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Virulence Markers and Antifungal Susceptibility of Vaginal Yeast Isolates from Contraceptive and Non-contraceptive Users in Uyo, Nigeria

***Akinjogunla OJ, Divine-Anthony O, Akpan MM, Ogbonna FC.**

Department of Microbiology, Faculty of Science,
University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria

***Author for Correspondence:**papajyde2000@yahoo.com

Abstract

The occurrence, virulence markers and antifungal susceptibility of vaginal yeast isolates from contraceptive users (CUs) and non-contraceptive users (NCUs) were determined using standard techniques. Five species of candida comprising *C. albicans, C. tropicalis, C. glabrata, C. krusei* and *C. parapsilosis* were isolated from the high vaginal swab (HVS) samples from CUs and NCUs. *Cryptococcus neoformans* was obtained only from HVS samples from CUs. There was no statistically significant difference (P ? 0.05) between the occurrences of yeast isolates among the CUs and NCUs. All HVS samples from the CUs aged < 20 yrs had isolates, while 80.0 %, 75.0 % and 60.0 % HVS samples from CUs with age groups of 21-25 yrs, 26-30 yrs and >31 yrs had yeast isolates, respectively. Among the NCUs, the highest and lowest occurrences of isolates were obtained from age group of 21-25 yrs and > 31 yrs, respectively. More than 62.5 % yeast isolates were sensitive to fluconazole, ≥ 32.5 % isolates were nystatin resistant, while between 50.0 % and 65.0 % isolates were sensitive to clotrimazole and itraconazole. *C. neoformans* and *C. tropicalis* displayed high sensitivity to clotrimazole and itraconazole, respectively. *C. albicans* (n=6), *C. tropicalis* (n=1) and *C. glabrata* (n=2) exhibited weak haemolytic activity, 50.0 % *C. parapsilosis* exhibited weak lipolytic activity, while *C. albicans* (n=9) and non-*albicans Candida* species (n=11) showed positivity for protease production. Though a large number of yeast isolates were sensitive to the antifungal drugs, intermittent antifungal susceptibility testings are necessary for monitoring trends of antifungal resistance among the pathogenic vaginal yeasts.

Key Words: Yeast, Contraceptive, Susceptibility, Antifungal, Virulence, Nigeria

INTRODUCTION

Vaginal yeast infections have gradually increased over the last few years, causing considerable increase in morbidity, mortality and affecting the well-being of women (Asticcioli et al. 2009). The yeasts of the genus *Candida* and *Cryptococcus* are opportunistic and invasive in individuals whose defense mechanisms are compromised, suppressed and are capable of causing infections ranging from superficial to life threatening systemic infections (Talaro and Talaro, 1996; Kim and Sudbery, 2011; Deepa et al. 2015). Although *C. albicans has* continued to be the most predominant *Candida* species causing invasive fungal infections in humans, the numbers of yeast infections caused by non-albicans *Candida* (NAC) species have also significantly increased over the last two decades (Pfaller and Diekema, 2007).

Steroids, use of spermicides, t-cell dysfunction pregnancy, perfumed feminine hygiene sprays, high dose estrogens and contraceptives are associated with *Candida* overgrowth, leading to symptomatic infections in women (Erdogan and Rao, 2015). The contraceptives universally used for birth control are oral pills, injectable contraceptives-Depo-Provera and cervical caps (Egbe et al. 2011). The contraceptives containing oestrogen and progesterone hormone increase the glycogen in the vagina which are converted into lactic acid by lactobacilli, consequently, overgrowth of *yeast* species occurs due to decreased pH. The susceptibility of yeast isolates to antifungals is frequently unpredictable and an increasing resistance of yeast strains especially *Candida* spp and *Cryptococcus neoformans* to azole due to the over-expression of efflux proteins which act by pumping the drug out of the cell at a rate faster than the rate at which drug enters the cell have been reported (Akinjogunla and Eghafona,

2012). The adhesins and invasins on the cell surface, yeast hyphal morphogenetic transformation, extracellular hydrolytic enzymes such as proteases, lipase, and haemolysins secreted and act synergistically under favourable conditions by the yeast isolates contribute to their pathogenicity (Silva et al. 2011). The haemolysin *aids the yeast to lyse* host erythrocytes and strip iron from haemoglobin molecules (Manns et al. 1994; Akinjogunla et al. 2017; 2018). The proteinase facilitates adherence and phenotypic switching of *yeast* by hydrolyzing the peptide bonds in proteins (Naglik et al. 2003). The study determined the virulence markers and antifungal susceptibility of yeast isolates from HVS of contraceptive and non- contraceptive users.

