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Prevalence and Antifungal Susceptibility patterns of *Candida* Isolated on CHROMagar^{TM}Candida at a Tertiary Referral Hospital, Eastern Uganda

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JBK and JSI participated in the conception of the idea, data analysis and writing of the manuscript. Author WJJ participated in the processing of the samples and writing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Pregnant women are susceptible to vaginal candidiasis and maternal vulvovaginal candidiasis is a major risk factor for colonization and/or infection of the infant. The purpose of this study was to determine the prevalence and antifungal patterns of albicans and non-albicans *Candida* among pregnant women attending a tertiary referral hospital.
Methods: Vaginal discharge- cotton swabs were self-collected from pregnant women clinically proven to have vulvovaginal candidiasis at the antenatal clinic of a tertiary referral hospital between January and July 2018. Microscopy and culture on Sabouraud's Dextrose Agar with chloramphenicol was done on the vaginal discharge-cotton swabs. Confirmatory fungal identification was done using CHROM agar^{TM} Candida. Antifungal susceptibility testing was carried out using the standardized Kirby Bauer method.
Results: *Candida* were isolated from 50.81% (126/249) of the swabs and included *C. albicans* (80.16%, 101/126), *C. glabrata* (19.05% (24/126) and *C. krusei* (0.79%, 1/126). *Candida albicans* showed resistance to amphotericin B (70.63%, 89/126), clotrimazole (11.9%, 15/126), nystatin

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(3.17%, 4/126), fluconazole (23.02%, 29/126), and itraconazole (17.46%, 22/126). Among the *non-albican Candida* species, *C. glabrata* showed resistance to fluconazole (100% ,24/24), amphotericin B (100% ,24/24), clotrimazole (14.29%, 18/24), nystatin (1.59%, 2/24), and itraconazole (18.25%, 23/24). C. krusei showed resistance to fluconazole (100%, 1/1), amphotericin B (100%,1/1), and itraconazole (100%, 1/1).

Conclusion: The candida species commonly associated with VVC in Eastern Uganda are *C. albicans C. glabrata* and *C. krusei*. Antifungal resistance was highly prevalent among the candida isolated. The use of CHROMagarTMCandida media for identification of clinically relevant Candida should be adopted instead of conventional methods that are tedious and time consuming such that treatment is based on laboratory evidence.

Keywords: Vulvovaginal candidiasis; antifungal susceptibility.

1. BACKGROUND

Maternal vulvovaginal candidiasis is a major risk factor for Candida colonization and infection of the infant and has been linked to perinatal morbidity and mortality in the infants [1]. *C. albicans* is the most common and clinically relevant pathogen that is responsible for 85-90% of the VVC cases [2,3]. The formation of a germ tube is necessary for successful colonization of the vaginal mucosa [4]. Moreover, this phenotype switching phenomenon is associated with alterations in antifungal susceptibility patterns [5] and yet pregnant women have a limited spectrum of drugs that they can use especially in the later stages of pregnancy [6]. More recently, due to resistance to antifungals, non- albicans *Candida* have emerged as clinically relevant causes of Candidiasis [7]. Most prevalent among these is *C. glabrata* [2,3,8-10] which is highly resistant to the commonly used azole antifungals [7]. *C. krusei* infections are commonest among patients with hematologic malignancies [11] and those of advanced age [12].

There is scanty data on prevalence and antifungal patterns of *Candida* in Uganda [13] mainly due to the thin laboratory infrastructure. Infections due to *Candida* are often treated empirically because the conventional identification methods for yeasts are tedious and long [14]. This study piloted the use of a chromogenic media for identification of Candida to species level and determined the antifungal patterns of Candida isolated on CHROMagar^{TM}Candida.

2. MATERIALS AND METHODS

2.1 Study Setting

This was a cross sectional study carried out among pregnant women attending the antenatal clinic at the Mbale Regional Hospital (MRRH), Uganda between January and July 2018. Only consenting pregnant women with confirmed vulvovaginal candidiasis were enrolled into the study.

2.2 Sample Collection and Transportation

Self-collected vaginal discharge -cotton swabs from pregnant women were transported in sterile tubes in temperature -monitored boxes to the clinical microbiology laboratory and processed within 3 hours of collection.

2.3 Laboratory Testing

2.3.1 Microscopy

Microscopy on the vaginal discharge-cotton swabs to observe suspect yeast cells was carried out by two laboratory technologists as previously described [15]. There was a 100% agreement between the two microscopists and the observance of yeasts on microscopy corresponded with the growth of yeasts on culture.

