

RESEARCH PAPER

PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA SPECIES ISOLATED FROM WOMEN ATTENDING A GYNAECOLOGICAL CLINIC IN KUMASI, GHANA

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ABSTRACT

Candidal vulvovaginitis causes extreme discomfort and affects the well being of women. The aim of this study was to determine the prevalence of Candida infections among women attending gynaecological clinic at the Komfo Anokye Teaching Hospital (KATH) in Kumasi and the antifungal susceptibility patterns of the Candida species isolated. A total of 186 high vaginal swabs (HVS) were collected from women suspected of having candidal vulvovaginitis between March and June 2009. Wet mount preparations in potassium hydroxide (KOH) and Gram-stained smears were performed directly on specimens. Cultures for Candida species were done using sabouraud dextrose agar (SDA) (Oxoid, UK) at room temperature (25 - 28°C). Antifungal susceptibility of the Candida species to amphotericin B, fluconazole and itraconazole was assessed using the ATB FUNGUS 3 test kits (bioMerieux, Marcy-l'Etoile, France). Of the 186 HVS cultured, 39 yielded Candida species giving Candida prevalence in HVS during the study period as 21%. Candida species isolated from HVS specimens were Candida albicans (n=19, 48.7%), Candida glabrata (n=7, 17.9%), Candida tropicalis (n=4, 10.3%) and Candida dubliniensis (n=4, 10.3%). Other species isolated were Candida sake (n=2, 5.1%), Candida krusei (n=2, 5.1%) and Candida parapsilosis (n=1, 2.6%). Susceptibility of the isolates to antifungal agents ranged from 66.7% to 87.2% with MICs ranging from ≤ 0.125 mg/l to 8mg/l. Amphotericin B was the best performing antifungal agents with sensitivity of 87.2%.

Keywords: *Antifungal susceptibility, Candida species, yeasts infections, Fluconazole, vulvovaginitis*

INTRODUCTION

Yeasts infections of the vagina, commonly known as candidal vulvovaginitis, is a female infection primarily common during the fecund period (Odds, 1988; Sobel, 1992). The prevalence of candidal vulvovaginitis in the world is estimated in the range of 5 to 20% (Collier *et al.*, 1998). Depending on the age, locality and

socio-economic status, the frequency of vaginal *Candida* isolates has been reported to be between 5 and 48.4% in women (Abu-Elteen *et al.*, 1997). The prevalence of vulvovaginitis increases in groups such as pregnant or diabetic women, those using oral contraceptives or on long-term antibiotic treatment (Sobel, 1992). *Candida albicans* is the most common etiologi-

cal agent of vulvovaginitis but other yeast species such as *Candida glabrata*, *Candida krusei* and *Candida tropicalis* are also encountered (Sobel, 1997). There are no recent published data in the literature to ascertain the true prevalence of candidal vulvovaginitis in Ghana. The aim of this study therefore was to determine the prevalence of vulvovaginal candidiasis among women attending the Komfo Anokye Teaching Hospital in Kumasi and the antifungal susceptibility patterns of the *Candida* species isolated from these women.

MATERIALS AND METHODS

The study was conducted at the microbiology laboratory of the Komfo Anokye Teaching Hospital with the approval of the Committee on Human Research, Publications and Ethics (CHRPE) of the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology (KNUST) and the Komfo Anokye Teaching Hospital (KATH). High vaginal swabs routinely delivered at the microbiology laboratory for mycological examination were further used for this research work. Wet mount preparations in KOH and Gram-stained smears were performed directly on HVS and then observed using the light microscope for yeast cells and hyphae. The samples were cultured on sabouraud dextrose agar (SDA) (Oxoid, UK) and then incubated at room temperature (25-28°C) for 2 to 3 days. *Candida* yeast cells isolated from the samples were identified to the species level using the germ tube test and the API ID 32 C test kits. The isolates were stored as suspension in 20% glycerol broth at -70°C.

