





Pattern of *Candida* Species Isolated from Patient with Vulvovaginal Candidiasis in Pregnancy

Indah Purnamasari¹, Evy Ervianti¹ , Damayanti¹ , Budi Prasetyo², Linda Astari¹, Pepy D. Endraswari³, M. Yulianto Listiawan¹, Cita Rosita Sigit Prakoeswa¹

¹Departement of Dermatology and Venereology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Departement of Obstetric and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

³Department Medical of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia/ Dr. Soetomo General Academic Hospital, Universitas Airlangga Hospital, Surabaya

ABSTRACT

Background: Vulvovaginal candidiasis (VVC) is one of the most prevalent genital tract diseases in women. The identification of the isolated *Candida* species is critical for guiding empirical antifungal therapy. **Purpose:** To determine the *Candida* species that cause vulvovaginal candidiasis in pregnancy at the Obstetrics and Gynecology Outpatient Clinic, Dr. Soetomo General Academic Hospital, thus providing scientific and therapeutic benefits. **Methods:** Pregnant woman receiving antenatal care (ANC) at Dr. Soetomo General Academic Hospital Obstetric and Gynecology Outpatient Clinic. Subjects were enrolled in a cross-sectional study during 3 months between March and May 2021. Vaginal swab samples were taken from subjects after informed consent. A specific color change in CHROMagar cultures and VITEK 2 compact were used to identify *Candida* species. **Result:** During the study period, 15 pregnant patients were diagnosed with VVC. The majority of the women in the study were pregnant women of sexually active age who complained of a whitish body and genital irritation. There were 18 *Candida* isolates from CHROMagar cultures, with *C. non albicans* (61.1%) predominating over *C. albicans* (38.3%). With details, *non albicans* were *C. glabrata* (54.4%), *C. dubliniensis* (36.3%), and *C. parapsilosis* (18.1%). **Conclusion:** This study revealed a high prevalence of *Candida non-albicans* species causing VVC. Culture investigation and identification of *Candida* are critical to avoid diminished susceptibility to antifungal treatments and recurrence.

Keywords: vulvovaginal candidiasis, species pattern, pregnancy, sexual and reproductive health care.

Correspondence: Evy Ervianti, Departement of Dermatology and Venereology, Faculty of Medicine, Universitas Airlangga, Surabaya, Dr. Soetomo General Academic Hospital, Jl. Prof. Dr. Moestopo Street no 47, Surabaya, Indonesia, evy-e@fkg.unair.ac.id.

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BACKGROUND

Vulvovaginal candidiasis (VVC) is one of the most prevalent illnesses diagnosed in women seeking gynecological care, and it is caused by abnormal yeast growth in the mucosa of the female genital tract.^{1,2,3} Vulvovaginal candidiasis is usually caused by physiological or non-physiological alterations that disrupt the balance between *Candida* vaginal colonization and the host environment.⁴ Women with a well-estrogenized vagina have a higher rate of vaginal colonization, especially during pregnancy.⁵

Vulvovaginal candidiasis affects around 75% of all pregnant women at some point during their lives, with roughly 50% experiencing a recurrence or recurrent infection. The distribution of the various *Candida* species found in VVC-affected women varies greatly based on the study regions and populations. According to reports, *Candida albicans* is responsible for 80–90% of VVC cases, whereas other species of the genus are responsible for 10–20%. The most frequently occurring *non-albicans* species include *Candida*

glabrata, *Candida tropicalis*, *Candida krusei*, and *Candida parapsilosis*.⁶

In recent years, it has been reported that the species causing VVC has shifted from *Candida albicans* to *Candida non-albicans*, which is more usually found as the cause of VVC in pregnancy. Recurrent VVC (VVCR) infection is typically linked to *C. non-albicans* (*C. glabrata*) infection.^{7,8} The complications of VVC in pregnancy can occur before and after birth. Several studies have shown that *C. albicans* and *C. glabrata* produced intra-amniotic infections, which resulted in membrane rupture, premature labor, and spontaneous abortion.⁹ The increase in VVC cases caused by *C. non-albicans* species is concerning, as these species are more resistant to routinely used antifungal medicines, are difficult to treat, and are linked to relapse.¹⁰ This study was conducted in order to characterize the pattern of *Candida* as a cause of VVC in pregnancy, in order to improve the success of VVC treatment and prevent complications in the mother or infant.