MATERIALS AND METHODS

Collection of Samples

One hundred and twenty (120) high vaginal swab (HVS) samples from contraceptive users (n=60) and non-contraceptive users (n=60) attending different hospitals / laboratories in Uyo, were aseptically collected from January to August, 2017. Verbal informed consent of each participant was obtained prior to sample collection. The samples were appropriately labelled, kept on ice immediately and transported to the microbiology laboratory for mycological analyses.

Mycological Analysis of Samples

Each HVS sample was aseptically inoculated onto each plate of Sabouraud Dextrose Agar (SDA) supplemented with 05.g /L chloramphenicol and aerobically incubated at 35°C for 48 hrs. After incubation, the plates with positive yeast growth were subcultured onto fresh plates of SDA, aerobically incubated at 35°C for 48 hrs. The yeast isolates were maintained on SDA slant at 4°C, characterized and identified based on their cultural and morphological characteristics. The *Candida* species were subcultured onto plates of CHROM agar Candida (Difco BBL., USA), aerobically incubated for 48 hrs at 35°C, and pigmentation was observed and used for species differentiation. Gram staining, germ tube, chlamydospores production, sugar fermentation and assimilation tests were also carried out. The *Cryptococcus* spp were subjected to urease test and capsule staining using India ink.

Antifungal Susceptibility Testing of Yeast Isolates

In vitro susceptibility of the each yeast isolate to itraconazole (ITR, 10 µg), fluconazole (FLU, 25 µg), ketoconazole (KET, 10 µg), clotrimazole (CLO, 10 µg), nystatin (NYS, 100 units) and voriconazole (VOR, 1 µg) was determined by disc diffusion method (CLSI, 2012). Suspension (10 µl) of each yeast isolate, prepared directly from an overnight agar plate using physiological saline, visually adjusted to turbidity of 0.5 McFarland Standard, was inoculated and spread over the dried surface of each plate containing Glucose - Methylene Blue - Mueller Hilton Agar (GMBMHA, composition: 0.5 g/mL methylene blue, 2 % glucose and Mueller Hilton Agar) using sterile pipettes. The antifungal discs were aseptically placed onto the surface of each GMBMHA plate and incubated for 48 hrs at 35 °C. Inhibitory zones after incubation were observed and measured in millimetre. The interpretation of the measurement as sensitive (S), dose dependent susceptible (DDS) and resistant (R) was made as follows: ITR, NYS and VOR (S: ≥ 16, DDS: 10-15, R ≤ 9), FLU (S: 19, DDS: 15≥18, R 14), KET (S: ≥ 30, DDS: 23-29, R 22) and CLO (S: ≥ 20, DDS: 12-19, R 11). ≤

Detection of Haemolysin Producing *Yeast* Isolates

Suspension (10 µL) of each *yeast* isolate, adjusted to turbidity of 0.5 McFarland Standard, was spot inoculated onto plate of human blood SDA (3% glucose, 5 % human blood and SDA) and aerobically incubated for 48 hrs at 35 ºC. Translucent zone around the yeast isolate was considered positive for haemolytic activity. The Haemolytic Index was obtained by dividing the diameter of the colony by the diameter of the colony plus translucent zone (in millimeters) and the results obtained were interpreted as follows: Weak activity (0.64 to 0.99) and strong activity (≤ 0.63) (Price et al. 1982).

Detection of Proteinase Producing *Yeast* Isolates

Suspension (10 µL) of each *yeast* isolate was spot inoculated onto plates of gelatin agar (1% gelatin and SDA) and aerobically incubated for 48 hrs at 35 ºC. Transparent zones around the *yeast* isolates indicated production of proteinase. The Proteolytic Index was obtained by dividing the diameter of the colony by the diameter of the colony plus the transparent zone the results obtained were interpreted as stated above (Price et al. 1982).

Detection of Lipase Producing *Yeast* Isolates

Suspension (10 µL) of each *yeast* isolate, adjusted to turbidity of 0.5 McFarland Standard, was spot inoculated onto plate of Tributyrin-SDA (1% Tributyrin and SDA) aerobically incubated at 35 ºC for 48 hrs. Clear zone around the colony indicated the production of lipase. The Lipolytic Index was obtained by dividing the diameter of the colony by the diameter of the colony plus the transparent zone and the results obtained were interpreted as stated above.

