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Identification and Characterization of *Candida* on CHROMAgar™ in Pregnant Women of Multan, Pakistan

Sidra Manzoor[1], **Mubashar Aziz**[2] **and Ahsan Sattar Sheikh**[3*]

[1]*Department of Molecular Biology and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan*

[2]*Department of Pathobiology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan*

[3]*Institute of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Pakistan*

[*]**Corresponding author:** Ahsan Sattar Sheikh, Institute of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Pakistan, Tel: +92-0322-4058338; E-mail: assheikh@bzu.edu.pk

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Abstract

Introduction: Infections to vagina with fungi/yeast are ten times more common during pregnancy because of hormonal and biochemical fluctuations. Increased use of antibiotics and presence of high levels of reproductive hormones during pregnancy also stimulate yeast colonization with *Candida* species. These two contributes significantly to be an important risk factor in Vulvovaginal Candidiasis (VVC) and Urinary Tract Infections (UTI), a complication during pregnancies. Overall occurrence of UTI/vaginal yeast infections increases with progression of pregnancy and maximizes by third trimester worldwide.

Study parameters: In the current study, pregnant women were look for presence of UTI by *Candida* species in different trimesters within the city of Multan. The total of 70 women with different gestational stages, attending gynecologists, aged 18-39 years were included in the investigation. A clean catch urine samples were cultured on CHROMAgar™ *Candida*, a selective medium, for the isolation and presumptive identification of yeast species. Of all samples, 25 women (36%) were positive for *Candida* yeast infection (symptomatically and asymptomatically). Colonies were identified as *C. albicans* (48%), *C. krusei* (16%), *C. tropicalis* (16%) along with some mixed infections with *C. parapsilosis* and *C. krusei*. Two of the mixed cultures (8%) were isolated with *C. albicans* and *C. tropicalis*, one (4%) with *C. albicans* and *C. krusei*, one (4%) with *C. tropicalis* and *C. krusei*.

Results: The highest incidence, 36% (n=9/25), of candiduria was recorded in the age group of 30-35 years. The colony counts in urine were also studied in different gestational stages and age. Total of 23% isolations share high counts (>1000 CFU/mL). While other 33% have intermediate (<600 CFU/mL) and 30% isolates were with low colony counts (<150 CFU/mL). The species distribution in these positive specimens reveals increased percentages of non-albicans species with increasing gestational stage.

Conclusion: From these findings it is concluded that incidence of candiduria in pregnant women were higher in 2nd and 3rd trimester of pregnancy with an increase in mixed *Candida* infections in young women. It is recommended to educate the group so that less young individuals are affected.

Keywords: Pregnancy; Infection; *Candida albicans*; Non-albicans species

Introduction

Vaginosis in pregnancy is most commonly linked to infection by *Candida* species [1]. In female, *Candida* is also part of normal flora and become opportunistic pathogen. In these women with Vulvovaginal candidiasis (VVC), most common problem and affects women two or more episodes yearly [2-4], can also involve urinary tract [5,6]. The infection accounts for 40-50% of all women cases within their childbearing age [7]. Accurate prevalence of these infections are quite difficult to assess as one third to three quarter of affected women 'feel' asymptomatic [7,8]. Recent randomized screening in pregnant women indicates complications like candidiasis, bacterial vaginitis, and/or trichomonasis, are on the increase that could result in spontaneous preterm birth in 46% of pregnancies by 15-19 weeks of gestation [9]. It has been reported that about 75% females develop it at least once in their lifetime [10]. *Candida albicans* is most frequently (90%) isolated form compared to other non albicans species [11]. Recent reports on *Candida* infections suggests that there is a shift in colonization pattern by *C. galabrata* [12] and other non-albicans species within developed countries [13]. From data, it is estimated that *Candida galabrata* account for 4-5% in these country individuals. While other non albicans species appear to be still less common that includes *Candida tropicalis* and *Candida parapsilosis* [14].