The most commonly implicated non-albicans species include *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis*.⁴ The most frequently non-albicans species include *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Candida parapsilosis*.

METHODS

This study was conducted *in vitro*. The subjects were enrolled in a cross-sectional study and the data was obtained by total sampling for 3 months from March to May 2021. They are all pregnant patients who came for antenatal care (ANC) at the Obstetric and Gynecology Outpatient Clinic of Dr. Soetomo General Academic Hospital. Inclusion criteria for subjects were pregnant patients diagnosed with VVC having at least

one of the VVC symptoms (vaginal discharge like cheese or cracked milk, vaginal itching, erythema, dyspareunia) and fungal positivity (*pseudohyphae* and/or *blastospores*) by Gram staining smears. Exclusion criteria were pregnant patients with a diagnosis of VVC who were treated with antifungal therapy, intravaginally or systemically, within the last 2 weeks and with no colony growth on CHROMagar media. Study protocols were explained to subjects who were willing to take part in the study and sign the informed consent. Specimen samples were collected from vaginal fluor and were cultured on CHROMagar media. The cultures were cultivated for 36-48 hours before species identification. In this study, 18 isolates of *C. albicans* and non-*albicans* were discovered. Species identification by looking for the typical color changes of *Candida* species on CHROMagar media and VITEK 2 compact. This research has received ethical approval from the Ethics Committee of the Dr. Soetomo General Academic Hospital Surabaya (0156/KEPK/III/2021)

RESULT

This study obtained 15 pregnant patients with VVC who came for antenatal care (ANC) at the Obstetric and Gynecology Outpatient Clinic of Dr. Soetomo General Hospital Surabaya, from March to May 2021. The characteristics of the subjects are indicated in table 1. Data recording in this study included basic data characterized by patient, chief complaints, physical examination, and laboratory examinations. Most of the age groups are 25-44 years, which is the sexually active age range. The majority of subjects were in the 3rd trimester of pregnancy (73.3%), followed by the 2nd trimester of pregnancy (26.7%). No subject was in the 1st trimester of pregnancy.

Table 1. Characteristics of subject

Characteristics	Age group	n	%
Age	18 – 25 years	2	13.3
	26 – 35 years	12	80
	36– 45 years	1	6.7
	46 – 55 years	0	0
	56 – 65 years	0	0
Gestational age	1 st Trimester	0	0
	2 nd Trimester	4	26.7
	3 th Trimester	11	73.3

