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Candida colonization of the vagina of HIV-seropositive pregnant women and their seronegative counterparts at selected health-care centers in Akure, Ondo State, Nigeria

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Abstract

Background: *Candida* colonization of the vagina is a risk factor in pregnancy. *Candida* isolates have been implicated in adverse pregnancy outcomes. The study determined the incidence of *Candida* species recovered from the vagina of HIV-seropositive and HIV-seronegative pregnant women that attended antenatal clinics in Akure, Ondo State between November 2014 and December 2015.
Materials and Methods: Two hundred and forty pregnant women aged 19–43 participated in the study, which included 114 HIV-seropositive subjects with mean age 31.81 years and 126 HIV-seronegative subjects with mean age 29.05 years as controls. High vaginal swab was collected from each subject using sterile cotton-tipped applicator, streaked onto Mycological Agar - supplemented with streptomycin. Each sample was incubated 24 h for yeast and 72–120 h for the growth of molds. Yeast colonies that grew on Mycological Agar were picked and studied. Thereafter, colonies resembling *Candida* were identified using sugar assimilation and fermentation. *Candida* isolates were further speciated using *Candida* Ident Agar, modified. Antifungal resistance profile was identified with azoles, polyenes, echinocandins, flucytosine, and griseofulvin drugs. Antifungal resistant assay was determined by disc and agar well diffusion.
Results: Altogether, 157 *Candida* isolates were recovered from HIV-seropositive and HIV-seronegative subjects. *Candida albicans* constituted 46.5%, *Candida dubliniensis* and *Candida glabrata* 15.3% each, *Candida krusei* 12.1%, *Candida* spp. 5.7%, and *Candida tropicalis* and *Candida pseudotropicalis* 2.5% each. Antifungal resistance was widespread with azoles, polyenes, echinocandins, flucytosine, and griseofulvin.
Conclusion: *C. albicans* was the predominant isolate recovered (17.2% HIV-seropositive and 29.3% HIV-seronegative subjects). Widespread antifungal resistance seems high and suggests possible abuse of these drugs.

Keywords: Antifungal resistance, *Candida* isolates, high vaginal swab, HIV, incidence

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Introduction

Women's genital tracts consist of residents' microflora some of which are useful to health and some exist as commensals and can become opportunists.[1] Vaginal infections are perhaps the most common health problems accosting women and have been increasingly linked to a growing array of serious health risks.[2] Vaginal infections are among the most common reasons why women visit hospitals in most developed and developing countries on the globe.[3] The majority of these vaginal infections are also transmitted through sexual intercourse.[4] A few microbial agents are recognized as etiologic agents of vaginal infections among which *Candida* species often play a prominent role.[5] Vaginal candidiasis is associated with an increased risk of delivery complications.[6],[7] Some studies have reported vaginal candidiasis among pregnant women to be more symptomatic than in nonpregnant women [8] while other investigators have reported a higher prevalence of asymptomatic infection during pregnancy.[9] Studies have revealed that different *Candida* species and types colonize the vagina, and prevalence rates rise from at least 20% of all women to 30% in pregnancy.[10] The increase and severity of vaginal candidiasis in women have been shown to sustain pregnancy-related factors including immunologic alteration among sufferers, increased estrogen level as well as increased vaginal glycogen production which are linked to pregnancy complications. Many studies in developed countries have reported vaginal candidiasis in HIV-infected pregnant patients [11] but we are not aware of similar and comparable studies in our environment hence our study, which was designed to investigate the prevalence of vaginal candidiasis among HIV-seropositive pregnant women that attended antenatal clinics in Akure, Southwestern Nigeria between November 2014 and December 2015 and whose high vaginal swabs (HVSs) were screened and cultured for *Candida* isolates in the third trimester along with HIV-seronegative pregnant counterparts that served as control. Furthermore, we characterized *Candida* isolates recovered and determined the antifungal resistant profile. We believe that results and information obtained from this study will enable clinicians to better manage HIV-positive patients and improve them. In addition, the findings will provide baseline data for the management of *Candida* and mold infections in this group which hitherto is unavailable in this environment.

