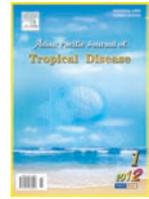




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi:10.1016/S2222-1808(12)60011-5

Genotyping and fluconazole susceptibility of *Candida albicans* strains from patients with vulvovaginal candidiasis in Jos, Nigeria

Nnadi Nnaemeka Emmanuel^{1*}, Orazio Romeo², Ayanbimpe Grace Mebi¹, Ojogba Okolo Mark³, Fabio Scordino², Enweani Ifeoma Bessy⁴, Giuseppe Criseo²

¹Department of Medical Microbiology, University of Jos, Jos, Plateau State, Nigeria

²Department of Life Sciences "Marcello Malpighi", Section of Microbiological, Genetic and Molecular Sciences, University of Messina, Messina, Italy

³Department of Medical microbiology, Jos University Teaching Hospital, Jos, Plateau State, Nigeria

⁴Faculty of Health Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

ARTICLE INFO

Article history:

Received 22 November 2011

Received in revised form 27 November 2011

Accepted 20 December 2011

Available online 28 February 2012

Keywords:

Candida albicans

Genotypes

Sensitivity

Fluconazole

Polymerase chain reaction

ABSTRACT

Objective: To investigate the fluconazole susceptibility of the genotypes of *Candida albicans* (*C. albicans*) strains isolated from patients with vulvovaginal candidiasis (VVC) in Jos, Plateau State, Nigeria. **Methods:** A total of one hundred and seventy seven (177) *Candida* isolates were examined. The strains were obtained from 320 female patients with VVC and were identified using both phenotypic and molecular methods. Their genotypes were determined, based on the presence or absence of a transposable intron in the 25S rDNA. **Results:** Eighty four (84) strains were recognized as being *C. albicans* and all the 84 *C. albicans* strains resulted to be genotype A. Antifungal susceptibility testing showed that 13 of those isolates (15.48%) were resistant to fluconazole. **Conclusions:** Based on these data, we concluded that *C. albicans* genotype A was common among VVC patients in Jos, and resistance to fluconazole is quite high. To our knowledge, this is the first study that reports *C. albicans* genotypes in Jos and its resistance to fluconazole.

1. Introduction

Vulvovaginal candidiasis (VVC) is a common vaginal infection, affecting up to 75% of women of child-bearing age at least once in their lifetime, and approximately 5% of them will have recurrent VVC. On the basis of the severity of symptoms, frequency, causative agents and host factors, VVC is usually classified as either uncomplicated (mild and sporadic) or complicated [recurrent, severe, or caused by non-*Candida albicans* (*C. albicans*) species][1–3]. Approximately 10–20% of women will have complicated VVC[1,4]. The most frequent cause of VVC is *C. albicans*, which is responsible for 70 to 90% of vulvovaginitis cases. Non-*C. albicans* species of *Candida*, predominantly *Candida glabrata*, are responsible for the remainder of cases[5]. Ten to 20% of women suffer complicated VVC in

their lifetime[2]. When properly diagnosed, uncomplicated VVC may be treated easily and reliably. However, complicated VVC often causes long-term physical and mental discomfort, significant economic burden from treatments, and considerable negative effect on sexual relations[2].

The genetic differences of *C. albicans* strains causing different conditions of VVC are still poorly understood. It is unknown whether genotypes of *C. albicans* strains correlate with severity or other conditions of VVC[5]. As the pathogenicity and antifungal susceptibility of *C. albicans* often vary among strains, identification of the disease-causing strains is crucial for diagnosis, clinical treatment and epidemiological investigation. Only few studies have been conducted to investigate the genetic diversity of *C. albicans* strains recovered from VVC and the correlation with antifungal susceptibility[6–8].

Recently, several new techniques have been explored to genotype the *C. albicans*, for example, polymerase chain reaction melting profile (PCR-MP)[7], high-resolution DNA melting (HRM) analysis[8], Multilocus sequence typing

*Corresponding author: Nnadi Nnaemeka Emmanuel, Department of Medical microbiology, University of Jos, Jos, Plateau State.

Tel: +234-8068124819; +234-8182803507

E-mail: nnadi.emmanuel@yahoo.com

(MLST), microsatellite length polymorphism (MLP)[9]. Although these genotyping techniques show high discriminatory potential at strain level, they are time consuming or highly expensive.

