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Full Length Research Paper

Prevalence and comparison for detection methods of *Candida* species in vaginal specimens from pregnant and non pregnant Saudi women

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Pregnancy represents a risk factor in the occurrence of vulvovaginal candidiasis. To investigate the prevalence rate of vaginal carriage of *Candida* species in Saudi pregnant and non-pregnant women, high vaginal swab (HVS) specimens (707) were examined by direct microscopy (10% KOH and Giemsa staining) and parallel cultured on Sabouraud Dextrose Agar (SDA) as well as on "CHROM agar Candida" medium. As expected, *Candida*-positive cultures were frequently observed in pregnant-test group (24%) than in non-pregnant group (17%). The frequency of culture positive was correlated to pregnancy (*P=0.047*), parity *(P=0.001*), use of contraceptive (*P=0.146*), or antibiotics (*P=0.128*), and diabetic-patients (*P<0.0001*). Out of 707 HVS examined specimens, 157 specimens were yeast-positive culture (22%) on Sabouraud Dextrose Agar or "CHROM agar Candida". In comparison, the sensitivities of the direct 10% KOH and the Giemsa stain microscopic examination methods were 84% (132/157) and 95% (149/157) respectively but both with 100% specificity. As for the identity of recovered 157 yeast isolates, based on API 20C biotype carbohydrate assimilation, germ tube and chlamydospore formation, *C. albicans* and *C. glabrata* constitute 80.3 and 12.7% respectively. Whereas rates of *C. tropicalis, C. kefyr, C. famata or C. utilis* were 2.6, 1.3, and 0.6% respectively. *Sachromyces cerevisiae* and *Rhodotorula mucilaginosa yeasts* were also encountered at a frequency of 1.3 and 0.6% respectively. Finally, among all recovered 157 yeast-isolates, strains resistant to ketoconazole were not detected, whereas 5% of the *C. albicans* and as high as 55% of the non-albicans yeast isolates (majority *C. glabrata*) showed resistance to fluconazole. Our findings may prove helpful for continuous determination of the existing vaginal candidiasis causative species during pregnancy, its lab-diagnosis and/or control and possible measures to minimize the incidence of the disease-associated pre-term delivery.

Key words: Vaginal candidiasis, *Candida* spp., pregnancy, risk factors, API 20C-yeast biotypes, Giemsa stain, antifungal agents.

INTRODUCTION

The rate of colonization with *Candida* spp. and symptomatic vaginitis is higher during pregnancy (CDC, 2010). *Candida* species are found in almost 30% of pregnant and in 15% of non-pregnant women (Kubota,1998) and *Candida albicans* infection occurs in vast majority (80 to 90%) of diagnosed true vulvovaginal candidiasis (VVC) cases (Boselli et al., 2004). Among the *Candida* species causing infections, *C. albicans, C. glabrata, C. parapsilosis,* and *C. tropicalis* account for 80 to 90% of fungal isolates encountered worldwide

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(Paulitsch, 2006; CDC, 2010). *Candida krusei* was also reported as the second most common agent of VVC. The increased incidence of *Candida* other than *C. albicans* species (non-albicans spp.) in VVC cases is not yet well established (Esmaeilzadeh et al., 2009) and it has been correlated to the extensive use of azole antifungal drugs that leads to the selection of more naturally resistant species, such as *C. glabrata* (Snydman, 2003). There is also accumulated evidence that mere *Candida* spp.-vaginal colonization may predispose for preterm birth, and clotrimazole prophylactic-treatment during pregnancy significantly reduces the disease-incidence (Czeizel et al., 2004).

Furthermore VVC affects between 70 and 75% of adult women during their lifetime, among which approximately 40–50% will experience further episodes and 5% will develop the recurrent VVC (Ringdahl, 2006; CDC, 2010). The most common predisposing risk factors are pregnancy, age and parity, history use of broad spectrum antibiotics and high estrogen content of oral contraceptives (Jindal et al., 2006; Fidel et al., 2003) as well as uncontrolled diabetes mellitus (Faraji et al., 2012). Unfortunately, none of these risk factors and/or symptoms either individually or collectively is precisely pathognomonic of *Candida* infection. The lack of specificity of symptoms and signs therefore precludes a diagnosis that is based on history and physical examination without the corroborative evidence of laboratory tests (CDC, 2010). Thus, over 80% patients, referred by physicians with a putative diagnosis of VVC were found to have some other cause of vaginitis, and therefore most patients fail to respond to antifungal therapy cause of incorrect diagnosis (Jindal et al., 2007). Therefore, rapid and specific detection and identification of *Candida* species will help to choose the suitable antifungal and improve patient care. *Candida* spp. in vaginal samples is still usually identified by microscopic examination (Jindal et al., 2006; Mohanty et al., 2007; Ahmad and Khan, 2009; CDC, 2010) of a wet mount with potassium hydroxide (10% KOH). This method detects budding yeast cells in only 50–70% of women with VVC and sometime fails to detect non-albicans spp. (CDC, 2010). Therefore, the present study deals with the prevalence of vaginal colonization by *Candida* species, and its risk determinants in pregnant and non-pregnant Saudi women, comparison for detection methods and fluconazole and ketoconazole antifungal activity against recovered yeast isolates as well as their API 20C biotypes and identity confirmation.