It is very difficult to ascertain the involvement of *Candida* species in vaginosis and later with candiduria. During vaginosis more than 50% of these individuals are asymptomatic and harbor endogenous vaginal microbiota [15]. This asymptomatic vaginosis very efficiently gets into urinary tract that could result without tissue invasion [16,17]. Milder signs and symptoms in urinary *Candida* infection don't permit proper diagnosis [18]. Factors like socio-economic variables, maternal smoking, other genital infections and short cervix contributes to increase colonization by *Candida* species [19]. These UTIs are also associated to number of gynecological and obstetric complications,

such as preterm birth [20], premature rupture of the membranes, chorioamnionitis [18], postpartum endometritis, inflammatory pelvic disease, intrauterine growth retardation [21], and low birth weight of the new born [1]. There are reports of transfer of *Candida* species from vaginal infection to new born also [5,22]. Increased UTI colonization and candiduria are believed to be due to higher incidences of vaginosis resulting from high estrogen levels, high glycogen content, and use of antifungal agents in treatment of VVC [21,23]. Identification of shared similar drug resistance markers in *Candida* and its species, from urine and vaginal, demonstrates their common ancestral origin [19]. Pregnancy is typically broken into three periods, or trimesters [20]. It has been elucidated with good conviction that *Candida* species can be cultured from the urine or vagina in more than >25% of pregnant women who are approaching term [24]. Prevalence survey in 228 hospitals from 29 European countries determined that just 9.4% of nosocomial UTIs were caused by *Candida* species [11]. During this period considerable high rates are observed with non albicans species. This isolation frequency of non-albicans species especially in some countries, are unclear and dubious of its selection [20]. This scenario is further compounded with presence of antifungal resistance in non-albicans *Candida* species that is a worry for future [25].

Material and Methods

Sample area and collection

Total of 70 randomly collected urine samples from pregnant women in different pregnancy trimesters was included in the study. The subjects were between 18 to 39 years. Sampling was done in local winter-spring season (more marriages in winter months). Urine was collected from antenatal patients' vising medical centers, maternity homes of Multan, Pakistan. Patients were inquired to complete a detailed questionnaire. The questionnaire includes demographic data (age, age at marriage, cousin marriage), number of pregnancies/deliveries, trimester, signs and symptoms related to *Candida* infection. The midstream urine was collected in sterile urine containers (China) and transferred to Micro/Molecular Biology Laboratory (MMBL) at IPAB, Bahauddin Zakariya University, Multan 60800 in a cooler packed with ice packs for further processing.

Media preparation and sample processing

CHROMAgar™ *Candida* was provided by CHROMAgar™, Paris, France. Media plates with antibiotics were made according to the manufacturer instructions. Prepared plates were stored in dark at 4°C till further use. Individual sample (1 mL) was centrifuged in sterile 1.7 mL microfuge tube (China) at 1400 g for 1 minute (Heareus, Germany). Samples having more than 10 WBC/HPF were selected for inoculation on to CHROMAgar™ *Candida* plates (0.1 mL of neat urine) with a pasture glass spreader in a clean bench surface (Dalton, UK). Plates were incubated (Memmert, Germany) at 36 ± 0.5°C for 48 hours. Colonies were identified according to manufacturer's instruction or described by Odds and Bernaerts.

Statistical analysis

Data was formulated from the questionnaire for all parameters and were analyzed for ANOVA and correlation of significance by SPSS 17 (SPSS Inc., USA).

Results

Isolation and identification

Direct presumptive identification of yeast species is a challenging task. Several brands of chromogenic media are available for rapid identification of yeast. This also includes CHROMAgar™ *Candida* [26,27]. This media provides straight forward advantage in time over conventional identification methods in mixed yeast infections. This is also important in significant clinical bearing [28]. In a total of 70 samples, only 25 samples (36%) turned positive for *Candida species* (Table 1).

The chromogenic substances in media help to identify *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis*. The *C. albicans* colonies were identified as green, *C. tropicalis* as steel blue, and *C. krusei* as fuzzy rose colored colonies. *C. parapsilosis* appears with pink to pale yellow colonies [29]. Other groups have also reported further success in differentiation of *C. dubliniensis* from *C. albicans* on CHROMAgar™ *Candida* medium which our group did not performed. From the total positive cultures, *C. albicans* (48%) share the maximum load (Table 1).

Non albicans *Candida* share just 32% in pure culture category. Mixed culture infections of *C. albicans* with either *C. krusei* or *C. tropicalis* (12% and 8% respectively) as non albicans were also recorded in the study.

