Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 1 Issue 6 June 2018

Research Article

Prevalence and Antifungal Susceptibility of Vaginal *Candida albicans* among Pregnant Women Attending Arua Regional Referral Hospital, Westnile Region of Uganda

Moses Ocan[1]*, Benjamin Tumushabe[2], Hellen Nakawunde[2], Jemmico Droma[2], Geoffrey Waiswa[2], Kalori Kirya[2], Hannington Baluku[3] and Wilson M Rwandembo[4]

[1]*Department of Pharmacology and Therapeutics, College of Health Sciences, Makerere University, Uganda*

[2]*Department of Medical Laboratory Sciences, Mbarara University of Science and Technology, Uganda*

[3]*Department of Medical Microbiology, College of Health Sciences, Makerere University, Uganda*

[4]*Uganda Institute of Allied Health and Management Sciences, Uganda*

***Corresponding Author:** Moses Ocan, Department of Pharmacology and Therapeutics, College of Health Sciences, Makerere University.

Received: April 16, 2018; **Published:** May 11, 2018

Abstract

Candida colonization's occur globally with the risk and effects varying in different populations especially among the immunosuppressed and pregnant women. This study was thus conducted to establish the prevalence and antifungal susceptibility of *Candida albicans* among pregnant women seeking antenatal care at Arua Regional Referral Hospital (RRH) in West Nile region of Uganda. A cross-sectional study design was used and pregnant women attending antenatal care at Arua RRH recruited into the study using systematic random sampling. Interviewer administered questionnaire was used in data collection. High vaginal swab (HVS) samples were collected from each participant for laboratory analysis. Germ tube test was done to identify *Candida albicans* colonization of the vagina. Antifungal susceptibility of the *Candida albicans* isolates was determined using VITEK® 2 Compact system. *Candida* colonization was present in a third, 35.5% (89/251) of the pregnant women who participated in the study. The majority, 27.5% (69/251) of the *Candida* colonization were caused by *Candida albicans*. Non-*candida albicans* species were present in 7.9% (20/251) of the pregnant women. Most, 51.4% (129/251) of the participants were 26-35 years of age. The majority, 91.2% (129/251) of the pregnant women reported to be aware of *Candida*. Fluconazole and Flucytosine each had *C. albicans* isolates that showed intermediate susceptibility. While all the 69 *C. albicans* isolates were susceptible to voriconazole, caspofungin and mucafungin antifungal agents. From the study, *C. albicans* isolates from pregnant women seeking antenatal care in Arua RRH are susceptible to common antifungal agents.

Keywords: Antifungal Agents; Candida Albicans; Susceptibility; Prevalence; Arua Regional Referral Hospital

Introduction

Candida albicans is a yeast which colonizes the mouth, skin, gastrointestinal tract and urogenital tract in healthy asymptomatic individuals [1]. There are over 40 species of *Candida* with *C. albicans* being the most prevalent in the population [2]. *Candida* albicans grows mostly in warm and moist parts of the body [3]. The microorganism has evolved into a commensal as well as an opportunistic pathogen. This implies that while it is present as part of normal mucosal micro flora, *C. albicans* retains the ability to establish an infection in its host. The ability of a microorganism to detect and respond to environmental cues that signal an invasion opportunity is a potent survival mechanism of *C. albicans* [4].

Vaginal *C. albicans* is a common pathogen among women of reproductive age and is responsible for up to 75% of the colonizations in this population [5]. In addition to causing localized colonization, *C. albicans* is able to establish systemic infection in its host. Once the organism crosses into the bloodstream of the patient it readily invades and flourishes in different organs of the body including the liver, kidney, heart and brain. This invasion by *C. albicans*of the major body organs is potentially life-threatening. Systemic fungal infections are most common in patients with compromised immune system, such as HIV/AIDS patients or those that are on immuno-suppressive therapies [6]. More than half of the women of reproductive age will experience at least one episode of *C. albicans* infection each year [5].