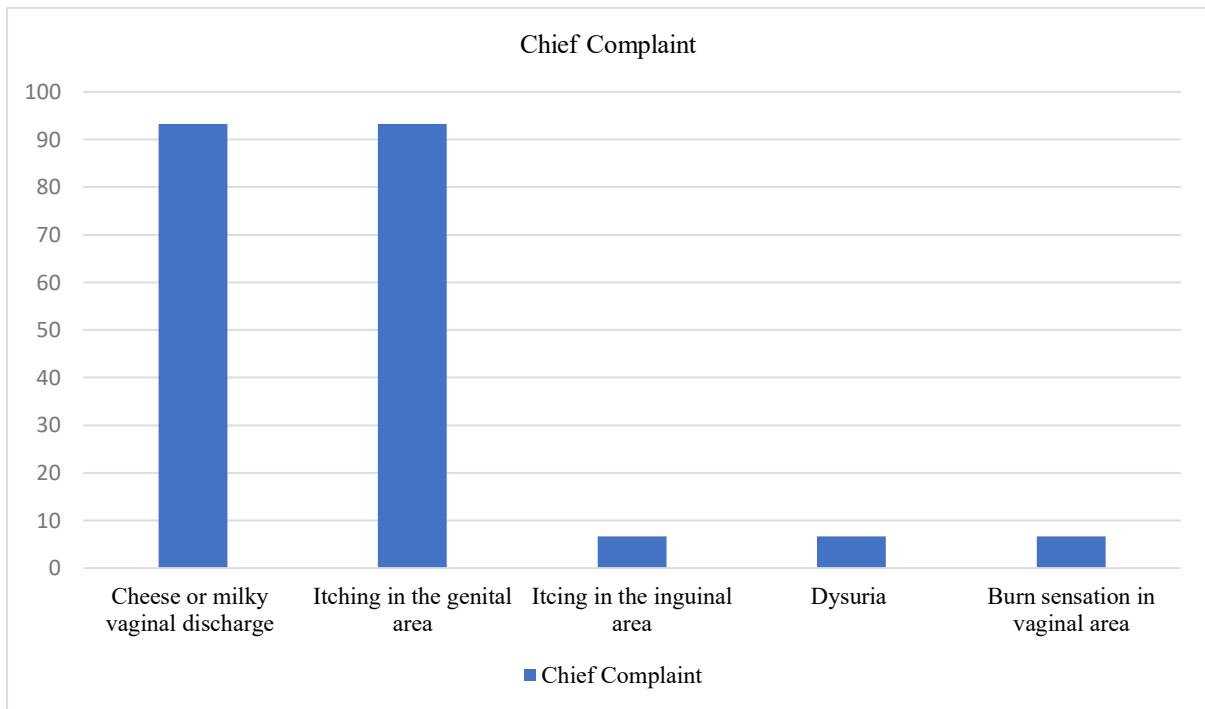


Figure 1. Distribution of subjects' chief complaints.

The distribution of microscopic examination with Gram staining is shown in figure 2. The most favorable results were positive *blastospore* and *pseudohyphae* (80%), and 20% were positive *blastospore* and negative *pseudohyphae*.

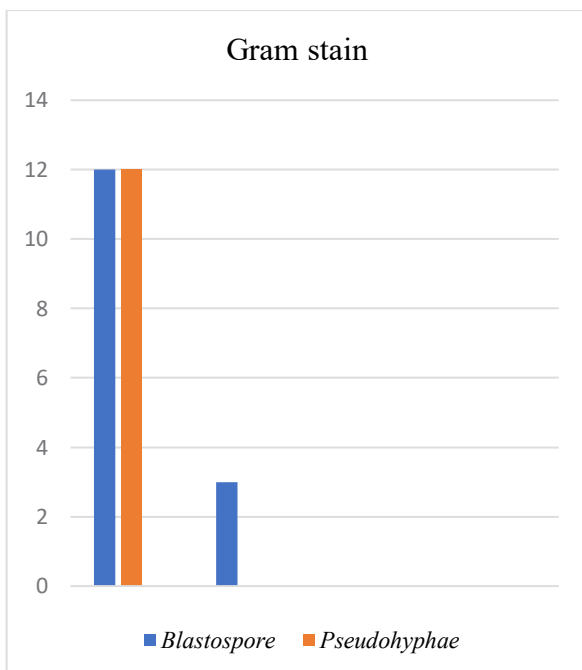


Figure 2. Gram staining laboratory result.

Identification of *Candida* species in this study used the conventional *CHROMagar* method at 35°C for 48h for the production of species-specific colors and also the VITEK 2 Compact examination. Four *Candida* species were isolated and identified,

identified : *Candida albicans*, *Candida glabrata*, *Candida dubliniensis*, and *Candida parapsilosis*. Figure 3 shows that there were 18 *Candida* isolates, of which 7 (38.8%) were *C. albicans* and 11 (61.1%) were *non-albicans*. *Candida non-albicans* isolates consisted of 7 isolates of *C. glabrata* (54.1%), 4 isolates of *C. dubliniensis* (36.3%) and 2 isolates of *C. parapsilosis* (18.1%). There were 3 isolates found to have more than one *Candida* species.