Studying the genetic relatedness of clinical strains of *Candida* species that cause recurrent VVC may have significance in clinical management. Genotyping of strains may help to distinguish vaginitis relapse due to inadequate treatment or from de novo infection by a new strain. In this study, A PCR-based method with specific primers to amplify the regions of the transposable group I intron of 25S rDNA gene developed by MuCullough *et al*[10] which is easy and quick to be performed with low cost was used to characterize the genotypic distribution of *C. albicans* affecting women with VVC in Jos, Plateau State and to determine their susceptibilities to fluconazole.

2. Materials and methods

2.1. Isolates and type strains

Clinical isolates were obtained from patients attending sexually transmitted infection clinic at a hospital in Jos using a swab stick collected by a gynaecologist. A total of 320 women suspected to have VVC were sampled. The following type strains were used in the study, *C. albicans* CBS 7987, *C. dubliniensis* CBS 7988 as controls, fluconazole resistant *C. albicans* strains supplied by Iskander Kerati from Turkey.

2.2. Media and drugs

The media used for the study were sabourauds dextrose agar (SDA), corn-meal-Tween 80 agar (CMA) (containing 40 g corn meal, 10 mL Tween 80, 15 g agar and distilled water to make 1000 mL). CHROMagar *Candida* were prepared as recommended by manufacturer. Commercially prepared disc of 25 µg fluconazole disc (oxoid, England) were obtained from country sales representative.

2.3. Isolation and identification of isolates

Clinical isolates obtained were incubated on SDA slants, incubated, isolated and maintained at 4 °C. *Candida* species were identified by inoculation onto CHROMagar *Candida* (Paris, France), germ tube in serum at 37 °C for 2–3 h and chlamyospore formation was determined by inoculation on CMA and incubated at 25 °C for 4–7 days.

2.4. Fluconazole susceptibility test

A disc diffusion method was used for the study as described by Meis *et al*[11]. The test is based on the National Committee for Clinical Laboratory Standard (NCCLS) document M2-A6 employing a 25 µg fluconazole disc (Oxoid, England) and Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/mL methylene blue. Inoculum were adjusted to a 0.5 Mc farlands density standard. Plates were incubated aerobically at 35–37 °C for 18–24 h and read manually. Zone inhibition interpretive criteria for fluconazole disc testing[11] was based on zone diameters correlated with NCCLS recommended category break points for reference macro birth dilution method[12]. Fluconazole break points were: Susceptible(S) ≤ 8 µg/mL or ≥ 19 mm, susceptible-dose dependent (S-DD)= 16–32 µg/mL or 13–18 mm, and Resistant (R) ≥ 64 µg/mL or ≤ 12 mm. Quality control was done using CBS *C. albicans* control strains.

2.5. Genotyping of *C. albicans*

Genotyping was done according to the method previously described[10]. Genomic DNA of *C. albicans* were obtained following manufacturers instructions using yeast DNA kit. DNA solution was adjusted to 0.01 g/L. Primer pairs CA-INT-L (5'-ATAAGGGAAGTCGGCAAATAGATCCGTAA-3') and CA-INT-R (5'-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3') were used to amplify a DNA fragment that spans the site of the transposable intron in the 25S rDNA. Amplification was in a 25 mL volume containing 2.5 mL of 10 buffer (with Mg²⁺), 1 mL of 0.01 g/L genomic DNA, 0.5 mL of 10 mM deoxyribonucleotide triphosphate, 0.5 mL of 50 pmol/mL each primer and 0.25 mL of 5 U/mL Taq polymerase. PCR conditions were as follows: 94°C for 3 min, 94°C for 1 min, 65°C for 1 min and 72°C for 2.5 min, for 33 cycles; and 72°C for 10 min. The PCR fragments of the strains with a band of 450 bp were designated as genotype A, with a band of 840 bp as genotype B, and with bands of both 450 and 840 bp as genotype C.

3. Results

A total of 320 women were screened within the study period, out of which 177 *Candida* isolates were obtained. Further screening of these *Candida* isolates showed that 84 of them were confirmed to be *C. albicans*, representing about 47.46% of the isolates. Antifungal resistance study conducted on these *C. albicans* isolates revealed that 13 of the 84 isolates were resistant to fluconazole representing 15.48% of the total isolates tested.

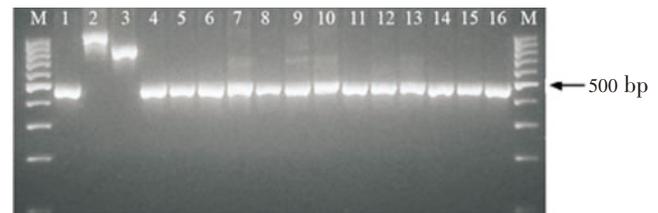


Figure 1. Molecular results obtained in this study. rDNA-based genotyping.