2.3.2 Fungal culture, identification, and susceptibility testing

The swabs were streaked on SDA with chloramphenicol (HiMedia laboratories Pvt Ltd. India) and cultured at 37°C for 48h and the resultant colonies gram stained to observe ovoid yeast cells and pseudohyphae. These were then regarded suspect *Candida*. A single colony was identified per patient.

Fungal identification was done using CHROM agar^{TM} Candida (CHROM agar Company, France). The sensitivity and specificity of this media for *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. glabrata* exceed 99% and out-performs conventional methods [16,17]. Only one isolate was identified from per patient. Antifungal susceptibility to anti-fungal agents fluconazole

(25µg), Itraconazole (10µg), clotrimazole (10µg), nystatin (100U), amphotericin B (100U) (Bioanalyze, Yenimahalle, Turkey) was performed using the kirby Bauer disc diffusion method and using 0.5 McFarland standard equivalent of inoculum. Mueller- Hinton agar with glucose (2%) and methylene blue (5 mg L^{-1}) was used and was supplemented with chloramphenicol (250 mg L^{-1}). Innoculum suspensions were incubated at 37ºC for 24hours. The diameters of zones of inhibition were measured in millimeters using a ruler[18]. The results were interpreted according to Clinical Laboratory Standard Institute (CLSI) M44A document [19] Commercially available control strains were used for each of the *Candida* species i.e *C.krusei ATCC* 6258, *C.albicans ATCC* 90028, *C.glabrata ATCC* 90030.

3. RESULTS

3.1 Sociodemographic Characteristics of the Study Participants

The average age of the participants in this study was 26.9 ± 2.3 yrs.

Of the 249 pregnant women that consented to participate in the study, 9.2% (23/249) were in the fist trimester, 41.4% (103/249) were in the second, while 48.9% (123/249) were in the third. Of these, 20.8% (52/249) had used antibiotics in the past two weeks.

3.2 Prevalence and Phenotypic Characterization of *Candida*

Candida were isolated from 50.81% (126/249) of the swabs and included *C. albicans* (80.16%, 101/126), *C. glabrata* (19.05% (24/126) and *C. krusei* (0.79%, 1/126). Of the 126 vaginal-discharge cotton swabs from as many women, from which *Candida* were isolated, 11.1% (14/126) were in the first trimester, 39.7% (50/126) were in the second, while 49.2% (62/126) were in the third. Of the *Candida* isolates, 80.16% (101/126) were *C. albicans,* 19.05% (24/126) were *C. glabrata* and 0.79% (1/126) were *C. krusei.*

3.3 Antifungal Susceptibility Patterns of Isolated *Candida* Species

Overall, all the isolates were non-susceptible to Amphotericin B, while 60.3% (76/126), 50% (63/126), 62.7% (79/126), and 48.4% (61/126) were non-susceptible to Itraconazole, Fluconazole, Nystatin, and Clotrimazole respectively. All the non-albicans *Candida* were resistant to itraconazole, amphotericin B, and fluconazole.

Candida albicans showed non-susceptibility to Itraconazole (50.5%, 51/101), amphotericin B (100%, 101/101), fluconazole (37.6%, 38/101), nystatin (57.4%, 58/101), and clotrimazole (39.6%, 40/101). Among the non-albicans *Candida* species, *C. glabrata* showed non-susceptibility to itraconazole (100%, 24/24), amphotericin B (100%, 24/24), fluconazole (100% ,24/24), nystatin (83.3%, 20/24), and clotrimazole (83.3%, 20/24). The *C. krusei* isolate showed resistance to itraconazole, amphotericin B, and fluconazole (Table 1).

4. DISCUSSION

This study revealed that *C. albicans* (80.6%, 101/126), *C. glabrata* (19.05%, 24/126), and *C. krusei* (0.79%, 1/126) were prevalent among pregnant women that had clinically confirmed vulvovaginitis, especially those in the third trimester (49.2%, 62/126).

All *Candida* isolated in this study were resistant to amphotericin B, and all non-albicans *Candida* were resistant to itraconazole, amphotericin B, and fluconazole.

The use of a chromogenic media has enabled the isolation to species level of clinically relevant *Candida* species in this setting and presents options for its adoption for routine clinical use. In addition to commonly reported *C. albicans*, this study has reported presence of multidrug resistant non-albicans *Candida* – resistant even to the commonly used antifungals. Pregnant women in the third trimester were mostly affected by VVC unlike a similar study in Peshawar which reported most infections in the second trimester [20].