Materials and Methods

The study was conducted between November 2014 and December 2015 at four different health-care centers in Akure South with an estimated population of 360,268 inhabitants, and Ifedore (with an estimated population of 289,838 inhabitants and a distance of 152 km from Akure South) Local Government Areas in Ondo State. All study participants, including controls, were pregnant women at their third trimester of pregnancy that attended the antenatal clinics of the selected health-care centers. The study was approved by the Ondo State Hospital's Management and Ethical Board. Information relating to each participant was obtained through verbal interview, questionnaire responses, and case files managed by the attending physicians.

Criteria inclusion

All participants were pregnant women at their third trimester of pregnancy who attended the antenatal clinic of each health-care centers and were registered and fully consented to the study. HIV-seropositive subjects participating in highly active antiretroviral therapy in accordance with the national guidelines on HIV/prevention of transmission from mother-to-child policy was also an inclusion criterion for HIV-seropositive subjects. There was no age restriction and subjects were required to keep all physician appointments throughout the study.

Exclusion criteria

Those subjects who did not fall into the above categories, i.e., subjects who did not comply with revisit and those who declined involvement in the study were not included in the study.

HIV screening among cohort

A 5 mL volume of blood was collected in a Sterile VACUETTE ® EDTA tubes K3 and sterile 38 mm × 0.8 mm needles from each participant. A small aliquot was applied onto the HIV-1/2 strip (Determine Test, Alere, London, England, UK) for the preliminary determination of HIV serostatus. Confirmatory test for HIV infection was performed using the Abbott enzyme-linked immunosorbent assay procedure (Abbott Laboratories, Chicago, IL, USA).

Isolation and identification of fungi (Candida and molds)

For the detection of *Candida* isolates and molds, an HVS sample was collected from each subject with the aid of sterile cotton-tipped applicator (Evepon Industrial Limited, Onitsha, Anambra, Nigeria) and streaked onto Mycological Agar - supplemented with streptomycin (Difco, Detroit, MI, USA). Samples were incubated for 24 h for *Candida* growth and between 72 and 120 h for the growth of molds. Each yeast colony that grew on the initial Mycological Agar was picked and studied. Furthermore, each colony resembling *Candida* was identified further using sugar assimilation and fermentation. Each *Candida* isolate was further speciated using *Candida* Ident Agar, Modified (Sigma-Aldrich St. Louis, MO, USA).

Disk diffusion method

Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml of methylene blue was used for the disk diffusion method. Each *Candida* cell suspension was adjusted to 0.5 McFarland standard (10^6 cells/mL) and was inoculated onto the plating medium with a sterile cotton-tipped applicator. Each antifungal disk was thereafter dispensed on agar plates and plates were incubated at 35°C for 24 h, after which each zone of diameter endpoint was measured and interpreted using the Clinical and Laboratory Standards Institute (CLSI), 2008 protocol. Each sample was carried out in triplicate.

Agar well diffusion method

The plating medium used was composed of Casitone agar (Bacto–Casitone 9 g), yeast extract 5 g trisodium citrate 10 g glucose 20 g, bacto-agar 15 g, phosphate buffer (pH 6.6) KH_2 $PO_4$1 g Na_2 $HPO_4$1 g in 1 L of distilled H_2O. Furthermore, each inoculum used was prepared from a 24 h culture for each *Candida* spp. on Mycological Agar (Difco

Laboratory, MI, USA) and adjusted to 0.5 McFarland standard (10^6 cells/mL). Sterile cotton-tipped applicator was dipped onto the inoculum and carefully spread onto the plating medium. Five wells of 6 mm in diameter were cut out of agar and 20 mL of antifungal agent was introduced into each well. Each plate used contained five antifungal agents. Plates were incubated at 35°C for 24 h. Thereafter, each zone of growth inhibition observed on the plates was measured and results interpreted using CLSI disc diffusion breakpoint. Each sample was carried out in triplicate.