Lane 1: *C. albicans* ATCC 10231 (genotype A); Lane 2: *C. dubliniensis* CD36 (genotype D); Lane 3: *C. stellatoidea* B4257 (genotype B); Lanes 4–16: Nigerian *C. albicans* isolates (random selected).

The determination of the genetic diversity among the *C. albicans* based on the presence or absence of an 25S rDNA transposable introns was used to differentiate between the various genotypes of *C. albicans*. *C. albicans* genotype B has an intron at the 25S rDNA (850 bp) while genotype A does not have and has a weight of 450 bp, *C. dubliniensis* also has an intron in the same location, but it is larger than that in *C. albicans* genotype B. PCR primer CA-INT-L gave a single product for *C. albicans* genotype A (450 bp) (figure 1) hence all the isolates obtained were Genotype A.

4. Discussion

The study revealed a prevalence of 47.46% for *C. albicans* among women with VVC, this shows that *C. albicans* is the dominant cause of VVC in Jos, Nigeria. A relatively high value when compared with the finding of Jombo *et al*[13] which revealed a prevalence of 20.1% for *C. albicans* infection in Jos. Xu *et al*[14] recorded a prevalence of 68.6%. The increase in prevalence for *C. albicans* recorded in this study when compared with studies conducted in Nigeria may be attributed to improved method of diagnosis, the increased

use of broad spectrum antibiotics.

An assay into the genotypic variation of *C. albicans* that is most prevalent in Jos, revealed that all of them belongs to the genotype A. Xu *et al*^[14] in their study showed that among 293 *C. albicans* isolates examined for genotypes, 203 isolates were identified as belonging to genotype A, Zhu *et al*^[15] in their study also revealed that among 500 isolates of *Candida* genotyped 365 (75%) belonged to the genotype A and 60% in another study^[16]. Our study is consistent with other studies which shows that majority of *C. albicans* from clinical sources belongs to genotypes A followed by genotypes B, C and D respectively^[17]. No other genotypes of *C. albicans* were observed in this study. This poses way for further research as to finding out the other genotypes of *Candida* involved in candidiasis in Nigeria as this is the first study documenting the genotype distribution of *C. albicans* in Nigeria.

The resistance of *Candida* to azole antifungals continues to be a significant problem in fungal infections^[18]. Resistance to these drugs can contribute to treatment failures. Resistance of *Candida* species to azole antifungals is the most prevalent type of resistance to antifungals. Vaginal *C. albicans* isolates have been found to be resistant to fluconazole and exhibited considerably higher resistance to itraconazole^[19]. *C. albicans* in this study revealed a high resistance to fluconazole, although other genotypes were not obtained in this study to compare sensitivity, however, other comparative study involving genotypes of *C. albicans* to azoles showed that genotype A has a less resistance to the azoles, than those of genotype B^[16,19]. Up to date, only a few researches have studied the genetic diversity of *C. albicans* strains recovered from VVC and the correlation between the antifungal susceptibility and gene diversity of *C. albicans*^[14,16,19,20].

A study conducted by Fan and colleagues^[5] to determine the genotypic variations of *C. albicans* in patients with various conditions of VVC revealed that genotypes of *C. albicans* strains correlate with the severity of VVC, and that the strains with the dominant genotypes are more virulent than others in causing VVC, and that strain differences may play a significant role in the aetiology of VVC. Zhu *et al*^[15] also confirmed that susceptibility varies among the various genotypes of *C. albicans*. Hence in the management of candidiasis it's important to take into consideration the genotype of the *Candida* strain causing VVC as genotypes show correlation to sensitivity.