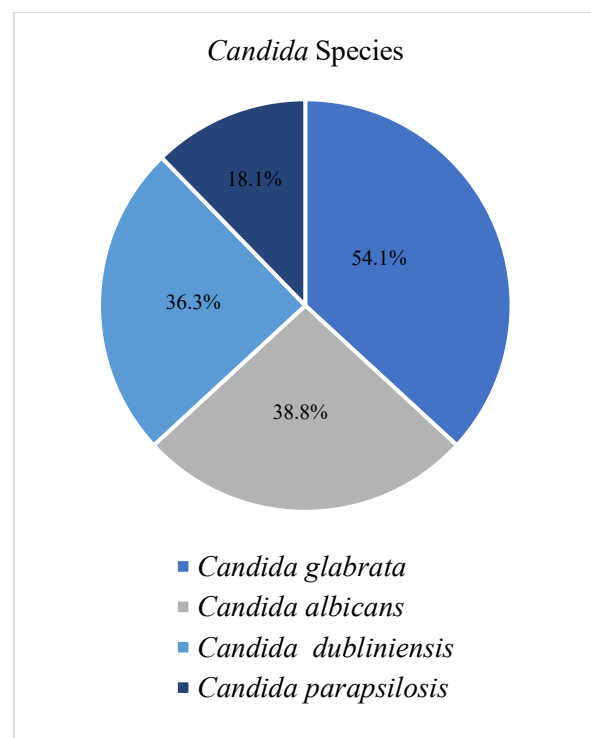


Figure 3. Identification of *Candida* species.