Statistical analysis

Statistical analysis of data obtained from the study was evaluated using paired *t*-test and analysis of variance (ANOVA) employing Statistical Package for Social Sciences (SPSS) (Chicago, IL, SPSS Inc., 2007) version 17.0 software for Windows ®. Statistical significance was considered as $P < 0.05$.

Results

[Table 1] depicts the profile of distribution of *Candida* isolates recovered from HVSs of HIV-seropositive and HIV-seronegative pregnant women. A total of 157 *Candida* isolates were cultured from the HVSs of the two cohorts (65/157 = 41.4% from HIV-seropositive subjects against 92/157 = 58.6% in HIV-seronegative subjects). When the two cohorts were compared, *Candida albicans* constituted 73 (27 [36.9%] from seropositive and 46 [63.0%] from seronegative subjects) followed by *Candida dubliniensis* 24 isolates consisting of 15 (62.5%) from seropositive and 9 (37.5%) seronegative subjects. *Candida glabrata* followed with 24 (9 [37.5%] seropositive against 15 [62.5%] from seronegative subjects) and *Candida krusei* constituted 19 isolates (8 [42.1%] from seropositive and 11 [57.8%] seronegative subjects). Furthermore, *Candida pseudotropicalis* was 4 (0 in HIV-seropositive against 4 [100%] in HIV-seronegative subjects). Similarly, *Candida tropicalis* constituted 4 (2 [50%] each for HIV-seropositive and HIV-seronegative subjects). With regard to *Candida* spp. which constituted 9 (4 [44.4%] in HIV-seropositive and 5 [55.6%] of seronegative subjects) [Table 1].

Table 1: Profile of *Candida* isolates cultured from high vaginal swab of HIV-seropositive and HIV-seronegative pregnant women

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[Figure 1] showed the distribution of *Candida* isolates from HVS of HIV-seropositive pregnant women. The figure showed the incidence of *C. albicans* was 42% followed by *C. dubliniensis* 23%, *C. glabrata* 14% while *C. krusei* isolates was 12% followed by *Candida* spp. and 3% of the total isolates was *C. tropicalis* [Plate 1 [Additional file 1]], [Plate 2 [Additional file 2]],[Plate 3 [Additional file 3]],[Plate 4 [Additional file 4]],[Plate 5 [Additional file 5]],[Plate 6 [Additional file 6]],[Plate 7 [Additional file 7]],[Plate 8 [Additional file 8]].

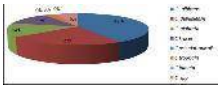

Figure 1: Distribution of *Candida* isolates cultured from high vaginal swab of HIV-seropositive pregnant women

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[Figure 2] also illustrates the pattern of distribution of *Candida* isolates among HIV-seronegative pregnant women which showed 50% *C. albicans* as 50% of the total isolates cultured, followed by *C. glabrata* 16% and *C. krusei* 12%. Similarly, 10% of the *Candida* isolates was *C. dubliniensis*, *Candida* spp. 6%, *C. pseudotropicalis* 4%, and *C. tropicalis* 0.2% [Plate 1],[Plate 2],[Plate 3],[Plate 4],[Plate 5],[Plate 6],[Plate 7],[Plate 8].