During pregnancy, the pH of vaginal secretions is range from 3.8 - 4.0 (Acidic) [7]. This helps to make the vagina resistant to bacterial invasion for the entire duration of pregnancy. However, the low vaginal pH (acidic) favors growth of *C. albicans* [8]. The high levels of estrogen and glycogen content in the vagina further supports proliferation of *C. albicans* during pregnancy [9]. Other factors influencing *C. albicans* infection among pregnant women include; emotional stress and suppression of immunity, eating habits especially foods with high sugar content, antibiotic use, and diabetes mellitus [10,11].

In the vagina, *C. albicans* colonization presents with different symptoms including, itching, inflammation (redness), occasional white patches on the skin of the vagina and thick white discharge [12]. These symptoms especially itching can cause physical discomfort among infected individuals.

There is a high likelihood of *Candida* colonization in most communities globally. However, information on actual prevalence of *C. albicans* infection colonization is not known especially in developing countries. This study therefore sought to establish the prevalence and antifungal susceptibility of *Candida albicans* among pregnant women seeking antenatal care in Arua RRH, in Westnile region of Uganda.

Materials and Methods

Study design, setting and population

This was a cross-sectional study done among pregnant women in West Nile region of Uganda attending antennal care in Arua regional referral hospital. The hospital is about 425 kilometers northwest of the capital city, Kampala. Antennal clinic of the hospital receives about 100 pregnant women daily and runs from Monday to Friday every week. The study used a questionnaire and microbiological culture and sensitivity tests during data collection.

Sample size: The sample size of participants was calculated using a formula by Kish Leslie. [13]. N = Z2 P (1-P)/d^2, where, Z = 1.96 for 95% confidence level, d = 5% error, p = prevalence of Candida colonization (prevalence of 79% was used according to a study by Abdallah., *et al* [14]. in Mbarara regional referral hospital in Western Uganda. After substitution, a sample size of 254 pregnant women was obtained.

Sampling criteria and data collection

The pregnant women waiting at the antennal clinic were recruited into the study using systematic random sampling method. On each day using sampling interval of three [3], 254 pregnant women were randomly approached for recruitment into the study. The questionnaire was developed using information from previous studies and was pre-tested among pregnant women attending antennal clinic at Mulago national referral hospital. Information from the pre-test was used to adjust the tool. Laboratory and questionnaire data was collected by third year medical laboratory students of Mbarara University of Science and Technology (MUST) who were first trained on data collection methods. Interviewer administered questionnaires were used to collect data. The tool consisted of questions on; socio-demographic information, prior use of antibiotics, and awareness of *Candida* infection among the study participants. At the end of each data collection day, questionnaires were reviewed by the lead researcher to assess for completeness.

Laboratory sample collection

After the interview, a high vaginal swab sample was collected by the clinician from each consenting study participant. Briefly, the vulva was cleaned with cotton wool soaked in isopropyl alcohol. A sterile vaginal speculum was inserted into the vagina then a high vaginal cotton wool swab was inserted into the posterior vaginal fornix making sure it does not touch the surface of the vulva. The swab was then rotated gently against the vaginal wall. The swab was then pulled back and put in a container and the speculum removed. The swab container was then uniquely labeled and then taken to the laboratory and examined immediately.

Laboratory procedures

Wet preparation, gram stain and culture of the high vaginal swab samples were performed to establish presence or absence of yeast in the sample. The sample was first examined using wet preparation, those which turned positive were further assessed using gram stain, and culture was then performed on the gram-positive samples.

Wet preparation briefly, new frosted glass slide was cleaned using isopropyl alcohol and labeled. A drop of normal saline was then placed on the slide and the high vaginal swab sample was emulsified on the slide. A cover slip was applied on the slide and mounted on the microscope and observed under X10 and X40 magnification for presence of yeast cells. Known positive and negative slides (066-P and 066-N) were generated and used at the Microbiology laboratory in Arua RRH as quality controls.