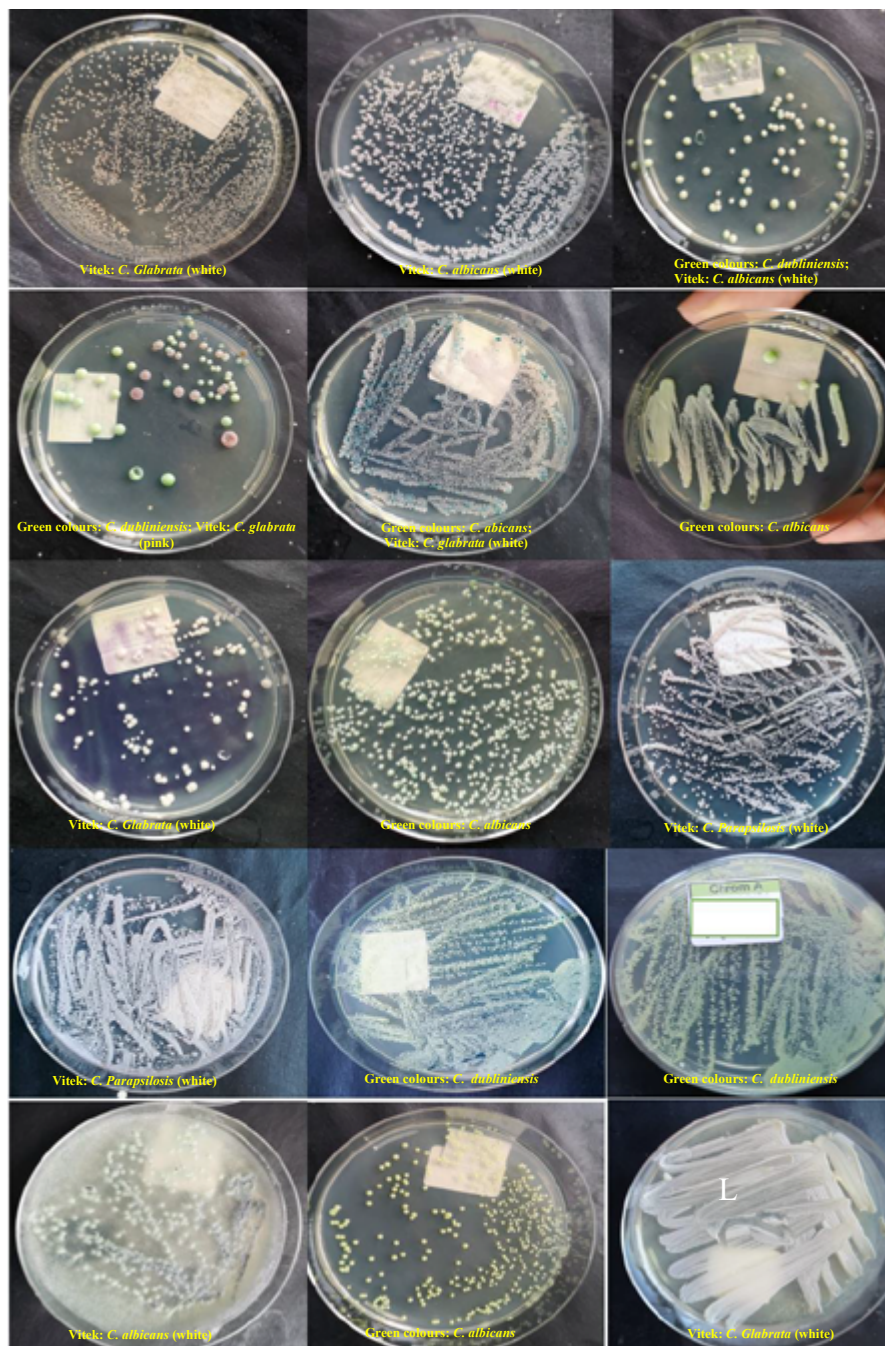


Figure 4. Identification of species-specific colors of *Candida* in CHROMagar media and Vitek 2 compact. On CHROMagar, *C. albicans* and *C. dubliniensis* showed a specific green color; unspecified color changes of *Candida* species identified with Vitek 2 compact.

DISCUSSION

Vulvovaginal candidiasis (VVC) is the second most prevalent cause of vaginitis globally. There is about 20% VVC risk for non-pregnant women, and it rises to 30% during pregnancy.^{4,2} In 85-95% of cases, vaginal candidiasis is caused by *Candida albicans*. Non-*albicans*, such as *Candida glabrata*, *Candida tropicalis*, and others, cause vulvovaginitis in less than 10% of cases, with fewer clinical symptoms.^{11,2,3} Candidiasis during pregnancy has been linked to a higher risk of pregnancy problems such as early membrane rupture and poor pregnancy outcomes.

There's some evidence that eradicating *Candida* during pregnancy can reduce the incidence of preterm births and late miscarriages.¹²

Age is a very important factor in the incidence of VVC, which is very rare before the age of menarche, then increases dramatically at the end of the second decade of life and peaks in the two decades after.¹³ In our study, the highest number of positive cases was found to be in the 26-35 year of age group (80%), followed by 18-25 years (13.3%), and 36-45 years (6.7%). These results are consistent with the findings of two other epidemiological studies, both of which

found that the incidence of VVC was higher among women of reproductive age (20-40 years) who were sexually active than in women of menopausal age.⁴ This age group in this study are sexually active age groups, have a strong desire for children, and the condition of pregnancy produces a surge in the hormone estrogen, so that possible to increase the incidence of VVC in pregnancy.