Statistical Analysis

All statistical analyzes were performed using Statistical Package for the Social Science (SPSS, Version 20) software. Chi square ($?^2$) was calculated and P-value ? 0.05 was considered as significant.

RESULTS

Forty-six (76.7%) of the HVS samples from contraceptive users showed typical yeasty appearance on SDA, while 14 (23.3%) samples showed no evidence of yeast growth. *C. albicans* was the most commonly isolated species, accounting for 36.7 % (22/46) of the total isolates, followed by *C. glabrata* (16.6 %; 10/46), *C. tropicalis* (10.0 %: 6/46), *C. krusei* (6.7 %: 4/46), while *C. parapsilosis* and *C. neoformans* had (3.3 %: 2/46) each. Five species of candida comprising *C. albicans, C. tropicalis, C. glabrata, C. krusei* and *C. parapsilosis* were isolated from the HVS samples of non-contraceptive users. The specie with the highest percentage of occurrence was *C. albicans* having 30.0 %, while the NAC occurring in relatively low percentages of occurrences were: *C.* ically *tropicalis* (6.7%), *C. glabrata* (10.0 %), *C. krusei* (6.7%) and *C. parapsilosis* (3.3%). There was no statistically significant difference (P ? 0.05) between the occurrences of yeast isolates in the HVS of contraceptive and non- contraceptive users (Table 1). The results obtained showed that all (100 %) HVS samples from the contraceptive users (aged < 20yrs) had yeast isolates, while 80.0 %, 75.0 % and 60.0 % HVS samples from contraceptive users with age groups of 21-25yrs, 26-30 and >31yrs had yeast isolates, respectively. Among the non-contraceptive users, the highest occurrence of yeast isolates was obtained from age group of 21-25yrs (66.7 %), followed by the age group < 20 yrs with (57.1%), while the age groups of 26-30 yrs and >31yrs had 50.0 % and 40.0 %, respectively (Table 2).

Antifungal susceptibility results indicated that 62.5 % of the yeast isolates were sensitive to fluconazole, while 20.0 % were fluconazole resistant (Flu^r). ≥ 26 (32.5 %) yeast isolates were nystatin resistant (NYS^r), 8 (10.0 %) were dose dependent susceptible (DDS), while 46 (57.5 %) were sensitive to nystatin. Varied percentage susceptibilities of the yeast isolates to voriconazole were observed with *C. neoformans* showing 100 % DDS to voriconazole. Fluconazole showed high antifungal activity against *C. krusei* (75.0 %), while between 50.0 % and 62.5 % C. *parapsilosis, C. tropicalis* and *C. glabrata* were sensitive to fluconazole. Twenty-two *C. albicans* (55.0%) were sensitive to nystatin, 4 (10.0%) were DDS, while 14(35.0%) were NYS^r. A small percentage (20.0 %) of *C. tropicalis* was DDS to nystatin, while none of the *C. tropicalis* and *C. parapsilosis* was FLU^r. The percentage sensitivities of the yeast isolates to clotrimazole, ketoconazole and itraconazole were 50.0 %, 57.5 % and 67.5 %, respectively. Of the 80 yeast isolates tested, 22 (27.5 %) were clotrimazole resistant, 24 (30.0 %) were ketoconazole resistant and 14 (17.5 %) were itraconazole resistant. ≤ 25.0 % of the *C. albicans* and ≤ 50.0 % NAC species were DDS to clotrimazole. The *C. neoformans* and *C. tropicalis* showed high sensitivity to clotrimazole and itraconazole, respectively (Table 4).

