EPIDEMICS AND SUSCEPTIBILITY OF DRUGS USED ON VULVOVAGINITIS IN WOMEN IN FOUR STATES OF NORTH CENTRAL NIGERIA

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ABSTRACT

The occurrence of Vulvovaginal Candidiasis (VVC) and antifungal resistance to antifungal agents during the last two decades has increased.This study determined the specie distribution and their antifungal susceptibility pattern from cases of vulvovaginitis in women using contraceptives in four selected States of North Central Nigeria. A total of 1600 High Vaginal Swabs (HVS) were collected from women using contraceptive device from the study area. Inoculation and culture was made unto Sabouraud Dextrose Agar (SDA) medium and CHROM agar. Colonies were examined using direct, lactophenol cotton blue and Germ tube test. Susceptibility test to antifungals (Nystatin, Voriconazole, Fluconazole) was performed with commercially prepared antifungal disks. Five species of Candida were isolated from 710 women with Vulvovaginal Candidiasis. *Candida albicans* was the most frequent isolate which accounted for 43.23% of the species isolated. Out of the non-albicans *Candida* species 19.01% were *C. glabrata,* 15.77% for *C. tropicalis*, 8.87% for *C. parapsilosis* and *C. krusei* accounted for 13.09% of the total isolates. *C. albicans* was 100% susceptible to Nystatin, 92.1% susceptible to Voriconazole, and 71.0% susceptible to fluconazole while 28.9% was resistant to fluconazole. All isolates of *C. glabrata* were 100% susceptible to Nystatin, Voriconazole and Fluconazole. *Candida tropicalis*was susceptible to nystatin (53.5%), voriconazole (85.7%) and fluconazole (100%). However, some isolates of *C. tropicalis* were 46.4% and 14.3% resistant against nystatin and voriconazole. *Candida parapsilosis* was 100% susceptible to nystatin and fluconazole while 82.5% were susceptible to voriconazole. *Candida krusei* was 100% susceptible to Nystatin but 100% resistant to Voriconazole and Fluconazole. The need for confirmation of *Candida* species and routine antimicrobial susceptibility testing before initiation of therapy is highly recommended.

Key Words: Resistance, *Candida albicans*, Vulvovaginitis, Antifungal, Nystatin

INTRODUCTION

Vulvovaginal candidiasis (VVC) is a common fungal infection among adult women within reproductive ages. *V. candidiasis* affects millions of women every year and has been considered an important public health problem. In most developing countries such as Nigeria, *V. candidiasis* is still received with little attention since it is considered to be a trivial disease. However, *V. candidiasis* has been identified as a global issue of concern due to its association with direct and indirect economic costs, mental distress, pain, great discomfort, sexually transmitted infections, particularly HIV and ascending genital tract infection (Sobel, 2007; Bitew and Abebaw, 2018).

Candida species are the leading cause of mycoses worldwide with significant crude mortality and morbidity rates (Bruder-Nascimento *et al.,* 2010; Bhooshon*et al.,* 2015). *Candida* species are a part of the complex endogenous vaginal microflora; however, under morphogenesis inducing conditions, their number increases rapidly in the vulva and vagina causing profuse 'cottage cheese like' discharge, pruritus and frequent and uncontrollable urination.

Various antifungal drugs with different modes of action have been developed over the years. However, invasive fungal infections and antimicrobial resistance among Cand*ida* species involved in *V. candidiasis* continues to rise despite the introduction of newer antifungal drugs for the treatment of infections by *Candida* species (Pfaller,2012).The rise in antifungal drug resistance is serious public health concern and has been attributed to the prolong use and/or abuse of antimicrobial drugs and inadequate diagnosis. Hence, this study investigates the antifungal susceptibility pattern of *Candida* species from cases of vulvovaginitis in women using contraceptives in four selected States of North Central Nigeria thereby providing relevant information on antifungal drug susceptibility for *Candida* species which has become imperative for the detection of resistance as well as effective treatment for patients.

MATERIALS AND METHODS

High vaginal swabs (HVS)were collected from women with the assistance of a gynaecologist in the hospitals visited. A total of one thousand six hundred (1,600) HVS specimens were collected from women using different contraceptive methods between the age group of 18-45 years from the study area. Sampling was carried out between January, 2018 and December, 2018. The samples were collected by means of sterile swabs provided to each participant in the study area. The samples collected and placed in the swab stick containers in ice packs and transported to the laboratory for

analysis within one hour of collection for culturing.

