**Candida albicans virulence genes SAP1 and SAP6 associated with vaginitis among pregnant women attending a Nigerian tertiary hospital**

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Dear Editor,

Vaginal candidiasis (VC) in pregnancy is a distressing infection and has emerged as an important cause of neonatal infections. The clinical symptoms and manifestations of VC include cottage cheese-like vaginal discharge, swelling, pruritus, pain, irritation, burning sensation, dyspareunia, and dysuria [1]. The hormonal milieu of the vagina during pregnancy can enhance candidal colonization and serves as risk factor. Progesterone has suppressive effect on the anti-candidal activity of neutrophils, while oestrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida albicans*. Moreover, a large proportion of women with chronic recurrent candidiasis first experienced the infection during pregnancy. In pregnancy, VC has been related to emotional stress and suppression of immune system [2].

*Candida albicans* is both a commensal and a pathogen that can exhibit yeast, pseudohyphae and hyphae morphology. These morphological transitions promote colonization and invasion at different anatomical sites, which also occur in other *Candida* species. *C. albicans* possesses several virulence factors that are involved in hyphae formation, phenotype switching, cell adhesion and extracellular production of hydrolytic enzymes. Secreted aspartyl proteases (SAPs) are enzymes that are secreted by *Candida* species and are coded for by the SAP gene family (SAPI-SAP10). The SAP superfamly members have been demonstrated as virulence factors in opportunistic pathogens of the genus *Candida*, and SAPI and SAP6 have been known to be associated with vaginal candidiasis [2,3].

The study was carried out to describe the *Candida* species in vagina of pregnant women, their susceptibility to antifungal agents and the carriage of virulence genes. Pregnant women (390), attending antenatal clinic of Ekiti State University Teaching Hospital, Ado-Ekiti, Nigeria, between 2016 and 2017, were enlisted in the study. Isolation of *Candida* species from high vaginal swab of the subjects was carried out using Brilliance Candida Agar® (Oxoid, England). Antifungal susceptibility testing, on nystatin, fluconazole and voriconazole, was performed on the *Candida* isolates by disk diffusion method. Quick-DNATM Universal Kit (Zymoresearch, USA) was used for DNA extraction from pure cultures of the *Candida* isolates. The DNA of isolates with green colour on Candida CHROMO agar were subjected to polymerase chain reactions (PCR) using the *C. albicans* specific primers INT1-F:5'-AAGTATTTGGAAGGGAAGG-3' and INT2-R:5'-AAATGGGCAATAGAAAGGC-3', to distinguish *C. albicans* from *C. dublinensis* [4]. PCR amplification of virulence genes SAP1 and SAP6 of *Candida albicans* were carried out using the primers: SAPI (F:5'-TCATCAATTCCTGCTTCCATTTCAACA-3'; R:5'-CACCAGCTTACCAAGGAGTTTTAATGACGCA-3') and SAP6 (F:5'-CCGTTTTGAAAATTAATGCTGATG-3'; R:5'-GTGCATGAAGTGCTGACCTGAC-3') as described by Lima et al. [5]. Molecular identification of *Candida* species by PCR analysis and sequencing using the universal primers ITS4/ITS5 (5'-GGAAGTAAAAGTCGTAA-3'; R:5'-GTCGTAAGGAGTTCTGGAGC-3) as described by El-Naggar et al. [4].

The 390 healthy pregnant women enlisted in this study were 19–46 (30.93 ± 4.60) years old, with median and modal age of 26-32 years. *Candida* species were isolated from HVS of 260 (66.7 %) of the subjects. Based on phenotypical characteristics, the *Candida* species isolated were identified as *C. albicans* (46.8%), *C. dublinensis* (3.2%), *C. tropicalis* (0.8%), *C. krusei* (29.8%), *C. glabrata* (12.1%) and *C. parapsilosis* (7.3%). The *Candida* isolates showed high level of resistance to the three antifungal agents used; nystatin (50.4 %), flucanazole (79.7 %) and voriconazole (81.3 %). Candidal isolation increased with parity, with women after forth delivery recording 75 % compared to 59.6 % of women expecting their first delivery. HIV positive women...
FIG. 1. Alignment (A) and phylogenetic relationship (B) among the Candida species isolated from HVS of pregnant women.
recorded higher occurrence of Candida (75.0 %) than HIV negative individuals (66.4 %). Candidal isolation from HVS of the women was found to be significantly associated with vaginal itching (p < 0.001), vaginal discharge (p < 0.001), previous abortion (p = 0.022) and antibiotics usage (p = 0.044).

Following sequencing and BLAST, the isolates of C. albicans were found to belong to different nearest relatives, homology and accession numbers in NBCI data bank. The alignment of the nucleotides of 10 Candida isolates, as presented in Fig. 1, showed various sites of insertions, deletions, transversions and translocations. However, 7 out of the 8 C. albicans showed strong homology in their nucleotide sequence. The phylogenetic tree shows the ancestral closeness of the 10 isolates of Candida species.

The virulence genes SAP1 and SAP6 were detected in 68.75% and 87.50%, respectively among the C. albicans isolates and were found to be associated with vaginitis among the subjects. The prevalence of virulence genes SAP1 and SAP6 (68.75 and 87.50 %, respectively), present study is in tandem with the findings in earlier work of Lima et al. [5], who reported incidence of 69.25 and 84.61% respectively of SAP1 and SAP6 genes in C. albicans isolated from vulvovaginal infection and colonization in Brazil.

In conclusion, the study showed high prevalence of vaginal candidiasis among apparently healthy pregnant women with drug resistant Candida species carrying virulent genes. Perinatal transmission of such drug resistant organisms to new-borns can be of grave consequence.

Transparency declaration

There is no conflict of interest.

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