Of the 80 yeast isolates tested, only 42 (52.5

%) produced haemolysins (Table 5). Eighteen (18) of the haemolysin producers comprising *C. albicans* (n=12), *C. tropicalis* (n=2) and *C. glabrata* (n=4) exhibited weak haemolytic activity, while 24 (30.0 %) consisting of *C. albicans* (n=14), *C. tropicalis* (n=2), *C. glabrata* (n=6) and *C. krusei* (n=2) showed strong haemolytic activity. All the *C. parapsilosis and C. neoformans* were non-haemolysin producers (Table 5). Of the 80 yeast isolates evaluated *in-vitro* for lipase production, 38 (47.5 %) produced lipase, while 42 (52.5 %) were non-lipase producers. Twenty-two (22) of the lipase producers showed weak lipolytic activity and 16 yeast isolates showed strong lipolytic activity. None of the *C. neoformans* exhibited lipase activity, 50.0 % *C. parapsilosis* exhibited weak lipolytic activity, while 25.0 % *C. krusei* showed strong lipolytic activity (Table 6). Eighteen (18) *C. albicans* and 14 NAC showed positivity for protease production (Table 7). The NAC species comprised *C. tropicalis* (6/10, 60.0%) and *C. glabrata* (8/16, 50.0%). Of the 32 (40.0 %) protease producers, weak protease activity was observed in *C. albicans* (20.0%), *C. tropicalis* (20.0%) and *C. glabrata* (37.5%). while 16 (20.0%) isolates expressed strong proteolytic activity (Table 7).

Table 1: Occurrence of Vaginal Yeast Isolates among Contraceptive and Non-contraceptive Users

Vaginal Yeast Isolates (n=80)	Contraceptive Users (n=60) No (%) of Occurrence	Non-contraceptive Users (n=60) No (%) of Occurrence	X^2	P-value
C. albicans (40)	22 (36.7)	18 (30.0)		
C. tropicalis (10)	6 (10.0)	4 (6.7)		
C. glabrata (16)	10 (16.6)	6 (10.0)		
C. krusei (8)	4 (6.7)	4 (6.7)	2.05	0.843
C. parapsilosis (4)	2 (3.3)	2 (3.3)		
C. neoformans (2)	2 (3.3)	0 (0.0)		
Total	46 (76.7)	34 (56.7)		

Table 2: Age-wise Occurrence of Vaginal Yeast Isolates among Contraceptive and Non-contraceptive Users

Contraceptive Users			Non-contraceptive Users		
No of Samples Collected	No. (%) Positive for Yeast Isolates	No. (%) Negative for Yeast Isolates	No of Samples Collected	No. (%) Positive for Yeast Isolates	No. (%) Negative for Yeast Isolates
4	4 (100)	0 (0.0)	14	8 (57.1)	6 (42.9)
30	24 (80.0)	6 (20.0)	24	16 (66.7)	8 (33.3)
16	12 (75.0)	4 (25.0)	12	6 (50.0)	6 (50.0)
10	6 (60.0)	4 (40.0)	10	4 (40.0)	6 (60.0)
60	46 (76.7)	14(23.3)	60	34 (56.7)	26 (43.3)

DISCUSSION

The morbidity and mortality rates associated with yeast infections have been on the increase and candidal vulvovaginitis, a common female infection, predominantly occurs during their fecund period (Sobel et al. 2004). The

Table 3: Antifungal Susceptibility of Vaginal Yeast Isolates among Contraceptive and Non-contraceptive Users

		Flu conazole			Nystatin			Voriconazole		
Vaginal	**No of**	**S**	**DDS**	**R**	**S**	**DDS**	**R**	**S**	**DDS**	**R**
Yeast Isolates	Isolates	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
C. albicans	40	26 (65.0)	4(10.0)	10 (25.0)	22 (55.0)	4(10.0)	14 (35.0)	2 0(5 0.0)	10 (25 .0)	10(2 5.0)
C. tropicalis	10	6(60.0)	4(40.0)	0(0.0)	6(60.0)	2(20.0)	2 (20.0)	6(60.0)	0(0.0)	4 (40.0)
C. glabrata	16	10 (62.5)	2(12.5)	4 (25.0)	10 (62.5)	0(0.0)	6 (37.5)	8(50.0)	4(25 .0)	4 (25.0)
C. krusei	8	6(75.0)	0(0.0)	2 (25.0)	4(50.0)	2(25.0)	2 (25.0)	4(50.0)	0 (0 .0)	4 (50 .0)
C. parapsilosis	4	2(50.0)	2(50.0)	0(0.0)	2(50.0)	0 (0.0)	2 (50.0)	2(50.0)	0 (0.0)	2 (50.0)
C. neoformans	2	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)
Total	80	50 (62.5)	14 (17.5)	16 (20.0)	46 (57.5)	8(10.0)	26 (32.5)	4 0(50.0)	16 (2 0.0)	24 (30.0)