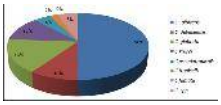

Figure 2: Distribution of *Candida* isolates cultured from high vaginal swab of HIV-seronegative pregnant women

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[Table 2] shows the profile of *Candida* isolates recovered in different age groups of pregnant mothers. The table showed at ≤ 19 years old, there was no pregnant woman in this age group and no *Candida* isolate was isolated from the HVS of HIV-seropositive pregnant women. However, among three pregnant women between 20 and 25 years age range, five *Candida* isolates were cultured consisting of three *C. albicans* and two *C. dubliniensis* indicative of a mixed infection. Ten subjects between age 26 and 31 years, 15 *Candida* isolates were cultured also made up of mixed cultures, 7 of which were *C. albicans*, 3 *C. glabrata,* 3 *C. dubliniensis*, and 1 each of *C. tropicalis* and *C. krusei*. Furthermore, among 23 subjects between 32 and 37 years age range, 39 *Candida* isolates were cultured consisting of also mixed infections, 15 of these were *C. albicans*, 8 were *C. dubliniensis*, 6 *C. glabrata*, 5 *C. krusei,* 4 were classified as *Candida* spp., and 1 belongs to *C. tropicalis*. Finally, between 38 and 43 years age range comprising five subjects, only six *Candida* isolates were cultured two each from *C. albicans, C. dubliniensis*, and *C. krusei* [Table 2].

Table 2: Distribution of *Candida* isolates cultured from high vaginal swab of HIV-seropositive pregnant women

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[Table 3] shows the distribution of *Candida* isolates from HVS of HIV-seronegative pregnant women in relation to age. Five *Candida* isolates were recovered at age ≤19 years from two subjects consisting of two *C. albicans* and 1 each of *C. dubliniensis*, *C. tropicalis* and *C. krusei* also indicative of mixed infection.

Table 3: Distribution of *Candida* isolates cultured from high vaginal swab of HIV-seronegative pregnant women in selected health centers

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However, for age group 20–25 years comprising 14 subjects, 22 different *Candida* isolates were cultured consisting of ten *C. albicans*, four each of *C. glabrata* and *Candida* spp., and two each of *C. krusei* and *C. dubliniensis*.

Furthermore, among 18 subjects between age range 26 and 31 years, 37 different isolates were recovered consisting of 18 *C. albicans*, 6 *C. glabrata,* 5 *C. krusei,* 4 *Candida dublinensis*, and 1 each of *C. tropicalis* and *Candida* spp. In addition, between age range 38–43 years, 1 subject had 2 different *Candida* isolates, each of which was *C. albicans* and *C. dubliniensis*. It must be noted that virtually all these groups showed mixed infections in their samples [Table 3].

[Table 4] shows the antifungal resistant profile of *Candida* isolates cultured from HVS of HIV-seropositive pregnant women. Of the 27 *C. albicans* isolates tested, all were each susceptible/sensitive to econazole, miconazole, ketoconazole, and clotrimazole. However, 4/27 (14.8%) isolates each was resistant to fluconazole, itraconazole, and caspofungin. Similarly, 9/27 (33.3%) was resistant to voriconazole and posconazole each, 10/27 (37.0%) was resistant to amphotericin B while 14/27 (51.8%) to griseofulvin. In addition, 25/27 (92.5%) of these isolates were resistant to flucytosine. Of the 15 *C. dubliniensis* tested, all were susceptible/sensitive to econazole, miconazole, ketoconazole, and clotrimazole and 3/15 (20%) each was resistant to fluconazole and itraconazole, whereas 6/15 (40%) was resistant to voriconazole, 4/15 (26.7%) each to posconazole and amphotericin B while 7/15 (46.7%) isolates were resistant to caspofungin. All 9/9 (100%) of isolates tested, however, were resistant to flucytosine and griseofulvin. The table also showed among *C. glabrata* tested, 5/9 (55.5%) isolates each was resistant to econazole, miconazole, fluconazole, voriconazole, amphotericin B, caspofungin, and griseofulvin; 4/9 (44.4%) isolates each was also resistant to ketoconazole, clotrimazole, itraconazole, and posconazole; and 6/9 (66.7%) to flucytosine. The results also showed *C. krusei*, 3/8 (37.5%) isolates each was resistant to econazole, miconazole, ketoconazole, clotrimazole, likewise fluconazole, itraconazole, voriconazole, and caspofungin while 5/8 (62.5%) isolates each were resistant to posconazole and amphotericin B. Similarly, 7/8 (87.5%) isolates were resistant to flucytosine and all (100%) the isolates tested were resistant to griseofulvin. Furthermore, for the two *C. tropicalis* isolates tested, 1 each (50%) was resistant to econazole, amphotericin B, flucytosine, griseofulvin, while all (100%) the 2 isolates each were sensitive to miconazole, ketoconazole, clotrimazole, fluconazole, itraconazole, voriconazole, posconazole, and caspofungin. Finally, of the 4 *Candida* spp. tested, 2/4 (50%) isolates each was resistant to econazole, miconazole, and ketoconazole. Similar trend was seen with clotrimazole, fluconazole, itraconazole, voriconazole, and griseofulvin. However, all the 4 isolates 4/4 (100%) each was resistant to posconazole, amphotericin B, caspofungin, and flucytosine [Table 4].