MATERIALS AND METHODS

This descriptive–analytic study was performed on 707 women referred to Medina Maternity and Children's Hospital (MMCH), Medina city, Saudi Arabia, who agreed to participate in this study. The MMCH is a 500 bed facility with all general and subspecialty medical services. The hospital provides primary, secondary care services for Saudi patients. It also provides tertiary care services to all Saudi citizens on referral bases. High vaginal swabs (two HVS of each subject) were collected from both pregnant and non pregnant women by a health care provider. The study included total of 707 women divided in two groups, 495 of pregnant women (test group) and 212 of non-pregnant women (control group). The study started from January to June 2012, and it was approved by MMCH ethical-committee and a written informed consent and questioners for possible risk factors was filled up and taken from each of all women who agreed to participate in the study.

To detect *Candida* spp. in examined specimens microscopically, one of the two HVS taken from each subject was extracted in a tube containing 0.5 ml of sterile saline, allowed to stand for few minutes then well vortexes to give uniform inoculums-suspension. A drop of the resultant suspension was placed on slide with a drop of 10% KOH (potassium hydroxide) then cover slip, followed by (wet mount) microscopic examination using low and high power for the presence of yeast pseudohyphae and budding (Jindal et al., 2006; Mohanty et al., 2007; Ahmad and Khan., 2009). In parallel, one drop of the above prepared inoculums was also placed onto another glass slide, and left in air to dry. The slides were then stained with Giemsa stain diluted 1:9 with phosphate buffer solution (pH 7.2) for 7 min. After rinsing with tap water, slides were allowed to air dry before microscopic (Figure 1) examination (Usanga et al., 2010).

To detect *Candida* spp. in examined specimens by culture, the 2nd high vaginal swab-specimen taken from each subject was directly streaked (one side) onto a surface of Sabouraud dextrose agar (Oxoid, UK) plates (SDA) and incubated at 37°C for 48 h. The cultures were separated into positive (growth of yeast) or negative culture (no yeast growth). The colonies of yeast cells (Figure 2) are opaque white to creamy (Esmaeilzadeh et al., 2009; Faraji et al., 2012). The same swab was then turned to the other side and streaked onto a surface of CHROM agar Candida–plate (BD company, Belgium) and incubated at 37°C for 48 h. CHROM agar Candida is a chromogenic medium with which a direct presumptive identification of *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. parapsilosis* can be made on the basis of defined colors (Figure 2) of colonies (Babic and Hukic, 2010).

The isolated yeast strains were further identified using the germ tube test, chlamydospore formation and API system 20C AUX (Biomerieux, France). *C. albicans* form germ tubes (Figure 3), as short lateral hyphen filaments without any constrictions; and on Corn meal agar (Hi-Media, Mumbai, India) it forms chlamydospre (Figure 3) among other morphology of budding cells, hyphae, and blastospores (Okungbowa et al., 2006; Babic and Hukic, 2010; Nwadioha et al., 2010; Mahmoudi-Rad et al., 2011). As for API system, a profile number based upon the overall reactions observed as well as pseudohyphae formation (+) or not(-) on inoculated Corn-meal agar plate, was constructed for each isolate. Identifications were obtained with numerical profile and enter the 7-digit to database (Version 4.0) (Ng et al., 2000; Chong et al., 2003).

The isolated yeasts (157 strains) were also tested for their *in vitro* susceptibility towards fluconazole and ketoconazole in accordance with the proposed guidelines for antifungal disk diffusion susceptibility testing of yeasts contained in the CLSI document M-44A (CLSI, 2004). The plates were incubated at 35°C, and inhibition zone diameters were measured after 24 and 48 h especially for *C. glabrata*. The interpretive criteria for the fluconazole disk test were as those previously described by CLSI (2004): dz ≥19mm-susceptible; 15<dz<18 mm-susceptible dose dependent and dz≤14mm-resistant. As for ketoconazole: dz ≥20mm-susceptible, 20<dz<10mm-intermediae and dz<10mm-resistant (Ng et al., 2000).