Each vaginal swab was inoculated onto to Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol and incubated at 37 ^{0}C for 24 – 48 hours. *Candida species* were identified through direct examination, lactophenol cotton blue examination with the microscope, colony morphology and germ tube test (Larone, 2002;Marinho *et al*., 2010; Menza*et al.,* 2013). Purified single culture from Saboraud dextrose agar (SDA) was inoculated on CHROM agar using an inoculating loop and incubated at 37^{o}C for 48 hours. *Candida* isolates were classified according to their colours on CHROM agar based on the manufacturer's protocol.

Antifungal susceptibility test was carried out on each fungal isolate using disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS, 2004). Fungal suspensions were prepared by emulsifying 3–5 pure cultures into mycological broth and adjusted to 0.5 McFarland standards. A sterile cotton swab was then dipped into the suspension and swabbed on surface of Mueller-Hinton agar plate. Standard antifungal discs were placed aseptically and the inoculated Mueller Hinton agar plates were incubated at 37^0 C for 24 hours. The diameters of zones of inhibition were measured in millimeters using a meter rule for each antifungal disc. Interpretation of all antifungal susceptibility were carried out and interpreted according to NCCLS 2004 document. The diameters of the zones of complete inhibition were measured using measuring calipers. The isolate zone of inhibition was reported based on NCCLS standard as Susceptible, Intermediate and Resistant. The antifungal drugs used were nystatin (10 mcg,) voriconazole (10 mcg) and fluconazole (10 mcg).

RESULTS

Five species of Candida isolated from 710 women with vulvovaginal candidiasis. *Candida albicans* was the most frequent isolate which accounted for 43.23% of the species isolated. Out of the non-albicans *Candida* species 19.01% were *C. glabrata,* 15.77% for *C. tropicalis*, 8.87% for *C. parapsilosis* and *C. krusei* accounted for 13.09% of the total isolates as shown in Table 1. The overall drug susceptibility pattern of Candia species against the three antifungal drugs tested is shown in Table 2.

C. albicans was 100% susceptible to nystatin, 92.1% susceptible to voriconazole, 71.0% susceptible to fluconazole and 28.9% resistant to fluconazole. All isolates of *C. glabrata* were 100% susceptible to nystatin, voriconazole and fluconazole. *C. tropicalis* was suscepti-

Table 1. Species distribution of Candida isolates from 710 patients with Vulvovaginal candidiasis

Species	Number of isolates	% of the total isolates
C. albicans	307	43.23
C. glabrata	135	19.01
C. tropicalis	112	15.77
C. parapsilosis	63	8.87
C. krusei	93	13.09
Total Isolates	710	100

(X^2 =41.61, df =3, P < 0.05)

ble to nystatin (53.5%), voriconazole (85.7%) and fluconazole (100%). However, some isolates of *C. tropicalis* were 46.4% and 14.3% resistant against nystatin and voriconazole. *C. parapsilosis* was 100% susceptible to nystatin and fluconazole. 82.5% were susceptible to voriconazole. *C. krusei* was 100% susceptible to nystatin but 100% resistant to voriconazole and fluconazole.

DISCUSSION

Several studies on the prevalence of *Candida* species have led to the general agreement that *C. albicans* is the most commonly isolated species in patients with vulvovaginal candidiasis. This study reveals that *C. albicans* had the highest occurrence of 43.23% and this may be due to the fact that *C. albicans* are capable of forming hyphae in the murine vagina, Candidalysin expression and consequently activating the NLRP3 inflammasome, which contributes to elicit to robust immunopathogenicity (Hubertine*et al.,* 2018).

Our finding of *C. albicans* as the most major species is in concordance with several studies carried out earlier by Jimoh *et al*.,(2016), Nnadi and Singh, (2017), Mnge *et al*.,(2017), Sasikala and Udayasri, (2018),Lavanya *et al*.(2019) who reported higher rates of 48.5%, 49.1%,45.4%, 46.1% and 42.8%in the

Table 2: *In vitro* antifungal susceptibility pattern of the isolates (n= 710)