Pregnancy-related vulvovaginal candidiasis rises in the third trimester, symptomatic recurrence is more common, and the therapeutic response is diminished.¹³ Pregnancy hormones increase with gestational age, one of which is HPL (*Human Placental Lactogen*), which affects metabolic processes, especially glycogen, interfering with vaginal pH and providing good nutrition for pathogenic microorganisms, one of which is fungi, making them more susceptible to infection if pregnant women do not practice good personal hygiene.¹⁴ According to this study, the majority of VVC patients (73.3%) occurred in the 3rd trimester of pregnancy, followed by the 2nd trimester (26.7%), and no occurred in the 1st trimester of pregnancy. This result in line with a study by Eryanti that found that the third trimester gestational age had the highest VVC incidence (44.4%), followed by the second trimester gestational age (37%), and the first trimester gestational age (18.5%).¹⁵

Vulvovaginitis is an inflammation of the vulva and vagina characterized by erythema and edema. The diagnosis of VVC can be suspected by the presence of complaints of white *cheesy* or *cottage cheese-like* flour, which is white like cracked milk, itching, vulvar pruritus, dyspareunia, dysuria, vulvar and vaginal erythema, and supported by the finding of *blastospores* and/or *pseudohyphae*.¹² In this study, the diagnosis of VVC was confirmed by history-taking and physical and microscopic examination with Gram stain. From anamnesis, the most common complaints were cheese or milk vaginal discharge (93.3%) and itching in the genitalia area (93.3%). These results are the same as a study by Kanagal reported in 118 pregnant patients who visited ANC. Of 50 patients diagnosed with VVC, 40% experienced vaginal discharge, 24% discharge accompanied by itching, and 18% vaginal burning.¹⁶ Gram stain has a sensitivity of 65% and a negative predictive value (NPV), as well as 100% specificity and a positive predictive value (PPV). On the other hand, Gram staining combined with wet preparation is regarded as the gold standard for the quick diagnosis of VVC.¹⁷

This study reveals a significant shift in the distribution of VVC-causing species in pregnancy; there are 3 species of *Candida non-albicans* identified, including: *Candida glabrata*, *Candida dubliniensis*,

and *Candida parapsilosis*. This study is supported by several studies in Asia, Africa, and India, *C. non-albicans* species were reported to be the most common cause of VVC, especially *C. glabrata* (29.5-50.4%).^{18,19} In general, *C. albicans* is also the most common species among pregnant women, although several studies have reported *C. non-albicans* species predominating. Low socioeconomic background, poor education, non-white race, and underlying medical conditions are more common in *C. non-albicans*.²⁰ *Candida glabrata* is mentioned only in the second and third trimesters.²¹ As an immunological evasion strategy, *Candida glabrata* can persist in macrophages, bypassing the innate immune response to pathogens. This adaptation to intracellular survival is linked to its ability to modify phagosomes, suppress reactive oxidative stress (ROS) production, and produce a minimum proinflammatory in order to avoid the toxic environment of phagolysosomes.²⁰ *Candida dubliniensis* has a genome that is quite similar to *C. albicans*, but the two species are very different in terms of pathogenicity. The absence of the agglutinin-like protein 3 (ALS3) precursor could be one of the causes of *C. albicans*' greater pathogenicity. The agglutinin-like protein 3 precursor gene has been identified in *C. dubliniensis* and is involved in epithelial adhesion.²⁰ *Candida non-albicans* species showed decreased sensitivity to commonly used antifungal treatments, especially azoles. This data demonstrated that *C. non-albicans* species have emerged as an important cause of infection in VVC, indicating that they cannot simply be seen as contaminants or non-pathogenic agents.²²

This study revealed a high prevalence of *Candida non-albicans* species that cause VVC in pregnancy. The increasing incidence of VVC cases in pregnancy caused by *C. non-albicans* has made the diagnosis and management of VVC more complex and difficult. It is preferable to screen all patients in the first/early second trimester in order to identify and treat positive cases as soon as possible in order to avoid preterm births caused by vaginal candidiasis. Culture analysis and identification of the causal species should be performed in VVC patients in order to improve the outcome of treatment, especially in those who have been treated with standard medication but have not seen a cure.

There were limitations to this study. Due to the fact that this study was conducted during the COVID-19 pandemic, it only included a small number of study subjects, and as a result, only a few isolates were found. Further studies are required for more subjects in order to learn more about the patterns of species that cause VVC in pregnancy.

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