Table 4: Profile of antifungal resistance of *Candida* isolates cultured from high vaginal swabs of HIV-seropositive pregnant women

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[Table 5] shows the antifungal resistant profile of *Candida* isolates cultured from HVS of HIV-seronegative pregnant women. Of the 46 *C. albicans* tested, none was resistant to econazole, miconazole, clotrimazole, and amphotericin B. However, 11/46 (23.9%) isolates were resistant to fluconazole, 13/46 (28.2%) to itraconazole, 30/46 (65.2%) to voriconazole, 25 (54.3%) to posconazole, and 40/46 (86.9) to flucytosine. The results also showed 26/46 (56.5%) was resistant to griseofulvin and 3/46 (6.5%) each to ketoconazole and caspofungin. Of the 9 *C. dubliniensis* isolates tested, all (100%) were sensitive to econazole, clotrimazole, ketoconazole, and amphotericin B. Similarly, 2/9 (22.9%) isolates each were resistant to miconazole, fluconazole, and itraconazole. In addition, 4/9 (44.4%) was resistant to voriconazole, and 5/9 (55.5%) to posconazole, while 6/9 (67%) isolates each was resistant to flucytosine and griseofulvin.

Table 5: Pattern of antifungal resistance of *Candida* isolates cultured from high vaginal swab of HIV-seronegative pregnant women in selected centers

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In addition, of the 15 *C. glabrata* isolates tested, 8/15 (53.3%) were resistant to econazole, miconazole, ketoconazole, and clotrimazole. Likewise, fluconazole, itraconazole, voriconazole, and caspofungin each while 12/15 (80%) of the isolates tested were resistant to posconazole and 13/15 (86.7%) isolates also were resistant to griseofulvin. Similarly, 14/15 (93.3%) of the isolates were resistant to flucytosine. The results also show of the 11 *C. krusei* isolates tested, 4/11 (36.3%) were resistant to econazole, miconazole, and ketoconazole; 2/11 (18.2%) of the isolates however were resistant to clotrimazole, and 8/11 (72.7%) isolates to fluconazole. However, 6/11 (54.5%) of the isolates tested were also resistant to itraconazole. In addition, 5/11 (45.4%) of the isolates were also resistant to voriconazole and posconazole each, but all (100%) isolates were resistant to flucytosine and griseofulvin. Of the two *C. tropicalis* isolates tested, 1/2 (50%) each was resistant to econazole, fluconazole, and itraconazole, while the two isolates were resistant to amphotericin B, flucytosine, and griseofulvin. Among the 4 *C. pseudotropicalis* isolates tested, all (100%) were resistant to econazole, miconazole, and fluconazole. Similarly, the four isolates were also resistant to itraconazole, voriconazole, posconazole, amphotericin B, caspofungin, flucytosine, and griseofulvin. The four isolates tested were however sensitive to ketoconazole and clotrimazole. Finally, of the 5 *Candida* spp. isolates tested, all isolates were resistant to econazole, miconazole, voriconazole, posconazole, amphotericin B, caspofungin, and flucytosine, while only 2/5 (40%) isolates were resistant to clotrimazole, fluconazole, itraconazole, and griseofulvin each and 4/5 (80%) to ketoconazole [Table 5].