As for statistical analysis, data were analyzed by SPSS 19 (Statistical Package for Social Science; release 19.0). The relationship between risk factors (pregnant, parity, contraceptive, antibiotic and diabetic) and microbiological results were compared. Chi-square test, Fishers exact test were used and a *P-value < 0.05*

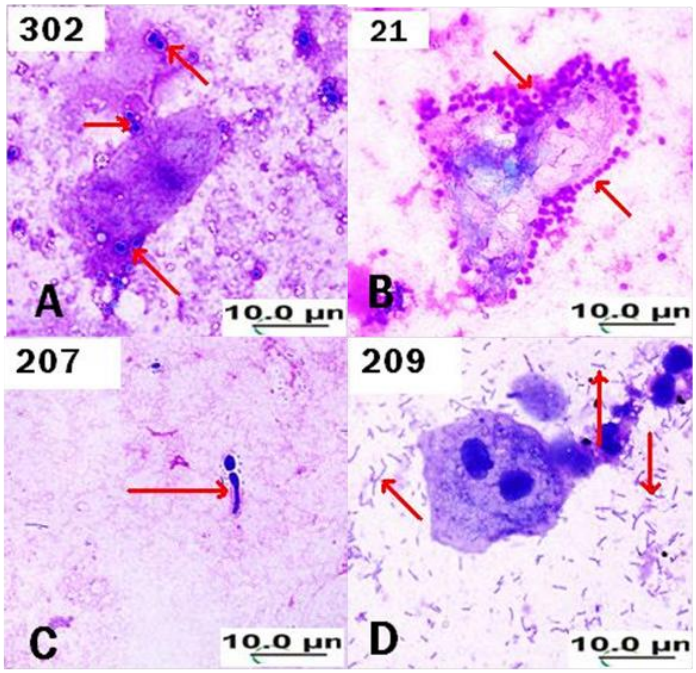

Figure 1. Phase contrast image in Giemsa slide of examined Lab.(numbers) specimens. (A) Yeast cells seen without lactobacilli. (B) Clue cells of various bacteria surrounding epithelial cells. (C) Yeast cell with short hyphae formation and few lactobacilli. (D) Only many lactobacilli around epithelial cells.

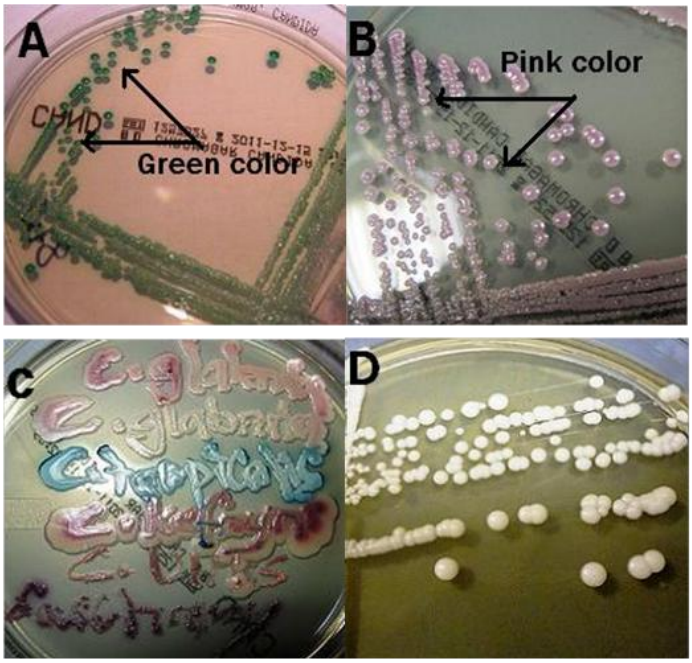

Figure 2. Detected color of *Candida* species on "CHROM agar Candida". (A) *C. albicans*, green colonies. (B) *C. glabrata*, pink colonies. (C) *C. glabrata*, pink colonies; *C. tropicalis*, blue-purple color; *C. kefyr*, pink color; *C. utilis*, green color; *S. cerevisiae*, dark pink color. (D) Positive culture of yeast-colonies on Sabouraud Dextrose Agar.