C. glabrata is intrinsically of intermediate resistance to fluconazole as a result of the induction of efflux pumps on exposure to azoles which are only fungistatic [21]. Globally, there has been a surge in MDR *C. glabrata* associated with prior fluconazole exposure [22]. In the African context were the cheaper azole antifungals are frequently utilized, resistance to multiple antifungals would be expected. Similarly, *C. krusei* are intrinsically resistant to fluconazole [23] and their emergence is a sign of clinical failure.

Table 1. Antifungal Susceptibility Patterns of Candida isolated from vaginal of pregnant women

Antifungal drug	*Species* of *Candida*, n (%)			
	***C. albicans* (n=101)**	***C. glabrata* (n=24)**	***C. krusei* (n=1)**	**Total (%, 95% CI) N=126**
Itraconazole				
Susceptible	50 (49.5)	0	0	50 (39.7, 31.6 – 48.4)
Resistant	22 (21.8)	23 (95.8)	1 (100)	46 (36.5, 28.6 – 45.2)
Intermediate	29 (28.7)	1 (4.2)	0	30 (23.8, 17.2 – 31.9)
Amphotericin B				
Susceptible	0	0	0	101 (38.5, 84 – 49.5)
Resistant	89 (70.6)	24 (100)	1 (100)	114 (90.5, 84 – 94.5)
Intermediate	12 (11.8)	0	0	12 (9.5, 5.5 – 15.91)
Fluconazole				
Susceptible	63 (62.3)	0	0	63 (50, 41.4 – 58.6)
Resistant	29 (28.7)	24 (100)	1 (100)	54 (42.9, 34.5 – 51.6)
Intermediate	9 (8.9)	0	0	9 (7.1, 3.8 – 13.02)
Nystatin				
Susceptible	43 (42.6)	4 (16.7)	0	47 (37.3, 29.4 - 46)
Resistant	4 (3.9)	2 (8.3)	0	6 (4.8, 2.2 - 10)
Intermediate	54 (53.5)	18 (75)	1 (100)	73 (57.9, 49.2 – 66.2)
Clotrimazole				
Susceptible	61 (60.4)	4 (16.7)	0	65 (51.6, 42.9 –60.14)
Resistant	15 (14.9)	18 (75)	0	33 (26.2, 19.3 – 34.5)
Intermediate	25 (24.8)	2 (8.3)	1 (100)	28 (22.2, 15.85 –30.2)

Treatment options for MDR *Candida* infections especially among pregnant women are limited with expert recommendations lacking in evidence [24]. Expert guidelines have few evidence-based data to guide their recommendations, especially for systemic infections [24]. MDR *C. glabrata* were isolated from vaginal discharge of pregnant women in this study setting. Colonization of the vagina with such strains has been associated with increased risk of morbidity and mortality in the infants. Such infections have been shown to have an elevated clinical failure rate when they cause systemic infections [25]. In such cases, it's recommended that liposomal amphotericin B is used in addition to managing the source of the infection [26]. However, in this study, they are also resistant to amphotericin B – a finding similar to that of an earlier study [13].

There was a high number of isolates showing an intermediate level of resistance to each of the antifungal drugs tested. This is an indicator of the emergence of high rates of resistance to antifungal drugs [7].

5. LIMITATIONS

The chromogenic media (CHROMagar™ Candida) used in this study is not 100% sensitive for all *Candida* species other than the commonest *Candida* species (*C. albicans*, *C. tropicalis*, and *C. krusei*).

7. CONCLUSION

The candida species commonly associated with VVC in Eastern Uganda are *C. albicans C. glabrata* and *C. krusei*. Antifungal resistance was highly prevalent among the candida isolated. Given the emergence of drug-resistant non-*Candida albicans* in the causation of VVC in this setting, there is need to change treatment approaches used in the management of VVC especially among pregnant women in the third trimester in this region. Effective therapeutic measures should be put in place to prevent the colonization of the newborn with MDR *Candida* strains. Further research is needed to fully understand the mechanisms of resistance among these strains, and their distribution in the population served by this hospital.

CONSENT

As per international standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval of the study was received from the MRRH research and ethics Committee (MRRH/12/2018) and the research and ethics committee of the School of Biotechnical and Biomedical Laboratory Sciences, Makerere University (SBBLS/JBK/2018).

DISCLAIMER

To the best of our knowledge, the findings of this study can be used as per the scope of the study and in light of the study limitations as clearly pointed out. We confirm that the experiments conducted in this study will yield the same results during repeated trials using the same reagents and detection platforms.To the best of our knowledge, the findings of this study, as obtained using the methods we employed, are valid for the study area and season.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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