[Table 6] shows the pattern of distribution of *Candida* and mold isolates cultured from HVSs of HIV-seropositive and HIV-seronegative pregnant women in relation to age. Overall, a total number of 41 subjects of HIV-seropositive subjects produced 65 *Candida* isolates resulting in 1.58 *Candida* isolate per subject while among 8 subjects, 9 mold (*Aspergillus niger*, *Aspergillus clavatus*, *Fusarium* spp., *Pityrosporum furfur*, *Rhizopus* spp., *Torulopsis glabrata, Syncephalastrum* spp., *Geotrichum candidum*, and *Leptothrix vaginalis*) isolates were cultured resulting in 1.12 mold isolate per subject. Similarly, among 51 HIV-seronegative subjects, 68 *Candida* isolates were cultured averaging 1.33

Candida isolates per subject compared with 14 subjects producing only 15 molds comprising 7 different species (*Penicillium* spp. 3, *Trichophyton* spp. 2, *Abisida* spp. 2, *A. niger* 4, *Entomophthora coronata* 1, *Aspergillus fumigatus* 2, and *Cryptococcus neoformans* 1) isolates averaging 1.07 mold isolate per subject. Statistical analysis on *Candida* isolates showed that there was no significant difference between the two cohorts ($P = 0.407$). Similarly, the results of the molds also showed no statistical significance difference ($P = 0.831$). The results also showed further analysis with regard to age range. Furthermore, at age ≤19 years, neither *Candida* nor mold isolates were recovered from HVS from HIV-seropositive pregnant mothers. However, among the 2 subjects in HIV-seronegative group, 3 *Candida* isolates were cultured but no mold isolates were recovered. Among 3 subjects aged 20–25 years, 5 *Candida* isolates were cultured from HIV-seropositive mothers, but no mold isolate was encountered. In contrast, among 14 HIV-seronegative subjects screened, 18 *Candida* isolates were cultured and also among 7 HIV-seronegative subjects, 8 mold isolates were cultured. Similarly, among 10 HIV-seropositive subjects aged 26–31 years, 15 *Candida* isolates were cultured and among 4 subjects, and 5 mold isolates were recovered. With regard to 18 HIV-seronegative subjects, 27 *Candida* isolates were recovered compared to 5 mold isolates cultured from 5 HIV-seronegative subjects. Furthermore, of the 23 HIV-seropositive subjects aged 32–37 years, 39 *Candida* isolates were cultured and among 3 subjects, 3 mold isolates were also recovered. In addition, among 16 HIV-seronegative subjects screened, 18 had *Candida* isolates and 2 had mold isolates. Finally, of the 5 subjects aged between 38 and 43 years, 6 *Candida* and 1 mold isolate was cultured from 1 subject while, 2 *Candida* isolates were cultured from 1 HIV-seronegative subject, and no mold isolate was recovered from the HVS [Table 6].

Table 6: Distribution of *Candida* and mold isolates cultured from high vaginal swabs of HIV-seropositive and HIV-seronegative pregnant women

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Discussion

Our study was designed to investigate vaginal colonization with yeasts particularly *Candida* species among HIV-seropositive and HIV-seronegative pregnant women. The study also recovered non-*Candida* isolates (molds) from some of these women HVSs. In addition, we determined the antifungal resistant profile of the isolates using standardized methods. Altogether, 240 pregnant women aged between 19 and 43 years participated in the study with a mean age of 30.43 years. These consisted of 114 HIV-seropositive with mean age 31.81 years and 126 HIV-seronegative subjects, mean age 29.05 years that served as controls. Altogether, 157 *Candida* isolates were cultured from the 240 women averaging (240/157 = 1.52%) *Candida* per subject. Among the 157 *Candida* isolates cultured, 65 (65/157) =41.4% were recovered from HIV-seropositive women and 92/157 = 58.6% from HIV-seronegative women, inferring statistical significant difference ($P = 0.001$). Prevalence studies indicate that *Candida* colonize the vaginal in at least 20% of all women rising to 30% in pregnancy.[10] Although some studies concluded that pregnant women were more likely to have a symptomatic vaginal infection with *Candida*, other studies found a high prevalence of asymptomatic infection only during pregnancy.[12]