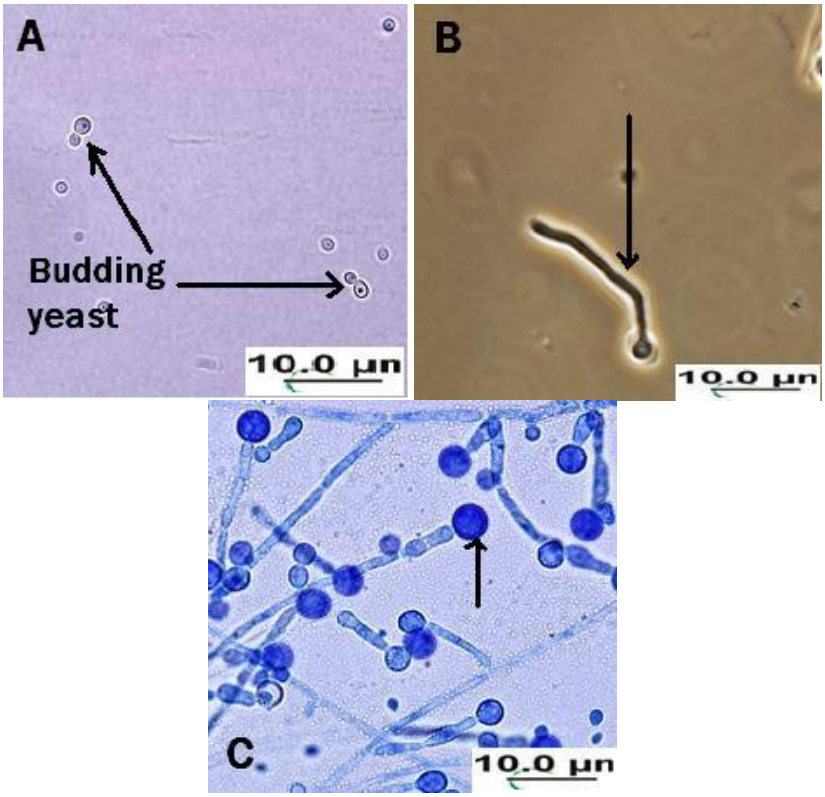

Figure 3. Phase contrast image. (A) Positive microscopic finding budding yeast in vaginal smear (10% KOH). (B) Germ tube identified on microscopic examination of *C. albicans* (100X). (C) Chlamydospores and pseudohyphae on microscopic examination *C. albicans* (lactophenol cotton blue 100X).

was considered as significant.

RESULTS

A total of 707 women referred to MMCH were enrolled in this study. The HVS specimens of only 157 (22.21%) yielded *Candida* positive culture (Table 1). To assess the effect of age on the prevalence of vaginal candidiasis, test group patients and control group ones were categorized into different age groups as presented in Table 1. The subjects women age varied from 18 years to 54 years. In both test and control groups, women of the 26-30 age group had the highest frequency of *Candida*-positive cultures followed by the 31-35 age group, whereas the women aged above 46 years showed the lowest frequency of *Candida*-positive samples. Statistical analysis (Chi-square test and Fishers exact test), showed significant difference with only the 41 to 45 age group (*P=0.026*). Further, the potential risk factors listed in Table 2 for positive VC were also evaluated. Out of 495 test group and of 212 control group specimens, *Candida* positive culture was detected in 120 and in 37 specimens respectively (Table 2). These findings indicate as expected that *Candida* spp. positive culture is frequently encountered among pregnant women as compared to those detected among non-pregnant patients, and this difference is statistically significant *(P=0.047*). Similarly higher incidence of *Candida* spp. positive culture was also observed (123, 25.89%) among those with parity than among those without parity (34, 14.65%), and this difference was also statistically significant (*P=0.001*). As shown in Table 2, there was no significant difference in the incidence of *Candida* spp. culture positive between women who previously used oral contraceptives and women who did not (27.52% versus 21.24%, *P=0.146*). Likewise in this study 215 women previously had taking antibiotics and 492 women did not. The frequency of culture positive for *Candida* spp. in the former group was (40, 18.60%), whereas in the later group it was (117, 23.78%), but again with no significant difference (*P=0.128*). In contrast, the rate of *Candida* spp. positive culture was higher in women with diabetes mellitus than in those without diabetes mellitus (53,06% versus 19.91%), and this differences was statistically significant *(P<0.0001)* (Table 2).

In comparison to the 157 *Candida* spp. detected culture positive (on SDA medium), the direct 10% KOH wet-

Table 1. Distribution of pregnant and non-pregnant women of different age groups.

Age group	Total number	No of women with *Candida* culture positive		Percent %
		Test group (Pregnant)	Control group (Non-pregnant)	
15-20	30	5	0	3.18
21-25	149	19	9	17.83
26-30	229	42	13	35.03
31-35	128	25	8	21.02
36-40	113	18	6	15.29
41-45*	44	10	0	6.37
46-50	8	1	0	0.64
51-55	6	0	1	0.64
Total	707		157	100

*Statistically significant P = 0.026.