Gram stain briefly, a smear (1 cm x 1 cm) of the high vaginal swab sample was made on a clean glass slide and labeled. The smear was then allowed to air dry on the staining rack. The smear was then flooded with 2% crystal violet for 60 seconds. The slide was then washed using clean gently flowing tap water. Lugol's iodine was then applied on to the smear for 30 seconds after which it was washed using clean running tap water. The smear was then decolorized by applying 50% acetone alcohol until the color of the crystal violet was not appearing. The smear was then counter stained with neutral red for 60 seconds and washed with running tap water. The slide was wiped with cotton wool and placed back onto the slide rack and left to air dry on the table in the laboratory. The slide was then mounted on a microscope and examined under oil immersion objective lens for presence of yeast cell. Positive result was indicated by presence of purple round/ovoid budding yeast cells.

Culture briefly, positive samples from the gram stain were each cultured on Sabouraud's dextrose agar (SDA) containing 2% chloramphenicol in a petri-dish. Fresh sabouraud Dextrose Agar (SDA) was prepared following the manufacturer's instructions and stored at 4oC in a fridge. On each day of use, the plates were removed from the fridge, uniquely labelled and allowed to attain room temperature. The sample was then streaked onto the surface of two Sabouraud Dextrose Agar (SDA) plates using plastic sterile loop. For quality control, known positive *Candida* sample was also plated and cultured alongside the samples. After plating, the petri dishes were then incubated in an oven at temperature of 37°C for 48 hours. After the incubation, occurrence of growth of yellow cream mucoid colonies on the plate with characteristic sweet smell confirmed presence of *Candida* yeast cells in the sample.

Identification of *Candida albicans*

A simple germ tube test was done to differentiate *Candida albicans* from other *Candida* species. Briefly, 12 x 75 mm test tubes were uniquely labeled. 0.5 ml of freshly prepared human serum was pipetted into the tubes. A sterile wooden applicator stick was used to lightly touch the surface of a yeast colony from the SDA culture plates. This was then transferred and emulsified in serum in the test tubes and covered loosely to allow aeration. The tubes were then incubated in an oven at 37°C for 3 hours. After incubation, a drop of the suspension was placed on a clean glass slide using a Pasteur pipette. A drop of lacto phenol cotton blue stain was then added on the slide to stain the yeast cells. The slide was mounted on a microscope and examined under x10 and x40 objective lens. Presence of sprouting yeast cells (tube like out growth) confirmed presence of *Candida albicans* in the sample. Those without tube-like outgrowth were reported as non-*Candida albicans* isolates.

The samples which had *Candida albicans* were transportation to Makerere University Clinical Microbiology laboratory for antifungal susceptibility test. Sabouraud broth was prepared and 1 ml was aliquoted into cryo vials and uniquely labelled. The cryo vials were then inoculated with the culture sample from the germ tube that contained *Candida albicans*. The vials were packed and transported in a cool ice box at 4°C under UN3373 classification. At the Clinical Microbiology laboratory, the isolates were sub-cultured on Chromager *candida* for identification and confirmation of *C. albicans* in the sample. All *C. albicans* colonies were blue in colour. The Isolates were then preserved in 20% glycerol and kept at -80°C until susceptibility tests were done.

Anti-fungal susceptibility procedure

Fresh cultures of *Candida albicans* isolates were prepared overnight. Briefly, 3 ml of normal saline (0.4%) was dispensed into 2 (two) separate glass test tubes. A colony of *C. albicans* was emulsified in sterile, distilled water in the first test tube. 500 µl of the mixture in test tube 1 (one) was pipetted and transferred to the second test tube. The foil cover of Vitek 2 machine cartridge was opened to remove the card. The tip of the card was placed in the mixture in the second test tube. The test tube was then placed on the rack, this was repeated until the cartridge of Vitek 2 machine was filled up. The cartridge was then transferred to incubation chamber for continual monitoring and reading for 24 hours. The Vitek 2 machine automatically reads and prints out the antifungal susceptibility test result after 24 hours of incubation. The susceptibility tests were interpreted according to CLSI M27-S4 guidelines [15] as shown in table 4. Standard strains of *Candida* NZRM 3394, *Candida albicans* ATCC 90028 and *Candida* parapsilosis, NZRM 4072T (ATCC 22019) were incubated alongside the study samples in the Vitek 2 machine.

