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Surveillance of *Candida albicans* in young women at a tertiary care Indian teaching hospital

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Abstract
Background: *Candida albicans*, normal flora of the vagina, is endogenous opportunistic yeast, which causes secondary infection in individuals with some underlying immune-compromised conditions. In this study, we investigated the prevalence and associated risk factors for *C. albicans* among young female attending at IMS and SUM Hospital, Bhubaneswar.
Materials and Methods: High Vaginal Swabs (HVS) specimens were collected from 284 female between the ages of 15 and 35 years attending at Outpatient department of obstetrics and gynecology. The participants also completed a simple structured questionnaire assessing demographic data and risk factors of candidiasis. Each HVS specimen was cultured on Sabouraud Dextrose Agar (SDA) containing appropriate antibiotics to suppress bacterial growth and incubated aerobically at 37 °C. C. albicans was identified by conventional microbiological techniques.
Results: The overall prevalence rate of *C. albicans* in the HVS specimen of 284 young women studied was 37.7%, with the highest rate of 58.53% observed among the patients between the age group 33-35 while the lowest prevalence (12.5%) was among those between ages 14-16 years. Analysis of the participants' response to the questionnaire indicates that *C. albicans* carrier rate may be associated with poor personal hygiene. Statistical analysis showed that prevalence rate of C. albicans among the patents from different locality was not significantly different in any of the regional sampled $P<0.05$.
Conclusion: This study indicates that the prevalence of vaginal candida colonization among female patents is highly significant. Predisposing factors such as the use of tight underwear, indiscriminate use of antibiotics should be avoided and the need for good and adequate personal hygiene should be encouraged.

Keywords: *Candida albicans*, female patents, prevalence and risk factors

1. Introduction
Vaginal candidiasis affects approximately 75% of women of child-bearing age [1]. Factors that predispose women to vaginal candidiasis include hormonal fluctua- tion, *i.e.*, during pregnancy, luteal phase of menstrual cycle, antibiotic uses and use of oral contraceptives [2]. Another 5% - 10% of seemingly healthy women suffer recurrent vaginal candidiasis without any predisposing factors [3]. It is much more common in pregnant women than in healthy women. Moreover, a large proportion of women with chronic recurrent candidiasis first present with the infection during pregnancy [3].
In pregnant women, vaginal candidiasis has been re- lated to emotional stress and suppression of immune sys- tem which step up the risk of *Candida* species over- growth and become pathogenic [5]. Other risk factors are associated with the eating habits of pregnant women of sugar rich containing food. The sugar increase ever more the threat of yeast infections powered by these sugary environments. Pregnancy induced hormonal modifica- tions, altered the vaginal context and made *Candida* more likely to grow beyond acceptable boundaries [6]. *Candida* is the agent most frequently implicated in the invasive vaginal candidiasis. The most common *Candida* species causing vaginal candidiasis is primarily *Candida albicans* followed by *Candida glabrata*, *Candida tropi- calis* and *Candida parapsilosis* [3].
In the recent years, the number of serious opportunistic yeast infections, particularly in immunocompromised patients, has dramatically increased [13]. Among them, *Candida* species accounts for a large number of serious opportunitistic yeasts in pregnant women.

Previous stud-ies done in Kenya on the isolation and identification of *Candida* species used different clinical samples from different study populations. These included HIV/AIDS patients and children with acute respiratory infections where *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei* among others were isolated and identified [14]. In developing countries, there is scanty data on vaginal candidiasis in women and the distribution of the vaginal *Candida* species [15]. In Kenya, there are no data that document the prevalence of vaginal candidiasis in pregnant women. The present study therefore intends to determine the prevalence of vaginal candidiasis among young women, identify the causative vaginal *Candida* species.

2. Material and Methods

2.1 Study Population and Sample Collection

The study population comprised of 284 female undergraduate patents attending OPD of gynecology department between the ages of 14 and 35 years who gave their informed consent to participate in the study. Those recruited for this study were young women patents, no complain of symptoms of urinary tract infections and those who were not on antifungal therapy at the time of sample collection, or who had not taken antifungal drugs within one month prior to sampling. The volunteered participants were divided into groups according to their age (14-16, 17-19, 20-22, 26-28, 29-31 and 23-25 years). The participants were enlightened on the purpose and importance of the study and were educated on how to obtain a High Vaginal Swab devoid of contamination with the vaginal orifice. A simple structured questionnaire assessing the demographic information, symptoms, and risk factors was also administered. Oral informed consent was obtained from all the volunteered participants and all personal information were kept confidential. The High Vaginal Swab (HVS) specimens were properly labeled and then transported in sterile containers to the laboratory for processing and cultivation.