Species	Antifungal agent	% Susceptibility of the isolates	% intermediate region of the isolates	% resistant of the isolates
C. Albicans (307)	Nystatin	307(100)	0 (0)	0 (0)
	Voriconazole	283(92.1)	24 (7.81)	0 (0)
	Fluconazole	218(71.0)	0 (0)	89 (28.9)
C. glabrata(137)	Nystatin	135(100)	0 (0)	0 (0)
	Voriconazole	135(100)	0 (0)	0 (0)
	Fluconazole	135(100)	0 (0)	0 (0)
C. Tropicalis (112)	Nystatin	60(53.5)	0 (0)	52(46.4)
	Voriconazole	96(85.7)	0 (0)	60(14.3)
	Fluconazole	112(100)	0 (0)	0 (0)
C. parapsilosis (63)	Nystatin	63(100)	0 (0)	0 (0)
	Voriconazole	52(82.5)	11(17.4)	0 (0)
	Fluconazole	63(100)	0 (0)	0 (0)
	Nystatin	93(100)	0 (0)	0 (0)
C. krusei (93)	Voriconazole	0 (0)	0 (0)	93(100)
	Fluconazole	0 (0)	0 (0)	93(100)

Plate 1: Growth of *Candida albicans* and *C. krusei* on CHROM agar

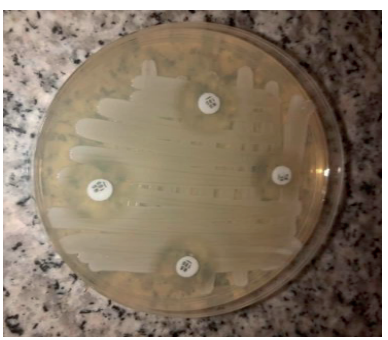

Plate 2: Susceptibility to antifungal discs on Mueller Hinton glucose methylene blue agar

United states, Nigeria, South Africa and India.

Candida albicans has been reported to be the most prominent species isolated from clinical samples of patients diagnosed with VVC; however, there has been a notable shift in the etiology of candidiasis with non-*albicans Candida* (NAC) species gaining prominence in recent times. Although *C. albicans* was the most common species isolated in this present study, the frequency of non-albicans *Candida* species isolated was 56.74% and this may be attributed to immunosuppression or uncontrolled diabetes. In separate studies conducted by Haleim *et al*., (2015) and Jhinuk *et al.,* (2015), the recovery rate of non-albicans *Candida* species were 57.5% and 62% in Iran and India respectively. Deorukhkar and colleagues also reported that NAC species accounted for over 60% of their isolates in a similar study conducted in India (Deorukhkar *et al.,* 2014).

Of the non-albicans *candida* species, *C. glabrata* had the highest prevalence of 19.01%.

This may be attributed to the ability of *C. glabrata* to survive in macrophages as an immune evasion strategy, thus avoiding the innate immune response to pathogens.

This adaptation to intracellular survival is related to its ability to prevent toxic phagolysosome environments by modifying its phagosome, suppressing Reactive Oxygen Species (ROS) production and producing minimal pro inflammatory response (Kasper *et al.,* 2015).

This may be attributed to the excessive use of azole drugs which has promoted drug resistance, hence, a higher prevalence in VVC patients. The studies of Trama *et al.,* 2005, Hasanvand *et al.,* 2017 and Gharaghani *et al.,* 2018 demonstrated that *C. glabrata* was the predominant yeast among the non-albicans *Candida* species with recovery rates of 14.3%, 20%, 7% in the United States of America and Iran.

The drug susceptibility profile of *Candia* species isolated in this study was tested against nystatin, voriconazole and fluconazole. All isolates showed varying susceptibility pattern to the three antifungal drugs. *C. albicans* was 100% susceptible to nystatin, 92.1% susceptible to voriconazole, and 71.0% susceptible to fluconazole. All isolates of *C. glabrata* were 100% susceptible to nystatin, voriconazole and fluconazole and this agrees with the findings of Bitew and Abebaw (2018).*C. tropicalis*was susceptible to nystatin (53.5%), voriconazole (85.7%) and fluconazole (100%). Similarly, *c. parapsilosis* was 100% susceptible to nystatin and fluconazole, 82.5% were susceptible to voriconazole. *C. krusei* was 100% susceptible to nystatin. The susceptible pattern to these antifungal drugs in this study may be an

indication that these drugs are still potent for the treatment of VVC.

C. albicans had a 28.9% resistance to fluconazole in this study. This resistance may be due to the fact that fluconazole is mostly administered as the first drug of choice in treating VVC, hence misuse of the drug may have resulted in resistance. This could probably be due to prolong use of fluconazole in treating VVC by the women. Our findings in this study do not correlate with the report of Ejike*et al.,* 2018 who recorded 0% resistance by all *Candida* species to fluconazole.In separate studies carried out in India, Ruchi *et al*., 2018 reported a higher resistance of 40.6% to fluconazole by *C. albicans* while Zaidi *et al*., 2018 recorded a lower resistance of 13.3% resistance to fluconazole by *C. albicans*.