	Pure culture				**Mixed culture**			
Age Group	*C. albicans*	*C. krusei*	*C. tropicalis*	*C. parapsilosis*	*C. albicans* *C. krusei*	*C. albicans* *C. tropicalis*	*C. krusei* *C. tropicalis*	*C. krusei* *C. parapsilosis*
18-23 Y (n=21)	2 (10%)	2 (10%)	1 (5%)	-	-	1 (5%)	1 (5%)	1 (5%)
24-29 Y (n=29)	4 (13%)	1 (3%)	-	-	2 (7%)	-	-	-
30-35 Y (n=19)	6 (32%)	1 (5%)	2 (11(%)	-	-	-	-	-
36-41 Y (n=1)	-	-	1 (100%)	-	-	-	-	-
	n=20				n=5			

Table 1: Relationship between age and *Candida* species infection in pregnant women.

Pregnancy trimester and *Candida* species

Breakup of pregnancy into trimester showed that most positive yeast infection occurred in the 3rd trimester (36%) (Table 1). Out of 15 positive cultures only 2 had mixed culture outcome (Table 2). In the 2nd trimester, highest number of specimens collected turned positive (47%). The species isolated and identified in all trimester specimens showed that 80% have single organism (Table 1).

	Age groups (Years)			
CFU/mL	**18-23**	**24-29**	**30-35**	**36-41**
<150 (n=9)	4	3	1	1
<300 (n=4)	2	-	2	-
<600 (n=10)	4	3	3	-
>1000 (n=7)	1	3	3	-

Table 2: Relationship between age and vaginal *candida* burden in pregnant women.

Interestingly, 60% of positive colorizations were within 3rd trimester (Table 2). Presence of *C. krusei C. albicans* or *C. tropicalis* or *C. parapsilosis* were identified in 4 cultures (20%) and 4 mixed cultures respectively (Table 2).

Age, *Candida* species and yeast burden

There were 15 *Candida albicans* cultures (60%) isolated that were either pure or mix with *C. krusei* or *C. tropicalis*. All mixed infections (20%) were, interestingly, present in all young age groups (18-23 years) and (24-29 years). In the middle age group, only single organism infection (predominantly *C. albicans*- 67%) occurred (Tables 2 and 3).

Talking about the yeast burden, most infections (>600 CFU/mL) were identified in middle age groups (24-29) years and 30-35 years) (Table 3). This group also is dominated by pure cultures by 2/3 positivity with *C. albicans*. Low yeast counts were predominantly noticed in only younger age groups (Table 3).

		Pure Culture				Mixed Culture			
Pregnancy (trimester)	**Infected n=25**	*C. albicans*	*C. krusei*	*C. tropicalis*	*C. parapilosis*	*C. albicans* *C. krusei*	*C. albicans* *C. tropicalis*	*C. krusei* *C. tropicalis*	*C. krusei* *C. parapilosis*
1st (n=11)	2 (18%)	-	-	1	-	1	-	-	-
2nd (n=17)	8 (47%)	4	2	-	-	1	1	-	-
3rd (n=42)	15 (36%)	8	2	3	-	-	-	1	1
Total=70	25	12	4	4	-	2	1	1	1

Table 3: Distribution of positive culture with trimesters of pregnancies.

Statistical analysis

ANOVA and correlation analysis on various factors indicated that yeast infections were highly significant (p=0.000 at 95% CI) to the pregnancy in this study. Analysis also correlates to *Candida* cell counts which is highly significant to pregnancy month (Pearson's Correlation 0.872 and significance of 0.000 at 0.01% CL) and trimester (Pearson's Correlation 0.447 and significance of 0.000 at 0.01% CL).

Data also specify that numbers of pregnancies are also significantly placed to the age and symptoms (Tables 4 and 5).

Pearson's Correlations (n=70)						
Variables		**Age**	**CFU/mL**	**Deliveries**	**Pregnancies**	**Candida Sp.**
Age	Pearson Correlation					
	Sig (2-tailed)					
CFU/mL	Pearson Correlation	1		-	-	-
	Sig (2-tailed)					
Deliveries	Pearson Correlation	0.422**	1	1	-	-

	Sig (2-tailed)	0	-			
	Pearson Correlation	0.506**	-	0.784**		-
Pregnancies	Sig (2-tailed)					
	Pearson Correlation	0	0.557**	0	1	
Candida Sp.	Sig (2-tailed)	-	0	-	-	1

Table 4: Pearson's correlation of *Candida* species.

