Profile of Candida Species in Vulvovaginal Candidiasis using Conventional Methods

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Abstract: Vulvovaginal candidiasis (VVC) is an inflammatory disease of vulva and vagina that caused by *Candida sp*.*Candida albicans* was the predominant cause of candidiasis. However, a shift toward non-*albicans Candida* species has been recently observed. These non-*albicans Candida* species demonstrated reduced susceptibility to commonly used antifungal drugs. Identification of the infecting *Candida* to the species level is of utmost importance for clinical microbiological services for prediction of likely drug susceptibility and to guide treatment. Conventional methods for the diagnosis of candidiasis are sensitive, low-cost, and although time consuming, are still considered the references standard for identification of yeast isolates. There were three conventional methods that is still the standard tests to identify the *Candida sp*. The tests are Sabouraud dextrose agar (SDA) thencornmeal-Tween 80 agar, carbohydrate fermentation test, and CHROM agar Candida (CAC). These tests usually give some specific colony, morphology andcolor for every *Candida sp*.

1 INTRODUCTION

Vulvovaginal candidiasis (VVC) refers to a disorder characterized by signs and symptoms of vulvovaginal inflamation in the presence of *Candida species*. It is the second most common cause of vaginitis symptom after bacterial vaginosis. It is estimated that at least 75% of healthy adult women will experience one episode of vulvovaginal candidiasis during their reproductive phase. The signs and symptoms of VVC include a thick cheese–like discharge associated with intense vaginal and vulvar pruritus, pain, burning, erythema, and/or edema (Kunduk and Garg, 2012; Moyes and Naglik, 2011; Sobel, 2008).

Candida sp are among the most common fungal pathogens. They are capable of initiating infections in both immunocompetent individuals and immunocompromised hosts. *Candida sp*, arecommensal organisms that normally colonize mucosal surfaces in an asymptomatic manner, but it also can become one of the most significant causes of disabling and lethal infection. *Candida sp* are responsible for various clinical manifestations ranging from mucocutaneous overgrowth to life threatening disseminated infections like candidemia. *Candida albicans* remains the most common causative agent for VVC in approximately 85%-95% of the cases. However,there is an alteration of species that cause VVC in the last decade as incidence of VVC due to non-*albicans Candidasp* is increasing. This rise could be due to an increase of over-the-counter antifungal use. The clinical manifestations of infections caused by non-*albicans Candidassp* are usually indistinguishable with VVC caused by *Candida albicans* except in its poor response to common anti-fungal drugs due to either inherent or acquired. Due to the changing epidemiology of *Candida*, physicians may no longer be able to make therapeutic decisions based on species levels study to enhance proper treatment for their patients (Hedayati et al., 2015; Deorukhkar et al., 2014; Farooqi et al., 2013; Richter et al., 2005; Fidel et al., 1999).

Species identification requires isolation and biochemical or physiological characterization. *Candida sp,* being non-fastidious organism, readily grows on laboratory media used for the isolation of fungus. Conventional methods, including Sabouraud

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dextrose agar(SDA), cornmeal-Tween 80 agar, carbohydrate fermentation test, and CHROM Agar Candida (CAC), are recommended to identify *Candida sp*to the species level. Each method has its own eminence at identifying Candida species. The formation of blastospore, pseudohyphae, hyphae and chlamydospores which aids identification of *Candida sp* requires the use of nutritionally deficient media like cornmeal agar Tween 80 as these nutritionally deficient media suppress the vegetative growth and promote sporulation. Carbohydrate fermentation test classifies Candida based on the color transformation on carbohydrate broth and gas formation on the tube. *Candida sp* metabolizes carbohydrates both aerobically (assimilation) and anaerobically (fermentation) (Mutiawati, 2016; Suyoso, 2013; Larone 2011). Lastly, enzymatic reaction methods using chromogenic substrates in Chromogenic agar (CHROM Agar Candida) medium has high sensitivity and specificity in differentiating *Candida* species in clinical samples. This media contains chromogenic substrates that react with enzymes secreted by yeast cells, resulting in various pigmentations. Using this medium, it is possible to identify *Candida* species based on color characteristics. CAC can differ one *Candida sp* to another *Candida sp* by its colony color. The presence of two colonies with different color indicates there were two species that grew on CAC.

2 METHODS

This was a cross-sectional descriptive study that identifies causes of VVC down to the species level by using conventional methods of fungal examination. The sample of this study were all VVC patients that fulfilled the inclusion criteria, and underwent examination at Sexually Transmited Infection Division, Dermatoveneorology Clinic of Dr.Soetomo Hospital, Surabaya. There were a total of 25 enlisted patients. The patients' data information in data collection sheet.

