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

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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)⁴-⁵. Approximately 10–20% of women will have complicated VVC⁴,⁵. 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 cases⁵. Ten to 20% of women suffer complicated VVC in their lifetime². 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 relations⁵.

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 VVC⁵. 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 susceptibility⁶-⁸.

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) analysis⁸, Multilocus sequence typing
(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 cultured 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 chlamydospore 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 inhibitory 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 point 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′–ATAAGGGAAGTCGGCAAAATAGATCTCCTGTA –3′) and CA–INT–R (5′–CCTGGCCTTGCTTTCGCTAGAT AGTAGAT–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: 948 °C for 3 min, 948 °C for 1 min, 658 °C for 1 min and 728 °C for 2.5 min, for 33 cycles; and 728 °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 66% 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 itraconazole19]. C. albicans in this study revealed a high resistance to fluconazole, although other genotypes where 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. albicans14,16,19,20].

A study conducted by Fan and colleagues5] 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.

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