2.2 Sample Cultivation, Isolation and Identification *C. albicans*

The HVS specimens were streaked directly onto Sabouraud Dextrose Agar (SDA) plates and incubated aerobically at 37 °C for 48 hours. Yeast growth characteristic colonial morphology of *C. albicans* (white to cream colony with a smooth border, pasty and moist appearance, Fig 1) was noted. The yeast cells were confirmed to be *C. albicans* by germ tube test (Fig 2).

Fig 1: Candida albicans on SDA

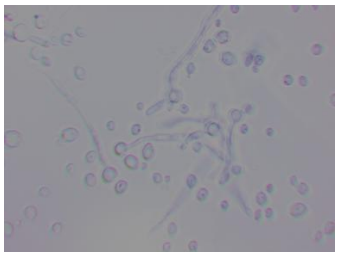

Fig 2: Germ tube formation of *Candia albicans*

2.3 Germ Tube Test

Germ tube experiment was used as a rapid tool for identification of *C. albicans*. Using a sterile wire loop, a small portion of a pure colony of *C. albicans* was harvested and inoculated in to sterile test tubes containing 0.5 ml of human serum. The resulting suspension was incubated aerobically at 37 °C for 3 hours. A drop of the yeast -serum suspension was placed on a clean microscope slide with 1 drop of cotton blue lactophenol stain and covered with a cover slip and examined microscopically, using the x10 and x40 objective lenses of the microscope. The appearance of small, sprouting tube-like outgrowths or filaments projecting from the cell surface confirmed production of germ tubes [4].

2.4 Statistical Analysis

Comparative prevalence rate of *C. albicans* among the patents from different hostels were statistically analyzed.

3. Results

In this study, out of 284 participants sampled, 107 were positive for *C. albicans* carriage, thus giving a total prevalence rate of 37.67%. There was even distribution of prevalence across the six different regions, thus carrier rate of *C. albicans* among the patents was not significantly different in any of the regions ($P<0.05$) Table 1. Considering the different age groups of the participants, the highest prevalence rate of 58.53% was observed in patents within the age bracket of 23-25 years while lowest prevalence rate observed (12.5%) was among the age group 17-19 years (Table 2).

Table 1: The Frequency and Distribution of *Candida albicans* among the Patents Residing in Different Hostels

Sample Source	Number Sampled	Number Positive	%
Khurda	50	17	34
Puri	55	21	38.18182
Pipili	52	20	38.46154
Jatani	30	12	40
Bhubaneswar	61	25	40.98361
Berhampur	36	12	33.33333
Total	284	107	37.67606

Table 2: Prevalence of *Candida albicans* According to Age of the Patents

Age Group (Years)	Number Sampled	Number Positive	(%)
14-16	8	1	12.5
17-19	34	7	20.58824
20-22	80	27	33.75
23-25	80	31	38.75
26-28	25	8	32
29-31	41	24	58.53659
33-35	24	9	37.5
Total	284	107	37.67606

It shows the elicited responses of patents extracted from completed questionnaires. Most patents whose HVS specimens yielded positive growth of *C. albicans* were those that make use of tight and nylon underwear (18.7%) as against those that wear cotton pants (7.0%). Also among patents who used vaginal douches, 15.5% of them were among those whose HVS yielded growth of *C. albicans*. Out of the patents who said they use water closet by squatting on it, 16.9% of them were among those who were positive for *C. albicans* carriage. Most patents responded that they washed their private parts with water after visiting the washroom. High prevalence rate of 30% was observed in patents who indicted that their toilet facility is very dirty agents 1.8% observed in those who said they make us of very clean toilet facility.

Table 3: Prevalence of *Candida albicans* in Relation to the Response of the Patents to Questionnaire

Hostels	**Khurda**		**Puri**		**Pipili**		**Jatani**		**Bhubaneswar**		**Berhampur**		**Prevalence**
Number Sampled	**n = 50(%)**		**n = 55(%)**		**n = 52(%)**		**n =30(%)**		**n= 61(%)**		**n = 36(%)**		**n=284(%)**
Variables													
Type of Underwear													
Nylon	9	(18.0)	9	(16.3)	11(21.2)		7(23.3)		12 (19.7)		5	(13.9)	53(18.7)
Cotton	3	(6.0)	5	(9.1)	3	(5.8)	1	(3.3)	5	(8.2)	3	(8.3)	20(7.0)
Cotton and Nylon	5	(10.0)	7	(12.7)	6	(11.5)	4	(13.3)	8	(13.1)	4	(11.1)	34(12.0)
Total positive	17 (34.0)		21(38.2)		20(38.5)		12(40.0)		25 (40.9)		12 (33.3)		107 (37.7)
Use of VD													
Yes	10 (20.0)		8	(14.5)	7	(13.5)	3	(10.0)	12 (19.6)		4	(1110)	44(15.5)
No	40 (80.0)		47(85.5)		45(86.5)		27(90.0)		49 (80.3)		32(88.9)		240(84.5)
Pattern of WC use													
Squat	8	(16.0)	8	(14.5)	9	(17.3)	6	(20.0)	11 (18.0)		6	(16.7)	48(16.9)
Sit	6	(12.0)	8	(14.5)	8	(15.4)	5	(16.7)	8	(13.1)	4	(11.1)	39 (13.7)
Stand	3	(6.0)	5	(9.1)	3	(5.8)	1	(3.3)	6	(9.8)	2	(5.6)	20(7.0)
Total positive	17 (34.0)		21(38.2)		20(38.5)		12(40.0)		25 (40.9)		12 (33.3)		107 (37.7)
Nature of WC													
Very clean	1	(2.0)	0	(00.0)	2	(3.8)	1(3.3.9)		1	(1.6)	0	(00.0)	5(1.8)
Quite clean	3	(6.0)	4	(7.3)	3(5.8)		2(6.7)		3	(4.9)	2	(5.6)	17(6.0)
Dirty	12(24.0)		17(30.9)		15(28.8)		9	(30.0)	21 (34.4)		10 (27.8)		84 (30.0)
Total positive	17 (34.0)		21(38.2)		20(38.5)		12(40.0)		25 (40.9)		12 (33.3)		107 (37.7)

