



Prevalence and risk factors for colonization of vaginal *Candida* in reproductive women of Iligan City, Philippines

Audrey Mae A. Putong, Lady Jane C. Fanuncio, Lucilyn L. Maratas*

Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Iligan City, Philippines

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Abstract

Candida albicans is a opportunistic fungal pathogen of the female genitourinary tract which is known to be a causative agent for vaginal thrush and candidiasis. With a definite lack of information about the prevalence of *Candida* amongst healthy women of reproductive age in the Philippines, the present study was performed to evaluate prevalence and associated risk factors for the vaginal colonization of *C. albicans*. One hundred seventy eligible individuals were recruited to be part of the study whose demographic, lifestyle and medical information were obtained through direct interviews using a semi-structured questionnaire. The self-collected vaginal specimens were obtained and were allowed to grow on Sabouraud Dextrose Agar (SDA) and incubated at ambient room temperature for seven days with a daily assessment of growth. Yeast-like colonies were isolated, purified and identified using phenotypic tests: micromorphology, germ tube test and the use of the chromogenic medium CHROMagar *Candida*. Ninety-eight samples yielded positive yeast cultures on SDA which is significantly higher than previously published studies. 150 yeast-like isolates were randomly picked and subjected to wet mount examination which showed 23% (35/150) of the isolates formed germ tubes and thus, presumed to be *C. albicans*. On the other hand, fifty-four isolates yielded green-colored colonies on CHROMagar *Candida* and were also presumptively identified as *C. albicans*. Statistical analysis showed no significant association among variables with the isolation of *C. albicans*. Statistical analysis showed no significant correlation between the isolation of *C. albicans* and the identified risk factors.

* **Corresponding Author:** Lucilyn L. Maratas ✉ lucilyn.lahoylahoy@g.msuiit.edu.ph

Introduction

Approximately 10-20% of asymptomatic healthy women harbor culturable *Candida* species and other yeasts in the vagina (Muzny and Schwebke, 2015; Denning *et al.*, 2018). *Candida albicans* is a normal, though minor, component of the human microbiota (Cassone and Sobel, 2016) which is also an opportunistic pathogen of the female genitourinary tract that is the predominant cause of vaginal thrush (Pidwill *et al.*, 2018). Key risk factors for *C. albicans* infection are immunosuppression, use of oral contraceptives, hormone therapy, previous antibiotic therapy, diabetes, pregnancy, genetic predisposition (Sobel, 2007; Brandolt *et al.*, 2017).

It is reported that there is an increasing number of *Candida* spp clinical isolates are resistant to antifungal reagents routinely used for the treatment of VVC (Brandolt *et al.*, 2017). Considering this and the lack of data about clinical isolates of *Candida* from women of reproductive age in Iligan City, this study aimed to identify the prevalence of *C. albicans* isolated from vaginal mucosa.

Methodology

Study population

One hundred seventy sexually active women of reproductive age were recruited to be the subjects of this study. The women agreed to participate by signing an informed consent form. Interviews were done prior to collection of vaginal swab samples. Demographical data was also collected which included personal, social and medical history.

Collection of vaginal swab samples

The self-collection of vaginal mucus were done by rubbing sterile cotton swabs on to the vaginal opening twice in a circular motion. The clinical sample was then placed in a properly labeled tube with Amies Transport Medium, a transport medium which will neither inhibit nor promote the growth of the desired specimen. The samples were then immediately brought to the laboratory and inoculated on to Sabouraud Dextrose Agar (SDA) for detection of yeast-like colonies. Incubation was at ambient room

temperature and visual inspection was done after 24- and-48 hours.

Isolation, purification and maintenance of suspected yeast isolates

The colonies which grew on SDA and exhibited the following colonial characteristics were presumed to be yeast isolates: opaque, white to yellow in color with an average size of three millimeters (mm) with smooth and convex traits (Larone,1995). These colonies were randomly picked and retreated twice on SDA to confirm its purity and viability.

Characterization and presumptive identification of the suspected yeast isolates using traditional methods

Cultural characterization was done according to the size and shape of the colony, edge, elevation, texture, color of the colony's surface and bottom after 24 and 48 hours of incubation (Larone, 1995). 24-hour-old yeast isolates were also morphologically characterized through wet mounts (for examination of purity as well as for observation of isolate morphology) (Rippon, 1974) and germ tube test (Baron *et al.*, 1994).

The isolates that produced germ tubes were presumptively identified as *C albicans*, otherwise it will be considered as germ tube negative.