Candida krusei was 100% susceptible to nystatin but 100% resistant to fluconazole and voriconazole. This result suggests the need for the synthesis of alternative antifungals while nystatin is used for treatment of VVC caused by *C. krusei*. *C. krusei* is reported to be intrinsically resistant to fluconazole (Lyon *et al.,* 2010; Alexander *et al*., 2017) and this was consistent with our findings as *C. krusei* showed 100% resistance to fluconazole and voriconazole. In a similar study, Khan *et al*., (2018) also reported that *C. krusei* showed 100% resistance to fluconazole in India. Mukasa *et al.,* 2015 recorded 71.43% resistance to fluconazole in Uganda.

Antifungal resistance to antifungal agents may be due to quantitative or qualitative modifications of target enzymes, low access of the drug to the target, or a combination of these mechanisms. Incomplete course of therapy may eliminate the more sensitive *c. albicans* and allow selection for more resistant species. The sale of antimicrobial medications is poorly regulated in Nigeria and is exacerbated by the influx of fake and adulterated drugs with little or no active ingredients, often available both in pharmacies and in the streets. The increased use of these antifungal drugs inappropriately may be responsible for resistance.

CONCLUSION

Candida identification to species level and antifungal susceptibility tests is rarely made in clinical settings in Nigeria owing to the fact that the procedure is relatively expensive, hence, patients are treated empirically based on their clinical symptoms. The need for confirmation of *Candida* species and routine antimicrobial susceptibility testing before initiation of therapy will go a long way in preventing the progression of drug resistance.

REFERENCES

Alexander, B.D., Procop, G.W., Dufresne, P., Fuller, J., Fothergill, A.W., Ghannoum, M.A., Hanson, K.E., Holliday, D. and Ostrosky Zeichner, L. (2017).*Performance Standards for Antifungal Susceptibility Testing of Yeasts*,1st ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 1–14.

Bhooshon, S., Gayal, A., Agrawa, A. and Verma, V. (2015).Prevalence and drug resistant of *Candida* species in pediatrics patients in Tertiary Care Hospital, North India. *J Microbiol Biomed Res.***1**: 1–6.

Bitew, A. and Abebaw, Y(2018). Vulvovaginal candidiasis: species distribution of Candida and their antifungal susceptibility pattern. *BioMedCentral Women's Health.***18**:94.

Bruder-Nascimento, A., Camargo, C.H., Sugizak, M.F., Sadatsune, T., Montelli, A.C. and Mondelli, A.L. (2010). Species distribution and susceptibility profile of *Candida* species in a Brazilian public tertiary hospital. *BMC Res Notes*. **3**: 1–5.

Deorukhkar, S.C., Saini, S. and Mathew, S. (2014). Non-*albicans Candida* Infection: An Emerging Threat. *Interdiscipline Perspectives onInfectious Diseases.*1–7.

Ejike, C. E., Agbakoba, N. R., Ezeanya, C. C., Emele, F. E., Oguejiofor, C. B. and Dirisu, J. (2018). Antifungal susceptibility and test for cure of *Candida* species among vulvovaginal candidiasis patients in a secondary care hospital, Nigeria. *African Journal of Clinical and Experimental Microbiology.* **19 (1)**: 30-37.

Haleim, M.M.A., El-Feky, A.M., Sayed, A., Ismail, D.K., Sayed, A.M. and Abdella, R.M.(2015). Provence of non albicans species associated with vulvovaginal candidiasisin Egyptian women. *International Journal* of *Advanced Health Sciences.***12**:304–13.

Hasanvand, S., Qomi, H.A., Kord, M. and Didehdar, M.(2017). Molecular epidemiology and in vitroantifungal susceptibility of Candida isolates from women withvulvovaginalcandidiasis in northern cities of Khuzestan Province.*Iran Jundishapur Journal of Microbiology.***10**(8):12804.

Gharaghani, M., Bahram, A., Marzie, T.S., Owrange, I.,Shahintaj, A., Fariba, M., Zohreh, B., Shohreh, R., Haniyeh, M. and Sadegh, N. (2018). Identification of *Candida* Species Isolated from Vulvovaginal Candidiasis Patients by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in Yasuj Southwestern Iran. *Jundis-*

hapur Journal of Microbiology. **11(8) :**e65359.