Table 2. Correlation of various risk factors of vaginal candidiasis.

Risk factor	*Number	*Candida* culture positive		*P-value*
Pregnancy				
Pregnant	495	120	24.24%	0.047
Non-pregnant	212	37	17.45%	
Parity				
Yes	475	123	25.89%	0.001
No	232	34	14.65%	
Contraceptive				
Users	109	30	27.52%	0.146
Nonusers	598	127	21.24%	
Antibiotics				
Users	215	40	18.60%	0.128
Non-users	492	117	23.78%	
Diabetics				
Yes	49	26	53.06%	<0.0001
No	658	131	19.91%	

*Total number of patients studied =707 and Total number of culture positive = 157.

mount detected yeast cells (Figure 2-A) only in 132 specimens (132/157, 84.08%) while the Giemsa stain method showed higher sensitivity and detected 149 specimens (149/157, 94.90%) of those proved *Candida* spp. positive culture, but trichomoniasis was not detected in any of the examined 707 HVS specimens. Though non significantly different, detection of positive yeast cells was higher among test group as opposed to the control group (Table 3). As compared to the gold standard method (culture on SDA medium), the direct microscopy methods showed 84 and 95% sensitivity for the 10% KOH and the Giemsa stain, respectively, while the specificity for both was 100% (Table 3).

As illustrated in Figure 1A-D and Table 4, Giemsa stain examination of 707 specimens revealed yeast with or without pseudohyphae form in 81 specimens (11.46%), bacterial cells surrounding epithelial cells (clue cells-presumptive of bacterial vaginosis) in 90 (12.73%) and also with yeasts in 16 specimens (2.26%) while in as much as 51% (364/707) of specimens, no special observation was detected.

As presented in Table 2 the test group showed 24.24% (120/495) *Candida*-culture positive whereas those of the control group revealed 17.45% (37/212). The results

Table 3. Sensitivity, Specificity and Predictive Value of direct microscopic examination by the 10% KOH and the Giemsa stain methods.

Type of the test	Sensitivity	Specificity	Positive Predictive value	Negative predictive value
10% KOH[*a]	84%(132/157)	100%	100%	95.7%
Gemisa stain[*b]	95%(149/157)	100%	100%	98.6%

*[a]P = 0.071; [*b] P = 0.081.(Compared to standard positive cultures on SDA medium).

Table 4. Microorganisms detected in the direct Giemsa stain-microscopic method.

Organism	Direct examination	Percent (%)
Yeast only	81	11.5
Bacterial cells attached to epithelial- cells (Clue-cells)*	90	13
Many lactobacilli	104	15
Yeast and clue cells*	16	2
Yeast and many lactobacilli	52	7
Lactobacilli in different forms	364	51.5
Trichomoniasis	0.0	0.0
Total	707	100%

*Clue –cells are presumptive of bacterial vaginosis.

seem to indicate that positive yeast culture is more frequently encountered among pregnant women, as opposed to the non-pregnant women, and this difference is statistically significant (*P=0.047).*

The present study also assisted the parallel direct cultivation of all HVS swabs from studied 707 subjects on SDA medium (Figure 2) as well as on "CHROM agar Candida" (Figure 2). CHROM agar Candida proved as efficient as SDA direct culture with all *Candida* spp. positive culture from both test and control groups with advantage of presumptive identification of the frequently isolated *Candida* spp. *C. albicans* isolates colony showed light green color, whereas isolates of *C. glabrata*, *C. tropicalis*, *C. kefyr*, *S. cerevisiae*, *C. famata*, *C. utilis* and *R. mucilaginosa* exhibited the colors of pink, blue-purple, pink, dark pink, off white-cream, light green and dark green, respectively (as exemplified in Figure 2). These findings suggest that the use of CHROM agar Candida seems an easy and reliable method for the presumptive identification of most commonly isolated *Candida* species in single and/or mixed infections especially *C. albicans*, *C. tropicalis* and *C. krusei* (Figure 2).

To differentiate the *Candida* spp., the isolated yeast strains (157) were further identified using the germ tube test, chlamydospore formation and API system 20C AUX (Biomerieux, France). So in this study 120 *Candida* positive culture were detected in the test group and 37 in the control group. Out of the test group, 120 isolates, 98 (82%) formed (+) germ tube test (Figure 3-B) while out of the control group 37 isolates 28 (76%) gave positive results, yet this difference was not significant. These findings strikingly confirm that *Candida albicans* is still the most frequently isolated fungal species from vaginal specimens.