Antifungal susceptibility of the *Candida* isolates to amphotericin B, fluconazole and itraconazole was assessed using the ATB FUNGUS 3 test kits (bioMerieux, Marcy-l'Etoile, France) according to manufacturer's instruction. The range of concentrations of different antifungal agents tested was at twofold dilutions and included: amphotericin B [AMB (0.5 - 16mg/l)]; fluconazole [FCA (1 - 128mg/l)] and itraconazole [ITR (0.125 - 4mg/l)]. The inoculum size was adjusted to turbidity stan-

dard equivalent to 2 McFarland. Results were read after 24 to 48 hours of incubation at 37°C. The yeast, *Candida parapsilosis* NCTC 3104, was used as quality control strain. Antifungal susceptibility results were analyzed using the interpretive criteria as recommended by Clinical and Laboratory Standard Institute (CLSI) for fluconazole [FCA ($S \leq 8\text{mg/l}$; I: 16-32mg/l and $R \geq 64\text{mg/l}$ for *Candida* species)]; itraconazole [ITR ($S \leq 0.125\text{mg/l}$; I: 0.25-0.5mg/l and $R \geq 1\text{mg/l}$ for *Candida* species)]. For amphotericin B, CLSI interpretive criteria for *Candida* are not available. However, it has been suggested that *Candida* isolates for which amphotericin B MICs were $\leq 1\text{mg/l}$ were considered susceptible and so it was used as cut off value to separate susceptible isolates from the resistant ones (CLSI, 2002).

RESULTS

Of the 186 high vaginal swabs cultured, 39 yielded *Candida* species (21%). *Candida* species isolated from high vaginal swabs were *Candida albicans* (n=19, 48.7%), *Candida glabrata* (n=7, 17.9%), *Candida tropicalis* (n=4, 10.3%) and *Candida dubliniensis* (n=4, 10.3%). Other species isolated were *Candida sake* (n=2, 5.1%), *Candida krusei* (n=2, 5.1%) and *Candida parapsilosis* (n=1, 2.6%). The distribution of different species of *Candida* among the different age groups is shown in Table 1. The results obtained also showed that the 15-20 year group had the highest frequency of *Candida* positive samples followed by the 26-30 year age group. The age groups 46-50 years and the above 51 year bracket had the lowest frequency of *Candida* (2.6%). Statistical analysis of the data using one sample t- test (SPSS version 17) indicates that there was significant difference in the prevalence among the different age- groups (p- value = 0.004).

The results of the antifungal susceptibility of the 39 *Candida* yeast isolates against amphotericin B fluconazole and itraconazole as determined by the ATB FUNGUS 3 test method are shown in Table 2 below.

Table 1: Distribution of different *Candida* species within different age groups

Age group	Yeast species							Total (%)
	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>	<i>C. dubliniensis</i>	<i>C. krusei</i>	<i>C. sake</i>	<i>C. parapsilosis</i>	
15-20	5.0	3.0	1.0	-	1.0	-	-	10(25.6)
21-25	4.0	-	-	-	-	-	-	4(10.3)
26-30	2.0	3.0	1.0	2.0	-	1.0	-	9(23.0)
31-35	-	1.0	1.0	1.0	-	1.0	1.0	5(12.8)
36-40	2.0	-	1.0	1.0	1.0	-	-	5(12.8)
41-45	4.0	-	-	-	-	-	-	4(10.3)
46-50	1.0	-	-	-	-	-	-	1(2.6)
≥51	1.0	-	-	-	-	-	-	1(2.6)
Total (%)	19 (48.7)	7(17.9)	4(10.3)	4(10.3)	2(5.1)	2(5.1)	1(2.6)	39 (100.0)

C.: *Candida*

DISCUSSION

Candida infection of the vagina is a common problem that causes significant morbidity and affects the well being of women (Asticcioli *et al.*, 2009). The results showed in the studied population *Candida* prevalence of 21% from high vaginal swabs. This is lower than the 40.6% and 51.5% reported by Enweani *et al.* (2001) among non-contraceptive and contraceptive users in Nigeria. The high prevalence recorded among the contraceptive users in Enweani's study could be attributed to the presence of oestrogen and progesterone hormones in the contraceptive that increased glycogen level in the vaginal fluid and hence promoted the overgrowth of *Candida* species (Rippon, 1988; Njoku-Obi *et al.*, 1976). However, the 21% prevalence reported in this study is consistent with findings from studies of the general female population (Gross *et al.*, 2007; Mohanty *et al.*, 2007; Falleiros de Padua *et al.*, 2003).

This study also showed that the 15-20 year age group had the highest frequency (n= 10, 25.6%) of *Candida* isolation from high vaginal swabs followed by the 26-30 year age group (n=9, 23.0%). Statistical analysis of the data using one- sample t- test (SPSS version 17) indicates

that there was significant difference (p-value = 0.033) in prevalence between the 15- 20 and 26- 30 year age groups. This finding contradicts that of Okungbowa *et al.* (2003) who reported in Nigeria that the 26-30 year age group followed by the 21-25 year group had the highest frequency of *Candida* positive samples in their study. Also Ako-Nai *et al.* (1993) reported in Nigeria that the age group 20-25years was the group in which genital candidiasis occurs most frequently. They suggested in their study that there could be a link between the disease and sexual activity.