Hubertine, M.E.W., David, J. L., Katherine, S. B., Glen, E. P., Brian, M. P. (2018). A comparative analysis of the capacity of the *Candida* species to elicit vaginal immunopathology. *Infection and Immunity.* 1-29 doi:10.1128/IAI.00527-18.

Jhinuk, B.M., Tapan, M., Jayanta, R. and Samir, K. S. (2015).“Changing Trends of Candida Isolates and their Antifungal Susceptibility Pattern in Vulvovaginal Candidiasis Cases o f Tripura, North East India.”*Journal of Evolution of Medical and Dental Sciences* **4(94)**; 15918-15922.

Jimoh, O., Inabo, H.I., Yakubu, S.E., Ankuma, S.J. and Olayinka, A.T. (2016).Prevalence and Speciation of Non-albicanVulvovaginal Candidiasis in Zaria.*Journal of Natural Science and Research.*.51–56.

Kasper, L., Seider, K. and Hube, B. (2015). Intracellular survival of *Candida glabrata*in macrophages: Immune evasion and persistence. *FEMS Yeast Research.***5**; 1-49.

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.

Larone, D.H. (2002). Medically important fungi: a guide to identification. (4th ed.), ASM Press, Washington, D.C. pp 409.

Lavanya, V., Pavani, P. and Kailasanatha, R. B. (2019). Speciation and antifungal susceptibility pattern of Candida isolates from vulvovaginitis patients attending a tertiary care hospital in South India. *IAIM* .**6(2):**62-68.

Lyon, G.M., Karatela, S., Sunay, S. and Adiri, Y. (2010). Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. *Journal of Clinical Microbiology.* **48**:1270–1275.

Marinho, S.A., Teixeira, A.B., Santos, O.S., Ricardo, F.C., Ferreira, R.F., Cherubini, K. and de Oliveira, I.S.D (2010).Identification of *Candida* spp. by phenotypic tests and PCR. *Brazilian Journal of Microbiology.***41**:286-294.

Menza N, Wanyoike W, Muturi, W.N (2013). Prevalence of Vaginal candidiasis and determination of the occurrence of Candida species in pregnant women attending the antenatal clinic of Thika district hospital, Kenya.*Open Journal of Medical Microbiology*. **:** (4), 1-9.

Mnge, P., Okeleye, B.I., Vasaikar, S.D. and Apalata,T. (2017).Species distribution and antifungal susceptibility patterns of *Candida* isolates from a public tertiary teaching hospital in the Eastern Cape Province, South Africa.*Brazilian Journal of Medical and Biological Research.* **5**;(6). doi.org/10.1590/1414431x20175797.

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. *Brazilian Microbiology Research Journal*. 322.

National Committee for Clinical Laboratory Standards.(2004). *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline.* NCCLS document M44-A [ISBN 1-56238-532-1]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Nnadi, D.C. and Singh, S. (2017). The prevalence of genital *Candida* species among pregnant women attending antenatal clinic in a tertiary health center in North-west Nigeria.*Sahel Medical Journal.***20**:33-37.

Pfaller, M.A.(2012). Antifungal drug resistance: Mechanisms, epidemiology, and consequences for treatment. *Am J Med.***125**:S3-13

Ruchi, G., Surinder, S., Amarjit, K. G., Amandeep, K. and Ivneet, K. (2018). Speciation, characterization and antifungal susceptibility pattern of candida species. *International Journal of Contemporary Medical Research*.**5(5)**:E1-E4.

Sasikala, G. and Udayasri, B.(2018). Speciation and antifungal susceptibility profiles of *Candida* isolates from vaginitis patients attending STD Clinic at a Tertiary Care Hospital. *Journal of NTR University of Health Science.***7:**94-97.

Sobel, J.D. (2007). Vulvovaginal candidiosis. *Lancet.***369**:1961-71.

Trama, J.P., Adelson, M.E., Raphaelli, I., Stemmer, S.M. and Mordechai, E. (2005).Detection of Candida species in vaginal samples in a clinical laboratory setting. *Infectious Diseases in Obstetrics and Gynecology.***13**: 63–67.

Zaidi,U. K., Abin, M., Richa, P. and Vijay,T. (2018**).**Antifungal Susceptibility Pattern of *Candida albicans* in Human Infections. *Open Biological Sciences Journal*. **4**: 1-6.