Interestingly, as the age of patient increases, chance of symptomatic rather asymptomatic infection also increases. This criterion of positively relate to number of pregnancies and deliveries (Table 5). Correlation dissection showed that in 1st and 2nd trimesters yeast burden is highly significant to pregnancy month and infecting *Candida* species (Tables 4 and 5). Comparison of same parameters in 2nd and 3rd trimester (most positive cultures) indicates that pregnancies correlates highly to symptoms and the month of pregnancy. *Candida* counts, however, were highly negatively rerated to *Candida* species and deliveries (Tables 5 and 6).

Pearson's Correlations (2 tailed)									
Variables	**Age**	**Symptoms**	**Trimester**	**Month**	**Hospital**	**Pregnancies**	**Deliveries**	**Candida Sp.**	**CFU/mL**
Age	1								
Symptoms	0.354**	1							
	0.001								
Trimester			1						
Pregnancy month	0.346**		0.811**	1					
	0.001		0						
Hospital				0.251*	1				
				0.021					
Pregnancies	0.615**	0.466**			0.307**	1			
	0	0			0.004				
Deliveries	0.372**	0.340**				0.823**	1		
	0	0.002				0			
Candida Species						-0.333**	-0.300**	1	
						0.002	0.006		
CFU/mL			0.398**	0.301**			-0.230*	-0.245*	1
			0	0.005			0.035	0.025	

Table 5: Pearson's correlations of trimester 2 and 3 (n=59).

ANOVA		
Candida Species	Between groups	0.792
	Within groups	
CFU/mL	Between groups	0.991

	Within groups	
Deliveries	Between groups	0.004*
	Within groups	
Pregnancies	Between groups	0.000**
	Within groups	

Table 6: Analysis of variance with age.

Discussion

During pregnancy, vagina is more susceptible to many infections because of its proximity, results in higher incidences of lower urinary tract colonization [16,17]. In a literature citation, candiduria indicates an asymptomatic presentation in patients, yet presence of yeast is noted in the urine as a serendipitous in routine urinalysis or urine culture [16]. These infections have lower cure rates [25] with more resistant species [20]. It has been demonstrated further that micro biota of vagina is of similar patterns as observed in urine [30]. Recent literature also highlights yeast drug resistance in non albicans yeast strains than in any other [4,22]. Thus, rapid identification of these yeast strains provides valuable information concerning treatment regimens in clinical settings [27,31]. Rein and colleagues [32] provided an outlined for infections in 60-90% women. In our study, total of 70 patients, 17 women were placed in 2nd trimester of pregnancy out of which 8(47%) were positive for *Candida* species (Table 2).

In 3rd trimester, one third (36%) specimens were positive. Jointly together, they share two third of women get candiduria during their antenatal days which is statistically important (Table 5). This is contradictory to recent finding where trimester doesn't have any adage [33]. In number of other studies, however, demonstrated that from the conception day till it reaches 2nd and 3rd trimester, *Candida* infections were recorded highest [5,25,34]. Similar conclusion was drawn from study of Rein et al. [32] where 10% of infections were in 1st trimester as compared to 36-55% in the 3rd trimester. Some other studies provide some controversies on rates of candiduria. These studies show that 3rd trimester has similar rates to that of 23-26 (2nd trimester) weeks of gestation [35]. This provides a positive backing to our study (Table 3).

CHROMAgar™ *Candida* has previously been reported to provide valuable results in identifying *Candida* species [2,26,27]. The accurate identification of *Candida* species on colony color and morphology provide fast and decisive offering for clinical treatment regimens [2,28]. Use of such culture medium also addresses the important issue of non albicans species which are emerging all around and is a matter of grave concern around the world [3,12,22]. In our hands, results conclude that all major pathogenic *Candida* species were conveniently identified on CHROMAgar™ *Candida*. This advantage of early identification is an essential step for clinician to use specific treatment protocols in lowering yeast burden in pregnant women. This provides lowering of complication during pregnancies [19,25]. In our study, *Candida albicans* was isolated in all cultures (either pure or mixed). The non albicans group shares; *Candida krusei* (28%), *Candida tropicalis* (20%), and *Candida parapsilosis* (4%) (Table 2). In a large multicenter study from Spain, *Candida albicans* were recovered as high as 68%, followed by *Candida galabrata* (8%) and *Candida tropicalis* (4%) [18]. The occurrence of such numbers has also been reported by da Silva et al. [17] from Brazil. Another large sample study from Iran, provided similar results as of our study results [16].