Keys: S: Sensitive DDS: Dose Dependent Susceptible; R: Resistant

Table 4: Antifungal Susceptibility of Vaginal Yeast Isolates among Contraceptive and Non-contraceptive Users

		Clotrimazole			Ketoconazole			Itraconazole		
Vaginal	No of	S	DDS	R	S	DDS	R	S	DDS	R
Yeast Isolates	Isolates	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
C. albicans	40	20(50.0)	10(25.0)	10(25.0)	24(60.0)	4(10.0)	12(30.0)	26(65.0)	6(15.0)	8(20.0)
C. tropicalis	10	4(40.0)	4(40.0)	2(20.0)	6(60.0)	0(0.0)	4(40.0)	8(80.0)	2(20.0)	0(0.0)
C. glabrata	16	10(62.5)	2(12.5)	4(12.5)	12(75.0)	2(12.5)	2(12.5)	10(62.5)	4(25.0)	2(12.5)
C. krusei	8	4(50.0)	0(0.0)	4(50.0)	2(25.0)	2(25.0)	4(50.0)	6(75.0)	0(0.0)	2(25.0)
C. parapsilosis	4	0(0.0)	2(50.0)	2(50.0)	2(50.0)	0 (0.0)	2(50.0)	2(50.0)	0(0.0)	2(50.0)
C. neof ormans	2	2(100)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)	0(0.0)
Total	80	40(50.0)	18(22.5)	22(27.5)	46 (57.5)	10(12.5)	24(30.0)	54 (67.5)	12(15.0)	14(17.5)

Keys: S: Sensitive DDS: Dose Dependent Susceptible; R: Resistant.

Table 5: Haemolysin Production and Activity of Vaginal Yeast Isolates

Vaginal Yeast Isolates	No. of Isolates	Haemolysin Producing Isolates No. (%) of Occurrence	Isolates with Weak Haemolytic Activity No (%) of Occurence	Isolates with Strong Haemolytic Activity No (%) of Occurence
C. albicans	40	26 (65.0)	12 (30.0)	14 (35.0)
C. tropicalis	10	4 (40.0)	2 (20.0)	2 (20.0)
C. glabrata	16	10 (62.5)	4 (25.0)	6 (37.5)
C. krusei	8	2 (25.0)	0 (0.0)	2 (25.0)
C. parapsilosis	4	0 (0.0)	0 (0.0)	0 (0.0)
C. neoformans	2	0 (0.0)	0 (0.0)	0 (0.0)
Total	80	42 (52.5)	18 (22.5)	24 (30.0)

isolation of *Candida spp* from the HVS in this study was in conformity with the reports of Okungbowa et al. (2003) who obtained *C. albicans and NAC species from HVS* in Southern part of Nigeria. *C. albicans* was the predominant yeasts, followed by *C. glabrata* and this agrees with Enweani et al. (2001) who reported *C. albicans* as the prevalent cause of invasive fungal

infections. *C albicans* remains the major *Candida* species, accounting for over half of all cases of candidal infections in the world and represents a grave public health challenge with increasing medical and economic importance (Pfaller and Diekema, 2007). The occurrence of *C. glabrata, C. krusei, C. tropicalis and C. parapsilosis in HVS corroborates the previous results of* Pfaller and Diekema (2007) who reported the prevalence of vaginal yeast infections caused by NAC. *The occurrence of C. neoformans in the HVS in this study differs from the results of Feglo and Narkwa (2012) who had no C. neoformans in the samples studied as this yeast* is rarely isolated from healthy individuals and does not appear to be a common human commensal.

The susceptibility of yeast isolates to antifungal drugs is often unpredictable, thus, testing individual yeast pathogens against the *ns* appropriate antifungal agents is often required. In this study, 20 % yeast isolates from HVS were FLU^r and this is dissimilar to the results of Ribeiro et al. (2000) who reported that all the 56 yeast isolates obtained from HVS were sensitive to fluconazole. Of the 8 *C. glabrata* isolated, 1 (12.5%) were DDS and ≥ 15% were FLU^r and this finding is similar to that of Richter et al. (2005) in USA where 15% of *C. glabrata* isolates were FLU^r. Although voriconazole has been shown to have an excellent *in vitro* activity against yeasts as reported by Mandras et al. (2009), yet our study contradicts this as candida isolates obtained were resistant to voriconazole. NYS^r *C. albicans* from HVS samples in our study were more than those obtained by Mukasa et al. (2015) in Uganda. Nystatin act by binding polyene to sterols in the yeast plasma membrane, resulting in a change in their permeability. Consequently, the fungal cells lose potassium,