Data Analysis

Data from laboratory investigations and questionnaire forms were entered into Excel spread sheet, and then subsequently exported to a statistical software package STATA version 12. Frequencies and proportions were determined using Univariate analysis. Association between socio-demographic characteristics and *C. albicans* colonization was assessed through bivariate analysis. Poisson regression analysis was used to establish predictors of *C. albicans* colonization and $P \leq 0.05$ was considered significant.

Ethics

The study protocol was reviewed and approved by the Ethics review committee of Mbarara University of Science and Technology. Permission to conduct the study in Arua regional referral hospital was obtained from the hospital administration. Written informed consent was obtained from the pregnant women attending antenatal clinic in Arua RRH prior to recruitment into the study. All pregnant women found with candidiasis or any other vulvovaginal colonizations were sent to the clinicians for further management.

Results

Demographic characteristics of pregnant women attending antenatal care at Arua RRH

Of the 254 pregnant women who were randomly approached for recruitment into the study, 251 consented to participate, representing a response rate of 94%. Over half of the respondents, 51.4% (129/251) were between 26-35 years of age. The majority of study participants, 91.2% (229/251) were in monogamous sexual relationships. Half, 50.4% (126/251) of the respondents operated small businesses. The majority of pregnant women, 91.2% (229/251) reported that they were aware of the signs and symptoms of Candida infection. Some of the study respondents, 2% (5/251) were taking medicines as a form of treatment for the symptoms related to fungal infection at the time of data collection (Table 1).

Characteristic	Description	Number of participants (n = 251)	Frequency (%)
Age (years)	15 - 25	84	33.5%
	26 - 35	129	52.0%
	36 - 45	27	10.9%
	≥ 46	11	4.4%
Occupation	Small business owner	126	50.4%
	Peasant farmer	76	30.3%
	Civil servant	24	9.6%
	Others	25	10.0%
Sexual relationship	Monogamous sexual relationship	229	91.2%
	Multiple sexual relationship	22	8.8%
Awareness about *Candida* colonization	Yes	229	91.2%
	No	22	8.8%

Table 1: Socio-demographic characteristics of pregnant women attending Arua RRH.

%: Percentage, n: Sample size, RRH: Regional Referral Hospital.

Prevalence and factors associated with vaginal *Candida* colonization among pregnant women attending antenatal care in Arua regional referral hospital

Of the 251 high vaginal swab samples analysed, 35.5% (89/251) had *Candida*. Of the 89 samples that had *Candida*, 77.5% (69/89) were *Candida albicans* while 22.5.9% (20/89) were none *C. albicans.* The prevalence of *Candida* colonization was higher, 17.1% (43/251) among women who were between 26-to-35 years of age compared to other age groups. Over a third, 31.5% (79/251) of the pregnant women with *Candida* reported having one sexual partner. *Candida* colonization was confirmed in 30.7% (77/251) of the pregnant women who reported being aware of the symptoms of *candida* colonization. However, there was no statistically significant association between *Candida* colonization and socio-demographic characteristics of the study participants ($P > 0.5$) (Table 2).

Factors associated with *Candida albicans* colonization among pregnant women seeking antenatal care at Arua RRH, Westnile region of Uganda

Of the pregnant women who had *Candida*, 32.6% (29/89) were between the ages of 26 - 35 years. The majority, 69.7% (62/89) of pregnant women who reported having a single sexual partner had *C. albicans* colonization. There was no statistically significant association between *C. albicans* colonization and the factors which were assessed ($P > 0.05$) (Table 3).

Variable	Description	Number of samples screened for *Candida* colonization (n = 251)				
		Negative n = 162 (%)	Positive n = 89 (%)	PR	95%CI	P-value
Age (years)	15-25	52 (20.7%)	32 (12.7%)	1.0		
	26-35	86 (34.3%)	43 (17.1%)	0.88	0.55 - 1.38	0.57
	≥ 36	24 (9.6%)	14 (5.6%)	0.97	0.52 - 1.81	0.92
Occupation	Business	82 (32.7%)	46 (18.3%)	1.0		
	Peasant	49 (19.5%)	23 (9.2%)	0.91	0.86 - 1.49	0.71
	Civil servant	15 (5.9%)	10 (3.9%)	1.14	0.58 - 2.26	0.71
	Others	16 (6.4%)	10 (3.9%)	1.02	0.51 - 2.17	0.69
Sexual relationship	One partner	135 (53.8%)	79 (31.5%)	1.0		
	Multiple partners	27 (10.8%)	10 (3.9%)	0.86	0.62 - 1.19	0.34
Aware of *Candida* colonization	No	10 (3.9%)	12 (4.8%)	1.0		
	Yes	152 (60.6%)	77 (30.7%)	1.03	0.52 - 2.09	0.92