In a comparative distribution of *Candida* isolates from HIV-seropositive and HIV-seronegative pregnant women at selected centers, the results revealed among 41 subjects, 65 *Candida* isolates were cultured averaging 65/41 = 1.58% *Candida* isolate per subject. *C. albicans* was the predominant isolates recovered from HVS of HIV-seropositive individuals followed by *C. dubliniensis* 15/25 = 23.1%, *C. glabrata* 9/65 = 13.85%, *C. krusei* 8/65 = 12.3% and *Candida* spp. and *C. tropicalis* accounted for 6.15 and 3.15, respectively. In contrast, 92 *Candida* isolates were recovered from 51 HIV-seronegative subjects averaging 92/51 = 1.80% *Candida* isolates per subject. *C. albicans* topped the list 46/92 = 50% followed by *C. glabrata* 15/92 = 16.3%, *C. krusei* 11/92 = 11.9%, and *C. dubliniensis* 9/92 = 9.8%. Furthermore, *Candida* spp. accounted for 5/92 = 5.4% while *C. pseudotropicalis* 4/92 = 4.3% and *C. tropicalis* 2/92 = 2.2%. Essentially, our study revealed similar organisms were recovered from the HVS of these cohorts which is of great interest. Among both cohorts, *C. albicans* was the predominant isolates cultured indicative of the fact that these strains were predominant during pregnancy among the *Candida* species. In a study reported by Kahn *et al.*,[12] *C. albicans* was recorded as the predominant etiological agent responsible for up to 90% of vaginal candidiasis a value that corroborates with our present finding (46.5%) which ranked the highest among other *Candida* species. Moreover, Masri *et al.*[9] findings showed the predominance of *C. albicans* in vagina colonization among pregnant women in a Malaysian tertiary-care hospital. It has been suggested that the virulence of *Candida* spp. is derived from extracellular enzymes which constitute important factors for the emergence of infections by *Candida*.[13],[14] Attributes such as the production of exoenzymes such as phospholipases and aspartyl proteinases by *C. albicans* have been considered as the major mechanisms of pathogenesis in penetrating host cells and inducing an inflammatory response that damage adjacent tissues.[15] Similarly, it is known that the pH of the mucosa,[16] environmental signals,[17] and others [16],[18],[19] have been implicated in the virulence capacity of *Candida* in modulating host defenses. In addition, some studies have shown phospholipase B, expressed at least two genes (*PLB1* and *PLB2*),[20],[21],[22] biofilm formation by *C. albicans* also contribute to the pathogenesis of candidiasis.[23],[24] Although *C. albicans* is a potential pathogen most commonly isolated from clinical specimens, many reports have documented the emergence of nonalbicans species as a nosocomial pathogens. *C. tropicalis*, *C. glabrata*, C. *krusei*, and *C. parapsilosis* have also been reported to cause nosocomial infections.[25] Sullivan *et al.*[26] reported similar enzymes from other species of *Candida* specifically *C. dubliniensis* that manifested their virulence through similar enzymes.