Key: VD: vaginal douche.

4. Discussion

It has being suggested that high incidence rate of *C. albicans* among young women may be due to increased physiological changes in estrogen and rich glycogen content of the vaginal mucosa there by providing adequate supply of utilizable sugar that support its proliferation [16]. Perhaps, this is the reason *C. albicans* is considered a major component of normal vaginal flora. Therefore, under certain favorable conditions such as use of vaginal douching, broad spectrum antibiotics or corticosteroids and other risk factors that increase the incidence of vulvo-vaginal candidiasis, *C. albicans* will proliferate and cause clinical candidiasis. In this study, we investigated the prevalence and associated risk factors of *C. albicans* among young women that were asymptomatic, of reproductive age and quite enlightened. The prevalence rate of 37.7% observed in the present study is comparable to similar findings in other parts of Odisha; 26.0% in Karu, Nasarawa State [17]; 53.3% in Ilorin Kwara State [18]; 28.0%, 52.5% and 27.9% reported in other parts of Africa [19-21]. However, our result is quite different from prevalence rate of 77.0% reported by [22] among HIV-infected women in Sagamu, Ogun state, Nigeria and the 70.0% reported by [23] among females of reproductive age in Kano, Nigeria which was higher than the prevalence rate recorded in this study.

In our study, there was an even distribution of carrier rate among patents from ages 14-35 years. These findings revealed that the infection was almost uniformly distributed in the subjected age groups indicating that *Candida albicans* is more frequent within the age range of 29-31 years and those within this age range were found to be sexually active. This could also be due to the fact that as girls mature, hormonal changes takes place thereby making them more vulnerable to *Candida albicans* infection and also as well as due to the high oestrogen content of the vaginal epithelia [24]. Many factors have been linked to the relatively higher predisposition of females to *C. albicans* colonization than their male counterparts. It has being indicated that the relatively short and straight anatomy of the female genitourinary tract and dynamic interrelationships between *Lactobacillus acidophilus* and other endogenous flora in the vagina contribute to candida colonization, others have associated female preponderance to candida colonization to unprotected sex, age, and genitourinary disorders as well as use of estrogen-based contraceptives (Hooton *et al*., 1996). Poverty, lack of water supply, sharing of panties is probably, additional implicated factors contributing to high incidence of candidiasis [18]. However, from the present study it was observed that host behavior, specifically improper use of WCs such as squatting on the WC seats coupled with poor sanitary conditions of washrooms may have impact on the prevalence rate of *C. albicans* among the study population. Although a direct association between candida colonization and risk factors was not vigorously pursued statistically, however, matching of questionnaire responses of the participants (Table 3) with their *C. albicans* carrier rate revealed that some of the patents whose HVS specimen tested positive for *C. albicans* engaged in some improper hygienic practices. For instance, some of the *C. albicans* carrier patents squat on WCs instead sitting on it; cleanse their vagina from back to front instead of from front to back, as well as use of vaginal douches, which possibly could have disadvantaged them in terms of *C. albicans* risk compared to their colleagues who observed proper personal

hygiene. Another factor that has been linked to candida colonization is nature of underwear. This study reveals that participants who use tight and nylon underwear had the higher prevalence rate of 18.7% when compared to 7% observed in those that use cotton underwear. The use of synthetic and tight underwear reduces airflow, which may increase moisture and warmth thereby encouraging yeast infections. Also some women have allergies to synthetic material that may cause health changes that encourage yeast infections [23].

In conclusion, this study indicates that prevalence of vaginal candida colonization among female patents is highly significant. Predisposing factors such as the use of tight and nylon underwear, indiscriminate use of antibiotics should be avoided. The practice of good personal hygiene will go a long way to prevent and reduce the spread of the infection.

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