Table 2: Factors associated with *Candida* colonization among study participants.

PR: Prevalence ratio; n = Sample size; CI: Confidence Interval; %: Percentage.

Variable	Description	Number of *Candida* isolates (N=89)				
		Non-*C. albicans* (n = 20)	*C. albicans* (n = 69)	PR	95%CI	P-value
Age (years)	15-25	5 (5.6%)	27 (30.3%)	1.0		
	26-35	14 (15.7%)	29 (32.6%)	0.80	0.47-1.35	0.40
	>36	1(1.1%)	13 (14.6%)	1.10	0.57-2.13	0.78
Occupation	Business	10 (11.2%)	35 (39.3%)	1.0		
	Peasant	7 (7.9%)	18 (20.2%)	0.9	0.5-1.57	0.69
	Civil servant	1 (1.1%)	8 (8.9%)	1.03	0.48-2.22	0.94
	Others	2 (2.2%)	8 (8.9%)	1.01	0.39-2.1	0.9
Sexual relationship	Multiple partners	3 (3.4%)	7 (7.9%)	1.0		
	Single Partner	17 (19.1%)	62 (69.7%)	0.87	0.40-1.91	0.74
Awareness about *Candida*	No	3 (3.4%)	9 (10.1%)	1.0		
	Yes	17(19.1%)	60 (67.4%)	1.03	0.52-2.09	0.92

Table 3: Prevalence of *C. albicans* colonization among study participants.

PR: Prevalence ratio; n = Sample size; CI: Confidence Interval; %: Percentage.

Susceptibility of *Candida* isolates obtained from the participants

All the *C. albicans* isolates (69) were sensitive to caspofungin, micafungin, and voriconazole. While intermediate susceptibility was found among *C. albicans* isolates to fluconazole and flucytosine antifungal agents (Table 4).

Antifungal Agent	M27-S4 breakpoints (µg/ml)			Percentage of *Candida albicans* isolates in indicated susceptibility category isolates, n=69 (%)		
	S	I	R	S	I	R
Fluconazole	≤ 2.0	4.0	≥ 8.0	66 (95.6%)	3 (4.4%)	0
Voriconazole	≤ 0.12	0.25 - 0.5	≥ 1.0	69 (100%)	0	0
Micafungin	≤ 0.25	0.5	≥1.0	69 (100%)	0	0
Caspofungin	≤ 0.25	0.5	≥1.0	69 (100%)	0	0
Flucytosine	≤ 4.0	8.0-16.0	≥ 32	67 (97%)	2 (3%)	0

Table 4: Susceptibility of *C. albicans* isolates to antifungal agents.

S=Sensitive; I=Intermediate; R=Resistant.

Discussion

Globally, vaginal candidiasis is common especially among pregnant women with *C. albicans* accounting for over 90% of the cases [16]. However, non-albicans vaginitis is clinically indistinguishable from that of *C. albicans* [17]. In this study, over a third of the pregnant women had *Candida* spp colonization with *C. albicans* being the most common species. The prevalence of *C. albicans* among pregnant women in our study is like that reported in previous studies [11,18]. In the general population, the incidence of *C. albicans* colonization has been shown to double among pregnant women especially in the third trimester [19]. The disproportionately high rate of *C. albicans* colonization among pregnant women is attributed to the elevated glycogen levels and hormonal changes during pregnancy [9]. Estrogen, a pregnancy hormone reduces the inhibitory effect of vaginal epithelial cells on the growth of *C. albicans*. While progesterone has suppressive effect on the anti-candida activity of neutrophils [20].