To the researchers knowledge this is the first study aimed at determining the genotypic variation among *C. albicans* and to test their sensitivity to fluconazole in Nigeria. Further work is therefore needed to investigate the presence of other genotypes and to correlate them to resistance. However, *C. albicans* genotype A is most prevalent among women with VVC in Jos, and with a high level of resistance to fluconazole.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Kimberly AW, Stuart MB. *Sexually transmitted diseases treatment guideline 2006*. Atlanta, GA: MMWR; 2006. [Online] Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5511a1.htm>. [Accessed on Oct 3, 2010]
- [2] Sobel JD. Vulvovaginal candidosis. *Lancet* 2007; **369**: 1961--1971.
- [3] Eschenbach DA. Chronic vulvovaginal candidiasis. *N Engl J Med* 2004; **351**: 851--852.
- [4] White DJ, Vanthuyne A. Vulvovaginal candidiasis. *Sex Transm Infect* 2006; **82**: 28--30.
- [5] Fan SR, Bai FY, Liao QP, Liu ZH, Li J, Liu XP. Genotype distribution of *Candida albicans* strains associated with different conditions of vulvovaginal candidiasis, as revealed by microsatellite typing. *Sex Transm Infect* 2008; **84**: 103--106.
- [6] Antonopoulou S, Aoun M, Alexopoulos EC, Baka S, Logothetis E, Kalambokas T, et al. Fenticonazole activity measured by the methods of the European Committee on Antimicrobial Susceptibility Testing and CLSI against 260 *Candida* vulvovaginitis isolates from two European regions and annotations on the prevalent genotypes. *Antimicrob Agents Chemother* 2009; **53**: 2181--2184.
- [7] Krawczyk B, Leibner-Ciszak J, Mielech A, Nowak M, Kur J. PCR melting profile (PCR MP)—A new tool for differentiation of *Candida albicans* strains. *BMC Infect Dis* 2009; **9**: 177--184.
- [8] Costa JM, Garcia-Hermoso D, Olivi M, Cabaret O, Farrugia C, Lecellier G, et al. Genotyping of *Candida albicans* using length fragment and high-resolution melting analyses together with minisequencing of a polymorphic microsatellite locus. *J Microbiol Methods* 2010; **80**(3): 306--309.
- [9] Garcia-Hermoso D, Cabaret O, Lecellier G, Densnos-Ollivier M, Hoinard D, Raoux D, et al. Comparison of microsatellite length polymorphism and multilocus sequence typing for DNA-based typing of *Candida albicans*. *J Clin Microbiol* 2007; **45**(12): 3958--3963.
- [10] McCullough MJ, Clemons KV, Stevens DA. Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea*. *J Clin Microbiol* 1999; **37**: 417--421.
- [11] Meis J, Petrou M, Bille J, Ellis D, Gibbs D. A global evaluation of the susceptibility of *Candida* species to fluconazole by disk diffusion. *Diag Microbiol Infect Dis* 2000; **36**: 215--223.
- [12] National Committee for Clinical Laboratory Standards. *Reference method for broth dilution antifungal susceptibility testing of yeasts. approved standard*. Villanova, PA: NCCLS; 1996.
- [13] Jombo GTA, Opajobi SO, Egah DZ, Banwat EB, Denen Akaa P. Symptomatic vulvovaginal candidiasis and genital colonization by *Candida* species in Niger. *J Public Health Epidemiol* 2010; **2**(6): 147--151.
- [14] Xu Y, Chen L, Li C. Susceptibility of clinical isolates of *Candida* species to fluconazole and detection of *Candida albicans* ERG11 mutations. *J Antimicrob Chemotherapy* 2008; **61**(4): 798--804.
- [15] Zhu X, Dong N, Wang Q, Cheng B, Zhu L, Tao J, et al. Genotyping and antifungal susceptibility of *Candida albicans* clinical isolates from anghzhou region in China. *Afri J Microbiol Res* 2011; **5**(25): 4406--4410.
- [16] Ge SH, Wan Z, Li J, Xu J, Li RY, Bai FY. Correlation between azole susceptibilities, genotypes, and ERG11 mutations in *Candida albicans* Isolates associated with vulvovaginal Candidiasis in China. *Antimicrob Agents Chemother* 2010; **54**: 3126--3131.
- [17] Bii CC, Kangogo MC, Revathi G, Wanyoike MW. Genotypes of *Candida albicans* from clinical sources in Nairobi Kenya. *Afr J Microbiol Res* 2009; **3**(9): 475--477.
- [18] Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. *J Clin Microbiol* 2005; **43**: 2155--2162.
- [19] Liu XP, Fan SR, Bai FY, Li J, Liao QP. Antifungal susceptibility and genotypes of *Candida albicans* strains from patients with vulvovaginal candidiasis. *Mycoses* 2009; **52**: 24--28.
- [20] Chong P, Abdul Hadi SR, Lee YL, Phan CL, Tan BC, Ng KP, et al. Genotyping and drug resistance profile of *Candida* spp. in recurrent and one-off vaginitis, and high association of non-albicans species with non-pregnant status. *Infect Genet Evol* 2007; **7**: 449--456.