The high prevalence of *Candida* isolation within the 21-30 age groups supports the reports of Enweani *et al.* (1987) which showed that 40.7% of total samples of asymptomatic female students of University of Jos, Nigeria had vaginal candidiasis. They reported that the 20-30 year age could be a vulnerable group of candidal vulvovaginitis probably due to use of contraceptives and increased sexual activity.

The most frequent *Candida* species isolated from high vaginal swab in this study was *Candida albicans*, followed by *Candida glabrata*. This finding compares favourably with that of

Table 2: Antifungal susceptibility test results as determined by the ATB FUNGUS 3 method

<i>Candida</i> species	No of isolates	<u>AMB</u>		<u>FCA</u>		<u>ITR</u>		
		S	R	S	R	S	R	
<i>Candida albicans</i>	19(48.7%)	15(44.1%)	4(80%)	14(53.8%)	5(45.5%)	15(55.6%)	3(33.3%)	1(33.3%)
<i>Candida glabrata</i>	7(17.9%)	7(20.6%)	-	2(7.7%)	5(45.5%)	-	5(55.6%)	2(66.7%)
<i>Candida tropicalis</i>	4(10.3%)	3(8.8%)	1(20%)	4(15.4%)	-	3(11.1%)	1(11.1%)	-
<i>Candida krusei</i>	2(5.1%)	2(5.9%)	-	-	2(100%)	2(7.4%)	-	-
<i>Candida dubliniensis</i>	4(10.3%)	4(11.8%)	-	4(15.4%)	-	4(14.8%)	-	-
<i>Candida sake</i>	2(5.1%)	2(5.9%)	-	1(3.8%)	1(9.0%)	2(7.4%)	-	-
<i>Candida parapsilosis</i>	1(2.6%)	1(2.9%)	-	1(3.8%)	-	1(3.7%)	-	-
Total	39(100%)	34(100%)	5(100%)	26(100%)	11(100%)	27(100%)	9(100%)	3(100%)

other studies in other parts of the world (Ellabib and ElJariny, 2001; Asticcioli *et al.*, 2009). However, in the study of Okungbowa *et al.* (2003) in Nigeria, it was reported that *Candida glabrata* was the commonest yeast isolated from both urinary and high vaginal specimens followed by *Candida albicans* and *Candida tropicalis*, a study in which urinary and high vaginal swabs isolates were combined.

Fluconazole is one of the first-line antifungal drugs that are used in the treatment of infections due to *Candida* species other than *Candida krusei* and some *Candida glabrata* isolates (Patel, 2000; Wolff *et al.*, 2000). In this study, it was found that none of the 19 *Candida albicans* isolated from high vaginal swabs was resistant to fluconazole even though 26.3% (5/19) were in the intermediate category. This finding is consistent with findings of Sobel *et al.* (2004) who reported no fluconazole resistance among the 401 *Candida albicans* recovered from women with candidal vaginitis in USA. Also Ribeiro *et al.* (2000) reported in Brazil that all the 56 *Candida albicans* tested against fluconazole were susceptible. However, another study in USA reported fluconazole resistance to be 3.6% among *Candida albicans* isolates collected from candidal vaginitis patients (Sobel *et al.*, 2003). The finding of 26.3% (5/19) of *Candida albicans* isolates in the intermediate category of the sensitivity testing could be considered alarming because it is an indication of the potential for development of fluconazole resistance.

Findings from the study showed 71.4% of *Candida glabrata* isolates were found to be in the intermediate category for fluconazole which confirms those reported in earlier studies that *Candida glabrata* isolates often generate a high fluconazole MICs (Pfaller, 1999; Arzeni *et al.*, 1997; Richter *et al.*, 2005). Resistance of isolates to itraconazole and amphotericin B ranged from 7.7 to 12.8% respectively. This compares with the result of Araj *et al.* (1998).

CONCLUSION

The prevalence of vulvovaginal candidiasis among women attending gynaecological clinic at the Komfo Anokye Teaching Hospital in Kumasi was 21%. *Candida albicans* was the most frequent yeast isolated from high vaginal swabs followed by *Candida glabrata*. Amphotericin B was found to be the best performing antifungal agents against the vaginal *Candida* isolates with susceptibility rate of 87.2%.

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