Presumptive Identification Using CHROMagar Candida

Pure cultures of yeast isolates were streaked onto CHROMagar *Candida* and observed for colonial growth and color after 24 hours of ambient incubation. Presumptive identification to the species level of the isolates was done based only on colonial color (Monami, 2000).

Statistical analysis

The results obtained were compiled by employing descriptive analysis of the data, chi-square and fisher exact test for categorical variables.

Results and discussion

Demography of the study population

The recruited members of the study population consisted mostly of housewives who belong to the average income bracket. The subjects have a mean age of 27 with 14% (24/170) of the subjects, pregnant. Table 1 summarizes the demographic information of the female subjects. Seventy-five percent (127/170) had taken antibiotics within the past six months as treatment of a diagnosed illness/es. However, only 48% (82/170) reported to have having experienced illnesses such as coughs, colds and fever. Other illnesses reported were urinary tract infections, kidney infection, hyperacidity and migraine. Twelve

percent (20/170) claimed to be occasional smokers and 54% were contraceptive users: 22% (37/170) took oral contraceptive pills, 23% (39/170) had intrauterine device and only 9% (15/170) were Depo-Provera users. Other lifestyle factors included in the survey were the preference of sweets (54%, 92/170), constant use of panty liners and the type and care of undergarments. Eighty-six percent (146/170) preferred wearing cotton undergarments rather than silk (4%) with only 28% used fabric conditioner in the care of undergarments. One hundred forty-six subjects constantly used panty liners (86%).

Table 1. Descriptive profile of the female population included in the study.

Variable	Number of individuals (Percentage)	
Pregnant	24	(14%)
With history of antibiotic use in the past 6 months	127	(75%)
Reported having medical conditions in the past 6 months	82	(48%)
Smoker	20	(12%)
Contraceptive user	91	(54%)
Included sweets as part of diet	92	(54%)
Used cotton undergarments	146	(86%)
Constantly use panty liners	146	(86%)
Used fabric conditioner	48	(28%)
Preferred tight fitting lower undergarments	123	(72%)

Detection of yeast-like colonies from the study population

Only ninety-eight specimens (78%) out of the 170 samples collected, yielded growth of yeast-like colonies on the primary isolation medium, SDA. 78%

yeast recovery rate is quite high compared to other published studies whose recovery rates ranged from 21% to 49.2% (Holland *et al.*, 2003; Guzel *et al.*, 2011; Brandolt *et al.*, 2017).

Table 2. Yeast-like colonization among the recruited individuals.

Variable	Number of individuals (Percentage)	
Pregnant	22/24	(92%)
With history of antibiotic use in the past 6 months	73/127	(57%)
Reported having medical conditions in the past 6 months	80/82	(98%)
Smoker	16/20	(80%)
Contraceptive user	49/91	(54%)
Included sweets as part of diet	81/92	(88%)
Used cotton undergarments	140/146	(96%)
Constantly use panty liners	30/146	(21%)
Used fabric conditioner	40/48	(83%)
Preferred tight fitting lower undergarments	118/123	(96%)

One hundred fifty presumed yeast-like isolates were randomly picked from these 98 positive vaginal cultures, subcultured twice on SDA to guarantee purified and viable isolates and were then maintained

on Sabouraud Dextrose broth. A distinct cultural trait of yeast is its colony size which is usually more than four millimeters (mm) in diameter and this was observed from all randomly picked isolates.

Carriage and colonization rates of yeast-like colonies from vaginal swab samples

Point-prevalence studies have reported that *Candida sp.* is isolated from the genital tract of 5%-20% of asymptomatic women (Sobel, 1988). However, several risk factors can be associated with increased rate of vaginal colonization of *Candida sp.*

Hormonal contraceptive use has been postulated to

change the composition of vaginal microbiome (Van de Wijgert *et al.*, 2011).

In fact, oral contraceptive pills has been documented to be a contributing factor to the emergence of yeast-like pathogens in the body for it may change the vagina biochemistry due to high dose of estrogens. Forty-nine individuals out of the 91 contraceptive users (54%) had positive vaginal cultures.

Table 3. Relative proportion of *Candida* species as identified by colonial color on CHROMagar *Candida*.

Species of <i>Candida</i>	Percentage of isolates
<i>Candida albicans</i>	36% (54)
<i>Candida krusei</i>	13% (15)
<i>Candida parapsilosis</i>	44% (66)
<i>Candida spp.</i>	7% (9)

Tight fitting clothes are also reported to facilitate yeast like growth due to poor ventilation. In fact in this study, 96% of the 123 individuals who preferred tight-fitting lower undergarments had yeast-like growth (Table 2).