The prevalence of vaginal *C. albicans* was high among women who were 26-to-35 years of age. This is similar to the findings of previous studies [21,22]. Although considered not to be sexually transmitted, *C. albicans* is known to spread in some cases through unprotected sexual intercourse. The reported high rate of sexual

activity [23] coupled with indiscriminate use of hormonal contraceptives in this age group could be responsible for the observed high prevalence of vaginal *C. albicans*. A study by Mikolajczyk [24] found that, inadequate personal hygiene, limited diagnostic facilities and inadequate knowledge are commonly associated with high prevalence of *C. albicans* in communities especially of developing countries. In pregnancy, candida colonization usually does not harm the fetus however, it causes distress to the mother including itching, burning sensation on the vulva, white vaginal discharge and redness [25]. If left untreated, vaginal candidiasis can lead to pelvic inflammatory disease which can cause scars in the fallopian tube and potentially result in infertility [26].

A previous study by Ocan., *et al.* [27] in northern Uganda reported high prevalence of antibiotic self-medication especially among high income earners. Inappropriate use of antibiotics may result in destruction of beneficial vaginal bacteria resulting in reduced vaginal immunity. This potentially increases the risk of *C. albicans* colonization in the population [24].

Our study also showed that a high proportion of pregnant women who reported being aware of the symptoms of *Candida* infection were confirmed to have vaginal *C. albicans*. However, these women had come to the hospital for antenatal visits and not to seek care for any symptoms of illness related to *Candida* infection. This is perhaps due to the fact that they could have been managing the symptoms by themselves at home. A previous study by Ocan., *et al.* [27] in northern Uganda showed that community members initiate self-treatment based on disease symptoms. However, due to high levels of illiteracy in the rural communities there is a high likelihood of misdiagnosis of the symptoms. A study by Rathod., *et al.* [28] showed that such a practice of initiating treatment based on disease symptoms may lead to mistreatment which could further worsen the disease due to delay in accessing appropriate care.

Antifungal susceptibility testing in our study revealed that 97% and 95.6% of the *C. albicans* isolates were susceptible to flucytocine and fluconazole respectively. This is similar to the 96% *C. albicans* susceptibility to fluconazole reported in a study by Mukasa., et al. [29] *C. albicans* was 100% susceptible to voriconazole, caspofungin and micafungin. However, three (3), two (2) *C. albicans* isolates had intermediate sensitivity to fluconazole and flucytosine respectively. There was no resistant *C. albicans* isolates. This could likely be due to the low levels of antifungal use especially in the rural communities. A previous study by Ocan., *et al*. [27] found only 0.3% prevalence of antifungal use in rural communities of northern Uganda. The current study showed that, voriconazole, caspofungin and micafungin are effective against *C. albicans*. However, the detected intermediate sensitivity could be indicative of a potential development of antifungal resistance among *C. albicans* in the population to flucytocine and fluconazole.

Conclusion

A third of the pregnant women reporting for antenatal care in Arua regional referral hospital have vaginal *Candida albicans*. All the *C. albicans* isolates were highly susceptible to currently used antifungal agents like fluconazole, micafungin, flucytosine, caspofungin and voriconazole. Public education programs like health promotions on safe hygiene and sexual practices among pregnant women could help in reducing the risk of colonization in communities. In addition, clinicians need to conduct regular screening for *C. albicans* colonization among pregnant women attending antenatal care in health facilities in the country.

Acknowledgements

We duly acknowledge the administration of Arua Regional Referral hospital for allowing us to conduct the study in their facility. In addition, the pregnant women who participated in the study and the department of Medical Microbiology, Makerere University for conducting the susceptibility tests.

Funding Statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of Interests

The authors report no conflicts of interest to declare. The authors alone are responsible for drafting and writing of the entire manuscript.

Ethical Approval

The study protocol was reviewed and obtained ethical approval from Mbarara University of Science and Technology Faculty of Medicine research committee.

Informed Consent

Prior to recruitment to the study, written informed consent was obtained from each participant.

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