Likewise all *Candida* isolates positive for Germ tube test were also positive for chlamydospore (Figure 3-C) formation on Corn meal agar and thereby assigned as *C. albicans*. It is observed that all assigned *C. albicans* exhibited primarily single terminal chlamydospore (Figure 3-C) and those of quadruple and / or cluster ones (presumably *C. dubliniensis*) were not observed.

Each of the 157 yeast isolates on SDA was further tested for its biotype assimilation on API system 20C AUX for comparison and identity confirmation. Obtained data (Table 5) revealed as much as 8 species of yeasts including *C. albicans* which was the most common species (80.25%) as expected among both test and control groups followed by *C. glabrata* (12.74). Other species were rarely encountered as *C. tropicalis* (2.55%), *C. kefyr* (1.27%), *S. cerevisiae* (1.27%), *C. famata* (0.64%), *C. utilis* (0.64%) and *R. mucilaginosa* (0.64%). Results (Table 5) seem to indicate that non-albicans spp. are more frequently encountered among test group as compared to that of the control group , though the difference is not significant (*P=0.319)*.

As illustrated in Table 6 the majority of *C. albicans* strains (109/126) gave a common biotype, while the other strains fall within other 5 biotype patterns. Whether these different biotypes exhibited different potential invasiveness or not remain to be clarified. The main differentiated key–sugars (ability + and /or inability -) to assimilate arabinose, addinotol, xyitol, maltose or trehalose. On the other hand, *C. glabrata* the second most encountered species exhibited very low bioactivity

Table 5. The frequency (%) of yeast-biotypes isolated from Test and Control groups as identified by API 20 C.

Specie	Test group (pregnant)*	Control group (non-pregnant)
C. albicans	98(81.7)	28(75.7)
C. glabrata	15(12.5)	5.0(13.5)
C. tropicalis	3.0(2.5)	1.0(2.7)
C. kefyr	1.0(0.83)	1.0(2.7)
S.cerevisiae	2.0(1.67)	0.0(0.0)
C. famata	0.0(0.0)	1.0(2.7)
C. utilis	0.0(0.0)	1.0(2.7)
R.mucilaginosa	1.0(0.83)	0.0(0.0)
Total	120(100)	37(100)

** P=0.319*

Table 6. Biotypes of yeast species using carbohydrates assimilation test.

Tests	Sugar assimilation												
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	-	-	-	-	-	-	-	-	-	+	+	-	+
2KG[*a]	+	+	+	+	+	+	-	+	-	+	-	-	-
L-Arabinose	-	-	-	-	-	+	-	-	-	+	-	-	-
D-Xylose	+	+	+	+	+	+	-	+	-	+	-	-	+
Adonitol	+	-	+	-	-	-	-	+	-	+	-	-	+
Xylitol	+	-	+	+	-	+	-	-	-	+	-	-	+
D-galactose	+	+	+	+	+	+	-	+	+	+	-	+	+
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Sorbitol	+	+	+	+	+	+	-	+	-	+	-	-	+
MDG[*b]	+	+	+	+	+	+	-	+	-	+	-	-	-
NAG[*c]	+	+	+	+	+	+	-	+	-	+	-	-	-
D-Cellobiose	-	-	-	-	-	-	-	-	-	+	-	-	-
D-Lactose	-	-	-	-	-	-	-	-	+	+	-	-	-
D-Maltose	+	+	-	+	+	+	-	-	-	+	+	+	+
D-Saccharose	+	+	+	+	+	+	-	+	+	+	+	+	+
D-Trehalose	+	+	+	+	-	+	+	+	-	+	-	+	+
D-Melezitose	-	-	-	-	-	-	-	+	-	+	+	+	+
D-Raffinose	-	-	-	-	-	-	-	-	+	+	+	+	+
Pseudohypae	+	+	+	+	+	+	-	+	+	-	+	-	-
API Identity	CA	CA	CA	CA	CA	CA	CG	CT	CK	CF	CU	SC	RM
Total isolates	109	6	5	3	2	1	20	4	2	1	1	2	1

*[a]Calcium2-keto-Gluconate; *[b] Methyl-D-Glucopyranoside;[*c] N-Acetyl-Glucosamine; CA, *C. albicans*; CG, *C. glabrata*; CT, *C. tropicalis*; CK, *C. kefyr*; CF, *C. famata*; CU, *C. utilis*; SC, *S. cerevisiae*; RM, *R. mucilaginosa* ; + , fermented and − , not fermented.

with positive assimilation of only glucose and trehalose. In contrast *C. famata* (torulopsis *Candida*) exhibited hyper bioactivity on most of tested API sugars including cellobiose and glycerol, while all other species did not assimilate cellobiose nor glycerol with the exception of *C. utilis* and *R. mucilaginosa* that showed positive assimilation for glycerol. Results in Table 6 also showed that lactose was only assimilated by 2 species *C. kefyr*, and *C. famata* indicating their positive B galctosidase activity (Table 6).

