection of the urogenital tract of females between the ages of 18 and 45 years old: A Case study of Patients receiving treatment in Ashford and Patrice clinic in Port Harcourt. *International Journal of environmental sciences.* **3**(4): 101-104.

[15]. Chijioke, A. N., Obijuru, C. E and Ohalete, C. V (2016). "High Prevalence of Candida albicans Observed in Asymptomatic Young Women in Owerri, Nigeria." *Biomedicine and Biotechnology.* **4**(1): 1-4.

[16]. Pfaller, M. A., Diekema, D. J and Sheehan, D. J (2006) Interpretive breakpoints for fluconazole and Candida revisited: a blueprint for the future of antifungal susceptibility testing. *Clinical Microbiological Reviews*; **19**:435–47.

[17]. Iroha, I. R., Esimone C. O., Neumann, S., Marlinghaus, L., Korte, M., Szabados, F., Gatermann, S and Kaase, M (2012). First description of Escherichia coli producing CTX-M-15- extended spectrum beta lactamase (ESBL) in out-patients from south eastern Nigeria. *Annals of Clinical Microbiology and Antimicrobials* **11**:19.

[18]. Khan, M., Ahmed, J., Gul, A., Ikram, A and Lalani F. K (2018). Antifungal susceptibility testing of vulvovaginal *Candida* species among women attending antenatal clinic in tertiary care hospitals of Peshawar. *Infection and Drug Resistance* **11**;447–456.

Nwankwo C. E. "Antifungal Susceptibility Profile Of Genitourinary Candida species AMONG Women Attending a Tertiary Hospital In Abakaliki, Nigeria." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 16(1), (2021): pp. 01-04.