However, lower incidences of *Candida albicans* are reported from Tanzanian pregnant women [36-38] where hygienic and socio-economic conditions may have played the differences. In North America, Europe and Latin American studies with CHROMAgar™ show that *Candida albicans* share the burden 51.5%, 47.8%, and 36.5%, respectively [18,23]. Taken together, in our study in this part of the world, we observed similar isolation rate as indicated in above mentioned studies. In Mexican study, on the contrary, other isolated species includes were *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, and *Saccharomyces cerevisiae* [18,19,23]. In many other studies on specifically candiduria, *Candida albicans* is shown to share 50-70% burden followed by *C. galabrata*, and *C. tropicalis*, which is the third most common species [39,40]. Similar findings were recorded in this study also except presence of *C. galabrata* which we did not follow because of antibiotic resistance [19,38].

In present study, we also looked at the nature of asymptomatic and symptomatic yeast infection as per reported worldwide for *Candida* species. We find little lower symptomatic reporting of candiduria that is reported in other parts of the world. This may be linked to less education in the females. Significance of trimesters of pregnancy were also ascertained in the investigation. Our data show that 36% of the collected samples were positive with non albicans species (Table 2). Importantly of these, most common non albicans species, was *Candida tropicalis* in our case study (n=9/25) (Table 1). Similar patterns were seen in other studies [3,5,39,40]. Portuguese data from Negri et al. [39] provide good support to the notion that non albicans strains are becoming more important day by day.

The age distribution data in the study show that *Candida* infection is predominantly present in middle age (30-35 years) group. Majority of these isolates were pure infection of *Candida albicans* (55.6%) (Table 1). This is in agreement with the results of American and Brazilian studies [13,40]. On the contrary, Venezuelan study provided similar good support to age groups for other *Candida* infections [41]. Our data also signifies to the fact that more non albicans species and mixed infections were isolated in 18-23-year group (Table 1). We also incline to infer from our data that non albicans species are on rise [2,10,17]. In this study, *C. tropicalis* is next most prevalent species after *C. albicans*. Repentigny and co-workers [42] suggested that higher cases of this species tend to be a result of patients being neutropenic which isn't been convincible address in literature. This high level of colonization of non albicans were observed in older subjects which is not seen in our study (Table 1). It is also documented that occurrence of nonalbicans species (up to 20-30% in some cases) are associated to recurrent candidiasis [12]. Quantification of viable yeast cell indicates that 23% of the isolates have higher than >1000 CFU/mL of urine. Other studies

have also provided similar results [43-45]. Of these 1/3 of positive specimens had intermediate numbers and 30% had low counts (<150 CFU/mL) (Table 3). Interestingly low yeast counts were detected in younger subjects with more non-albicans species.

Over and above, worldwide and our data indicates that infections with *C. krusei*, *C. tropicalis* and other non-albicans species have gone up [18,44]. Occurrence of this high rate is quite disquieting. Recent literature highlights the notion that *C. krusei* along with *C. tropicalis* are involved in invasive fungal infections [18,19]. Other studies demonstrate that these organisms also show resistance to ttariazoles with high mortality [4]. Presence of these species in relatively high number of young pregnant women from Punjab, Pakistan is alarming. Possible explanation to the scenario may be due to malnutrition, lower immunity levels and use of antifungal agents that needs to be further investigated like other workers have done in other parts of the world (Tables 1 and 2) [19,45].

Conclusion

This study, first of its kind in south Punjab, concludes finally that candidiasis in Pakistani women is at the same rate, same species distribution as per seen in other parts of the developing world [43]. A larger future project would provide more insight into *Candida* species infections in non-pregnant and pregnant (different trimesters) women would provide discernment in resident microbiota that could contribute to this pathological conditions.