Results of this study on the antifungal activity of tested agents (Table 7) indicated that all *Candida* spp. tested against ketocanazole were susceptible. While 94.4% of C*. albicans (*119/126) strains were also susceptible to fluconazole, whereas 55% of non-albicans spp. Exhibited resistance to fluconazole predominantly *C. glabrata*

Table 7. Comparative susceptibilities of *Candida albicans* and non-albicans spp. against fluconazole and ketoconazole.

Antifungal-agent	Classified as	*C. albicans* n=126 (%)	Non-albicans n=31 (%)
Ketoconazole	S	125 (99)	31 (100)
	I	1.0 (<1)	0.0 (0.0)
	R	0.0 (0.0)	0.0 (0.0)
Fluconazole	S	112 (90)	10 (33)
	S-DD	7.0 (5)	4.0 (12)
	R	7.0 (5)	17 (55)

S, susceptible; I, intermediate; R, resistant ; S-DD, susceptible dose dependent.

(17/31, 55%).

DISCUSSION

Candida spp. infection is one of the most common causes of vaginitis among women, second only to bacterial vaginosis, and pregnancy represents a risk factor in the occurrence of vulvovaginal candidiasis. Therefore, this study was initiated to determine the prevalence rate of vaginal colonization by *Candida* spp. in Saudi pregnant and non-pregnant women, their respective species identification, detection methods, correlation to possible risk factors, and their antifungal susceptibility patterns. Out of the overall 707 examined HVS-specimens, 157 yielded relatively high rate of positive (22.21%) yeast culture, despite the fact that women participated in this study where those who for routine examinations of vaginal secretion, independent of the presence or absence of VVC-symptoms. These findings are consistent with those previously reported from other countries as Germany (Mendling and Brasch, 2012), Italy (Consolaro et al., 2004), India (Jindal et al., 2006, 2007; Ahmad and Khan, 2009), Australia (Pirotta and Garland, 2006) and Nigeria (Nwadioha et al., 2010), as well as, Saudi Arabia (Al-Hedaithy, 2002).

Our results also indicated that *Candida* positive culture was significantly correlated with pregnancy (*P=0.047),* the increased number (malt-parity) of pregnancy *(P=0.001),* as well as women-child bearing age of (40-45 years versus same age but non-pregnant (*P=0.026*) which confirms previous findings by Akinbiyi et al. (2008).

As compared to the non-*albicans* spp., *C. albicans* was more frequently isolated from the pregnant women compared with non-pregnant women, presumably due to the opportunistic plasticity nature of *C. albicans* as triggered by estrogenized vagina and/or the high glycogen content during pregnancy. Yusuf et al. (2007) postulated that lactobacilli play a crucial role in the conversion of glycogen to lactic acid, hence decreasing pH of the vagina, thereby suppresses activities of the bacterial biota but favors the growth of yeast-species. *Candida* spp. -colonization may also be associated with genetic predispositions, for the absence or presence of secretors of Lewis antigens-glycoproteins that potentially may inhibit the binding of *Candida* to vaginal mucosa (Chaim et al., 1997). The increase of glycogen in the vaginal cells not only has a direct growth promotion of *Candida* but also facilitated its adherence to the epithelial cells (Barousse et al., 2004).

Likewise, our results strikingly showed that colonization of *Candida* spp. is more common in diabetic women *(P=0.0001)* than in non diabetic ones which confirms previous findings (Faraji et al., 2012). It has been reported that increased glucose levels in genital tissues enhance yeast adhesion and growth and that vaginal epithelial cells bind to *Candida* spp.- *cells* with greater propensity in diabetic women than in non-diabetic ones (Barousse et al., 2004).

Regardless of pregnancy, the observed increased frequency of *Candida*-positive cultures in women-group of age range 26 to 35 years old, agree with the results of other workers (Akinbiyi et al., 2008), who attributed that to the active behavioural sexual relations in this age group. Generally and in agreement with the study of Okungbowa et al. (2003), a lower frequency of Candida-positive cultures was observed among women aged above 41 years, but pregnancy at this age favours *Candida*- colonization.