Table 6: Lipase Production and Activity of Vaginal Yeast Isolates

Vaginal Yeast Isolates	No. of Isolates	Lipase Producing Isolates No. (%) of Occurence	Isolates with Weak Lipolytic Activity No (%) of Occurence	Isolates withStrong Lipolytic Activity No (%) of Occurence
C. albicans	40	22 (55.0)	14 (35.0)	8 (20.0)
C. tropicalis	10	6 (60.0)	4 (40.0)	2 (20.0)
C. glabrata	16	6 (37.5)	2 (12.5)	4 (25.0)
C. krusei	8	2 (25.0)	0 (0.0)	2 (25.0)
C. parapsilosis	4	2 (50.0)	2 (50.0)	0 (0.0)
C. neoformans	2	0 (0.0)	0 (0.0)	0 (0.0)
Total	80	38 (47.5)	22 (27.5)	16 (30.0)

Table 7: Protease Production and Activity of Vaginal Yeast Isolates

Vaginal Yeast Isolates	No. of Isolates	Protease Producing Isolates No. (%) of Occurence	Isolates with Weak Proteolytic Activity No (%) of Occurence	Isolates with Strong Proteolytic Activity No (%) of Occurence
C. albicans	40	18 (45.0)	8 (20.0)	10 (25.0)
C. tropicalis	10	6 (60.0)	2 (20.0)	4 (40.0)
C. glabrata	16	8 (50.0)	6 (37.5)	2 (12.5)
C. krusei	8	0 (0.0)	0 (0.0)	0 (0.0)
C. parapsilosis	4	0 (0.0)	0 (0.0)	0 (0.0)
C. neoformans	2	0 (0.0)	0 (0.0)	0 (0.0)
Total	80	32 (40.0)	16 (20.0)	16 (20.0)

sugar and phosphate ions, which leads to the impairment of glycolysis and cellular respiration. The resistance of *C albicans* and NAC species to ketoconazole and clotrimazole in this study substantiates the reports of Dias et al. (2011). *C. neoformans* was sensitive to itraconazole *in vitro and this corroborates the results of* Saag et al. (1999). Itraconazole acts by blocking the lanosterol 14 a-demethylase and the NADPH-dependent-3-ketosteroid reductase in *C. neoformans* (Casadevall and Perfect, 1998). The increasing resistance of *Candida* strain to azole might be due to the over-expression of efflux proteins which act by pumping the drug out of the cell at a rate faster than the rate at which drug enters the cell have been reported (Akinjogunla and Eghafona, 2012).

The pathogenicity of *Candida* species is attributable to tissue-damaging extracellular hydrolytic enzymes that act synergistically under favourable conditions (Silva et al. 2011). In our findings, lipase production was exhibited by 47.5 % yeast *isolates*, 40.0 % yeast isolates were proteinase producers, while 52.5 % yeast isolates showed positivity for haemolysin production. The occurrence of these virulence markers in *Candida spp from HVS corroborates the results of* Ying and Chunyang (2012). The production of protease and haemolysin by *C. albicans* in this study is in accordance with Odd (1988). In this study, 22.5 % *Candida* spp showed strongly positive haemolytic activity and this value was lower than 50.0 % *Candida* spp with strongly positive haemolytic activity reported by *Rossoni et al.* (2013). Haemolysin is a putative virulence factor secreted by *Candida spp that aids to lyse* host erythrocytes and strip iron from haemoglobin molecules (Manns *et al*. 1994). In our findings, 50 % of *C. glabrata* isolates from HVS showed proteinase production and this agrees with Sachin et al. (2012) whose studies showed the capability of *C. glabrata* to produce proteinase. Proteinase enzyme facilitates adherence and phenotypic switching of *Candida spp* by hydrolyzing the peptide bonds in proteins (Naglik et al. 2003).

CONCLUSION

Although a number of vaginal yeasts obtained from both contraceptive and non-contraceptive users were sensitive to the antifungal drugs tested, intermittent *in vitro and in vivo* antifungal susceptibility testing should be carried out so as to monitor trends of resistance to antifungal drugs among vaginal yeasts that are pathogenic and also among those that extracellular hydrolytic enzymes producers.

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