This subpopulation and those who reported to have had medical conditions yielded the highest incidence of yeast like growth (96% and 98% subsequently). Based on statistical analysis, no correlation was found between the the detection of yeast-like colonies and the the variables presented in Table 2 (with a P value of greater than 0.05).

Characterization and presumptive identification of yeast-like isolates using traditional methods

Cultural and Morphological Characterization: Pure cultures of 351 randomly picked yeast-like isolates were characterized using colonial growth traits.

All isolates generally exhibited a smooth, convex and white to cream-colored colonies. Upon examination under the low power magnification (LPO) only 150 (43%) of the isolates exhibited a circular-to-ovoid shape characteristic of yeast cells. Besides LPO, a further examination under high power objective (HPO) was also done to ensure the accuracy of the first observation.

Germ Tube Test: One hundred fifty presumed yeast isolates were then subjected to germ tube test, which is based on the observation that *Candida albicans* produces tube-like structures when incubated in serum for two-to-four hours, is simple, economical and has long been considered the reference method (Hoppe and Frey, 1999). Thirty-five out of the 150 isolates were found to be positive for the formation of germ tubes and were presumptively identified as *C. albicans*.

Prevalence of Germ Tube Positive Candida Among contraceptive users

Twenty out of the 91 contraceptive users had germ tube positive yeast isolates. 85% of the contraceptive users who professed to have sweet tooth had *C. albicans* and only 15% of pregnant women had *C. albicans*. The high colonization rates of *C. albicans* among those who included sweets as constant part of their diet may be attributed to the high glucose content in the body which in turn creates a favorable environment for yeasts. However, the carriage rates of *C. albicans* among gravid women does not conform to the idea that pregnancy would facilitate hormonal imbalance, which would induce yeast proliferation in the vagina. However, P value ($P \geq 1$) showed that there was no significant correlation among these variables with colonization of *C. albicans*.

Prevalence of Germ Tube Positive Candida Among Contraceptive Users

Of the 79 non contraceptive users, there were only 15 individuals who were colonized with germ tube positive *C. albicans*. The highest carriers of presumptively identified *C. albicans* were the women group who preferred cotton undergarments (80%) while the least colonized were the smokers (13%). Using one way correlation, statistical analysis showed no significant distribution with a P value of less than or equal than 1.

For the two subpopulations (contraceptive and non contraceptive users), antibiotic users had been the highest carriers of germ tube positive *Candida* (85% and 87%, respectively). The use of broad spectrum antibiotics destroys the normal microflora allowing yeast overgrowth (Catterall, 1971). Statistical analysis using Fischer's Exact Test showed that there is no significant correlation among these variables with the isolation of *C. albicans*.

CHROMagar Candida as a Medium for Presumptive Identification of Candida albicans

The CHROMagar *Candida* (CC), a proprietary product, was donated for evaluation by the CHROMagar Company, Paris, France. It was supplied as white powder in pre weighed batches for the preparation of 1000 milliliter (ml) volumes and was prepared according to the manufacturer's instructions. CHROMagar *Candida* is a selective and differential medium that allows selective isolation of yeasts and simultaneously identifies (by color reactions and colony morphology) colonies of *Candida albicans*, *Candida tropicalis*, and *Candida krusei* with a high degree of accuracy (Pfaller, 1996). Four distinguishing colony colors were observed among the 150 isolates (Table 3) corresponding to *C. albicans*, *C. parapsilosis*, *C. krusei* and *Candida* subspecies (spp). Thirty six percent of the isolates (54/150) yielded dark green colored colonies and were presumptively identified as *C. albicans* and came from 32 subjects (33%). Forty-two (43%) of the individuals harbored cream-colored isolates that comprised 48% of the total isolates and were identified as *C. parapsilosis*.

Twenty-one subjects (21%) were carriers of purple to violet colored isolates (1%) which were presumptively identified as *C. krusei*. Only nine isolates (6%) from three individuals had pink colored colonies and were classified under *Candida spp*.

Statistical analysis showed that there is a highly significant correlation among the variables of the individuals that exhibited the cream, green and purple colonies with p values 0.001, 0.1, 0.025 and 0.05 obtained respectively. However, pink colonies showed no significant distribution among given variables.

It is important to note that the presence of *Candida* does not always cause disease. However, *Candida* species possess certain virulence factors which contribute to the pathogenesis of fungal infections (Ekuma *et al.*, 2019)

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