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References

1. Aslam M, Hafeez R, Ijaz S, Tahir M (2008) Vulvovaginal candidiasis in pregnancy. Biomedica 24: 54-56.
2. Guzel AB, Ilkit M, Akar T, Burgut R, Demir SC (2011) Evaluation of risk factors in patients with vulvovaginal candidiasis and the value of ChromID Candida agar versus CHROMAgar Candida for recovery and presumptive identification of vaginal yeast species. Med Mycol 49: 16-25.
3. Sobel J (2011) Genital candidiasis. Medicine 38: 286-290.
4. Mendling W, Brasch J (2012) Guideline vulvovaginal candidasis (2010) of the German Society for gynecology and obstetrics, the working group for infections and infect immunology in gynecology and obstetrics, the German Society of dermatology, the board of German dermatologists and the German speaking mycological society mycoses 55: S1-S13.
5. Kauffman CA, Fisher JF, Sobel JD, Newman CA (2011) Candida urinary tract infections-diagnosis. Clin Infect Dis 52: S452-S456.
6. Sobel JD, Vazquez JA (1999) Fungal infections of the urinary tract. World J Urol 17: 410-414.
7. Fidal PF, Cutright J, Steele C (2000) Effects of reproductive hormones on experimental vaginal candidiasis. Infect Immun 68: 651-657.
8. Halbreich U (2009) Women's reproduction related disorders. J Affect Disord 1: 24-26.
9. Mann PA, McNicholas PM, Chau AS, Patel P, Mendrick C, et al. (2009) Impact of antifungal prophylaxis on colonization and Azole susceptibility of Candida species. Antimicrob Agents Chemother 53: 125026-5034.
10. Achkar JM, Fries BC (2010) Candida infections of the genitourinary. Clin Microbiol Rev 23: 253.
11. Babić M, Hukić M (2010) Candida albicans and non albicans species as etiological agent of vaginitis in pregnant and non-pregnant women. Bosnian J Basic Med Sci 10: 90-97.
12. Vermitsky JP, Self MJ, Chadwick SG, Trama JP, Adelson ME, et al. (2008) Survey of vaginal-flora candida species isolates from women of different age groups by use of species-specific PCR detection. J Clin Microbiol 46: 1501-1503.
13. Tortorano AM, Rigoni AL, Biraghi E (2003) The European confederation of medical mycology (ECMM) survey of candidaemia in Italy: Antifungal susceptibility pattern of 261 non albicans Candida isolates from blood. J Antimicrobiol Chemother 52: 679-682.
14. Szilagyi J, Foldi R, Gesztelyi R, Bayegan S (2012) Comparison of the kidney fungal burden in experimental disseminated candidiasis by species of the Candida parapsilosis complex treated with fluconazole, amphotericin B and caspofungin in a temporarily neutropenic murine model. Chemother 58: 159-164.
15. Watson CJ, Fairley CK, Grando D, Garland SM, Myers SP, et al. (2013) Associations with asymptomatic colonization with Candida in women reporting past vaginal candidiasis: An observational study. Eur J Obstet Gynaecol Reprod Biol 169: 376-379.
16. Mahmoudabadi A, Zarrin M, Ghanatir F, Vazirianzadeh (2012) Candiduria in hospitalized patients in teaching hospitals of Ahvaz, Iran. J Microbiol 4: 198-203.
17. Da Silva EH, Ruiz LS, Matsumoto FE, Auler ME, Giudice MC, et al. (2007) Candiduria in a public hospital of Sao Paulo (1999-2004): Characteristics of the yeast isolates. Rev Inst Med Trop Sao Paulo 49: 349-353.
18. Miceli MH, Diaz JA, Lee SA (2011) Emerging opportunistic yeast infections. Lancet Infect Dis 142: 142-151.
19. Badiee P, Hashemizadeh Z (2014) Opportunistic invasive fungal infections: Diagnosis & clinical management. Indian J Med Res 139: 195-204.
20. Kalkanci A, Guzel, AB, Khalil IIJ, Aydin M, Ilkit M, et al. (2012) Yeast vaginitis during pregnancy: Susceptibility testing of 13 antifungal drugs and boric acid and the detection of four virulence factors. Med Mycol 50: 585-593.
21. Roberts CL, Rickard K, Kotsiou G, Morris JM (2011) Treatment of asymptomatic vaginal candidiasis in pregnancy to prevent preterm birth: An open-label pilot randomized controlled trial. BMC Pregnancy and Childbirth 11: 18.
22. Kazi YF, Saleem S, Kazi N (2012) Investigation of vaginal microbiota in sexually active women using hormonal contraceptives in Pakistan. BMC Urology 12: 22.
23. Dias LB, Melhem MDC, Szeszs MW, Filho JM, Hahn RC (2011) Vulvovaginal Candidiasis in Mato Grosso, Brazil: Pregnancy status, causative species and drugs tests. Brazilian J Microbiol 42: 1300-1307.
24. Trofa D, Gacser A, Nosanchuk JD (2008) Candida parapsilosis, an emerging fungal pathogen. Clin Microbiol Rev 21: 606-625.
25. Fisher JF, Sobel JD, Kauffman CA, Newman CA (2011) Candida urinary tract infections--treatment. Clin Infect Dis 52: S457-S466.
26. Bouchara JP, Declerck P, Cimon B, Planchenault C, Gentile L, et al. (1996) Routine use of CHROMAgar Candida medium for presumptive identification of Candida yeast species and detection of mixed fungal populations. Clin Microbiol Infect 2:202-208.
27. Madhavan P, Jamal F, Chong PP, Ng KP (2011) Identification of local clinical Candida isolates using CHROMAgar Candida as a primary identification method for various Candida species. Trop Biomed 28: 269-274.
28. Odds FC and Bernaerts R (1994) CHROMAgar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. J Clin Microbiol 32: 1923-1929.
29. Fraisse T, Lachaud L, Sotto A, Lavigne JP (2011) Recommendations of the infectious disease committee of the French association of urology diagnosis, treatment and monitoring candiduria. Prog Urol 21: 314-321.