It has been shown that continuous deployment of antifungals in treating infections caused by *C. albicans* has led to the emergence of drug resistance resulting in cross-resistance to many unrelated drugs.[27] With regard to antifungal resistance, we tested 12 different antifungal drugs for their resistance. Of the 27 *C. albicans* isolates tested among the HIV-seropositive pregnant women, 4 each (4/27 = 14.8%) was resistant to fluconazole and itraconazole while 9 each (9/27 = 33.3%) was resistant to voriconazole and posconazole. Similarly, 25 (25/27 = 92.6%) of the *C. albicans* isolates tested were resistant to flucytosine, 14 (14/27 = 51.8%) to griseofulvin, 10 (10/37 = 37.0%) to amphotericin B, and 4 (4/27 = 14.8%) to caspofungin [Table 4]. In contrast, among the HIV-seronegative pregnant women encountered, 46 *C. albicans* isolates were tested. Forty (40/46 = 86.9%) were resistant to flucytosine, thirty (30/46 = 65.2%) isolates resistant to voriconazole, 26 (26/46 = 56.5%) isolates resistant to griseofulvin. Furthermore, 25 (25/46 = 54.3%) of *C. albicans* were also resistant to posconazole, 13 (13/46 = 28.3%) to itraconazole, 11 (11/46 = 23.9%) to fluconazole,

while 3 each (3/46 = 6.5%) to ketoconazole and caspofungin. Prolonged usage of antifungals in treating infections caused by *C. albicans* has been reported to cause the emergence of azole resistance,[28] which has been associated with induction of the ABC transporter family of multidrug efflux pumps that confer resistance to multiple azoles.[29] In addition, loss of function/mutation of ERG 3[30] has also been associated with azole resistance by preventing azole binding to the enzymatic site. However, Masri *et al*.[9] have reported the susceptibility of all *C. albicans* and *C. famata* isolates recovered from HVSs from pregnant women in Malaysia to fluconazole and that *C. glabrata* isolates showed dose-dependent susceptibility, an observation which is at variance with our finding that recorded resistance of these isolates to fluconazole [Table 4] and [Table 5]. This observation suggests susceptibility of *Candida* isolates to antifungal drugs may vary from region to region on the globe. Furthermore, our study also revealed antifungal resistance to echinocandin in *C. albicans*, which has been reported to be associated with point and intrinsic mutation that showed RHO1 as a positive regulator of β(1,-3) D-glucan synthase in specific regions of genes and mediated stress responses which conferred resistance on the cell.[31] Recently, filamentous fungi have been increasingly identified in disseminated and sometimes fetal opportunistic infections in HIV patients with AIDS, patients with hematologic malignancies and hematopoietic stem cell transplant recipients.[32] T-cell deficiency, neutropenia, and high-dose corticosteroids are major risk factors that predispose immunocompromised patients to fungi infections.[33]

Conclusion

The study revealed even distribution of *Candida* isolates in both HIV-seropositive and HIV-seronegative pregnant women in this study. The findings underscore the predominance of *C. albicans* in colonization of the vagina of these two cohorts (i.e., among HIV-seropositive pregnant women = 41.5% and 50% for HIV-seronegative pregnant women). The distribution of the *Candid* a isolates recovered was common (i.e., seven different *Candid* a species) were recovered from each of the cohorts. The results also revealed the high prevalence of antifungal resistances among the different *Candida* isolates most of which were resistant to multiple antifungal. Resistance to flucytosine was remarkably high and concerning. This underscores the ineffectiveness and probable abuse of this drug in this environment. Our study also revealed the recovery of coinfection with different molds species recovered from the HVSs (9 different isolates from HIV-seropositive and 15 for HIV-seronegative subjects) which is interesting and revealed the importance of fungi colonization in pregnancy. One interesting finding we encountered is *Candida* colonization and the distribution of *Candida* isolates may be a major factor predominating among women irrespective of their pregnancy status. Vaginal colonization with *Candida* among HIV-seronegative pregnant women was higher in this group than among HIV-seropositive pregnant women most probably due to HIV immunosuppression and/or microbial antagonism which favors the growth of indigenous microflora over pathogens an important aspect of innate immunity.

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Conflicts of interest

There are no conflicts of interest.

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Figures

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Tables

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