In the current study, there was no significant correlation between the incidence of *Candida*-culture positive from women who previously used oral contraceptives *(P= 0.146)* or antibiotics *(P=0.128)* and those who did not, whereas other studies showed significant correlation in this regard (Yusuf et al., 2007; Xu et al., 2008; Bahram et al., 2009). Also some reports found no evidence of an association between antibiotic-drugs and symptomatic VVC, whereas others concluded otherwise (Xu et al., 2008). This discrepancy is apparently due to differences in size of studied women subjects, duration and types of used contraceptives or antibiotics, as well as methods of yeast-detection.

Vaginal yeast infection is usually diagnosed on the basis of clinical symptoms and direct microscopic

examination and (Nyirjesy, 2008; Faraji et al., 2012). The microscopic examination of the clinical material is rapid, easy and may identify the presumptive etiologic agent, but vaginal culture is indispensable to confirm the diagnosis (CDC, 2010). In this study, direct 10% KOH microscopic-examination for yeast cells and pseudohyphae was found to be positive in 132 of the 157 women with positive culture, with a sensitivity of 84% and specificity of 100%. This finding is compatible with that of 65-85% and higher than that of 50% previously reported by other investigators (Jindal et al., 2007; Nyirjesy, 2008; CDC, 2010) respectively. In comparison, the Giemsa staining method revealed better sensitivity (95%) than that of KOH, probably due to the difference in the concentration of yeast cells in different vaginal secretions (Jindal et al., 2006) and/or better visualisation. The Giemsa staining method also provided additional valuable information for the presence or absence of trichomoniasis and clue cells (presumptive diagnosis of bacterial vaginosis) as well as different morphological forms of lactobacilli which largely affect and maintain the balance of vaginal microbiota. Based on these findings, therefore, we believe that Geimsa staining and/or culture should be explore as a method for routine microscopic examination of the vaginal secretions in lab diagnosis of symptomatic vaginitis, as it is rapid, easy and may identify the presumptive etiologic agent, especially in regions where facilities for PCR assays (Nyirjesy, 2008) are limited, and its high cost would preclude its use. Though PCR testing method is highly sensitive, specific and available commercially, its usefulness is also limited by the need to obtain PCR for the full spectrum of organisms that can cause VVC and therefore added expense relative to direct microscopy examination and/or culture (Nyirjesy, 2008).

This study also confirmed that “CHROM agar Candida” based on different colors and consistency of colonies, provides a rapid presumptive identification of common yeasts species. Although “CHROM agar Candida” appears to be a quite accurate in identify the most common *Candida* spp. it is not proposed as a substitute for standard identification protocols. The major advantage of “CHROM agar Candida” was its ability to detect the presence of more than one yeast-species (Nejad et al., 2011). Our results on the assimilation test of *Candida* species (API 20C AUX) revealed a common single biotype of *C. albicans* among other 5 biotypes detected, and confirmed the identity of recovered non-albicans spp. (*C. kefyr*, *C. famata*, *C. utilis*, *S. cerevisiae* and *R. mucilaginosa*). Whether these different biotypes of *C. albicans* vary in their proliferation and invasive abilities or not remains to be clarified. *C. albicans* was the most common species among the isolates (126, 80.25%) followed by *C. glabrata* (20, 12.73%), *C. tropicalis* (4, 2.5%), *C. kefyr* (2, 1.27%) and other species (5, 3.1%). Thus, the overall prevalence of non- albicans spp. was 19.75%. These findings are consistent with those previously reported by other researchers (Pirotta and Garland, 2006; Ahmad and Khan, 2009). Similarly, *C. glabrata* was the second most common species in the USA (Holland et al., 2003), Iran (Mahmoudi-Rad et al., 2011), India (Ahmad and khan, 2009) and also in Saudi Arabia (Al-Hadeithy, 2002).

Furthermore, our data on the antifungal activity of fluconazole against *C. albicans,* revealed that 88.1% of tested strains were susceptible. This sensitivity rate is more or less comparable with those rates of 87.5%, 80.9% and 89.5% previously reported by Citak (2005), Teseng et al. (2005) and Badiee and Alborzi (2011) respectively. In agreement with the study of Sabatelli et al. (2006), most of the detected resistant strains belong to *C. glabrata,* emphasizing, its greatest potential to acquire resistance to fluconazole (Sabatelli et al., 2006). In agreement with the findings of Ng et al. (2000) and Richter et al. (2005) our ketoconazole -susceptibility data showed that all yeast isolates were susceptible, and no observed remarkable difference in the susceptibility between *C. albicans* and non-albicans spp..

In conclusion the overall results of this study emphasize the importance of the different risk factors that play a role in *Candida*-colonization. The culture of vaginal discharge should be warranted because culture technique is more sensitive than direct smear. These findings may help in drawing strategies in preventing and controlling vulvovaginal candidiasis that may affect women and their newborns.

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