30. Zahra S, Seifi Z, Mahmoudabadi A (2012) Sensitivity of vaginal isolates of Candida to eight antifungal drugs isolated from Ahvaz, Iran. Jundishapur J Microbiol 5: 574-577.
31. Nadeem SG, Hakim ST, Kazmi SU (2010) Use of CHROMAgar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited settings. Libyan J Med 5: 2144.
32. Rein MF, Holmes KK (1983) Non-specific vaginitis Vulvovaginal candidiasis and trichomonasis. J Infec Dis 4: 281.
33. Parveen R, Azmi MA, Tariq RM, Mahmood SM, Hijazi M, et al. (2008) Determination of antifungal activity of Cedrus deodara root oil and its compounds against Candida albicans and Aspergillus fumigates. Pak J Bot 42: 3645-3649.
34. Moharram AM, Ati MG, Othman EOM (2013) Vaginal yeast infection in patients admitted to Al-Azhar University Hospital, Assiut, Egypt. J Basic App Mycol (Egypt) 4: 21-32.
35. Cotch MF, Hillier SL, Gibbs RS, Eschenbach DA (1998) Epidemiology and outcomes associated with moderate to heavy Candida colonization during pregnancy Vaginal Infection Prematurity Study Group. Amer J Obstet Gynaecol 178: 374-380.
36. Namkinga LA, Matee MIN, Kivaisi, K, Kullaya A (2005) Identification of Candida strains isolated from Tanzanian pregnant women with vaginal candidiasis. East African Medical Journal 82: 226-234.
37. Mujica MT, Finquelievich JL, Jewtuchowicz V, Iovannitti CA (2004) Prevalence of Candida albicans and Candida non-albicans in clinical samples during 1999-2001. Rev Argent Microbiol 36: 107-112.
38. Oliveira R, Azeredo J, Henriques M, Silva SC, Negri M, et al. (2010) Effect of itraconazole on Candida glabrata biofilm matrix CEB- abstracts in Proceedings 4th International Conference Biofilms IV: Communities bridging disciplines pp: 32.
39. Negri M, Martins M, Henriques M, Svidzinski T (2010) Examination of potential virulence factors of Candida tropicalis clinical isolates from hospitalized patients. Mycopathologia 169: 175-182.
40. Okungbowa FI, Isikhuemhen OS, Dede APO (2003) The distribution frequency of Candida species in the genitourinary tract among symptomatic individuals in Nigerian cities. Rev Iberoam Micol 20: 60-63.
41. Passos XS, Sales WS, Maciel PJ, Costa CR, Miranda KC, et al. (2005) Candida colonization in intensive care unit patients' urine. Mem Inst Oswaldo Cruz 100: 925-928.
42. Repentigny L (1992) Serodiagnosis of Candidiasis, Aspergillosis, and Cryptococcosis. Clin Infect Dis 14: S11-S22.
43. Carricajo A, Boiste S, Thore J, Aubert G, Gille Y, et al. (1999) Comparative evaluation of five chromogenic media for detection, enumeration and identification of urinary tract pathogens. Eur J Clin Microbiol Infect Dis 18: 796-803.
44. Nelson M, Wanjiru WM, Muturi W (2013) Prevalence of vaginal candidiasis and determination of the occurrence of Candida species in pregnant women attending the antenatal clinic of Thika District Hospital, Kenya. Open J Med Microbiol 3: 264-272.
45. Vidal C, Viasus D, Carratalà J (2013) Pathogenesis of invasive fungal infections. Cur Opin Infect Dis 26: 270-276.