The Inclusion criteria were VVC patients, aged 15 years or older, married or unmarried, willing to follow the research and sign the informed consent.The exclusion criteria were patients with negative culture result.

All women who attended Sexually Transmitted Infection (STI) Division, Dermatoveneorology Clinic of Dr.Soetomo Hospital, Surabaya were interviewed for medical history and clinically examined. Samples were taken from vaginal swab and checked for Candida and Gram stain examination. Patients diagnosed with VVC were included in inclusion criteria.

Consecutively, the samples were first grown in SDA, followed by cornmeal-Tween 80 agar and then performed on carbohydrate fermentation test. The results were available in 2-3 days. Therewere 6 carbohydratesused in this study: urea, dextrose, lactose, sucrose, maltose, galactose and trehalose. Positive result was marked bythe changing color of broth to yellow and the gas formation in the Durham tube. The other test was CHROM agar Candida (CAC), showed colony color in18 hours-3 days. Each color represents one specific species of Candida.

3 RESULTS

This study involved 25 female patients with VVC. The patients were predominated by 15-24 age group. Most patients were private employees and most of the patient's education were bachelor.

Most common duration of complaint was 1 month until 9 months with vaginal douching usage being the highest predisposition factor. Most patients admitted have not consumed any therapy for VVC yet. Clinical examination revealed edematous and erythematous vulva and vagina on all 25 patients. Direct examination from wet specimen and Gram stain were: wet specimen contained positive blastospore with negative pseudohifa 20%, positive blastospora with positive pseudohifa 48%, negative blastospora with negative pseudohifa 32%, no negative blastospore with positive pseudohifa(0%) and Gram positive blastospore with negative pseudohifa 16%, negative blastospore with positive pseudohifa 4%, positive blastospore with positive pseudohifa 52%, and negative blastospore with negative pseudohifa 28%.

There were various results for each conventional method. Cornmeal agar showed the specific formation of every *Candida*. The presence of terminal vesicles (chlamydoconidia) with pseudohifa and flower-like blastoconidia in cornmeal agar indicates that the fungi was *Candida albicans*. Other sample showing divaricated pseudohifa with oval blastoconidia indicates the presence of *Candida tropicalis*.

Table 1: Comparation between direct examination and conventional methods

Wet specimen & Gram stain	Conventional methods
Microscopic examination (wet specimen and Gram stain) can show fungal morphology (blastospore, pseudohyphae, and hyphae)	Conventional methods can reveal species of the fungi (*Candida sp* itself).

As for the carbohydrate fermentation test, for example, positive result on dextrose and trehalose means that the sample was *Candida glabrata*.

CAC revealed color of colony. All samples showed one type of colony color, meaning one *Candida sp* in every sample, and only 4 samples shown two colony color (*2 Candida sp*). Light green-colored colony is specific for *Candida albicans,* while dark green colony is specific for *Candida dubliniensis*. Purple-colored colony characterized the colony of *Candida glabrata*.

These three conventional methods from 25 samples revealed that 14 sample were positive for*Candida albicans*, and others were non-*albicans Candida sp*. Five samples were positive for *Candida glabrata*, 1 sample for *Candida parapsilosis* and 4 samples were positive for2 *Candida sp* (double infections). From those 4 samples,1 sample were positive for *Candida albicans* and *Candida glabrata*, 1 sample for *Candida albicans* and *Candida famata*, and 2 samples were positive for *Candida albicans* and *Candida tropicalis*. From the table 1 we can see the comparation between microscopic and conventional methods. Conventional methods revealed species of the fungi while direct microscopic examination can only show pseudohypha and blastospore.

4 DISCUSSION

This was a descriptive cross-sectional study that aimed to identify the causative agents of VVC. After 3 months sampling, 25 participants fulfilled the inclusion criteria. The result showed that most participants were in 15-24 age group. This is a reproductive age group, with high level of estrogen. Estrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida albicans* and also decreases immunoglobins in vaginal secretions resulting in increased vulnerability of women to vaginal Candidiasis. Most patients were private employees. This group of people tend to use vaginal hygiene products or vaginal douche that contained antiseptic, and prolonged misuse of antiseptic can cause VVC. Most patient education were bachelor; they might have better senses of personal vaginal hygiene than patients with lower education group So it made them went to hospital and checked their complaint and to get a proper treatment from the hospital (Fidel et al., 2000).

Most common duration of complaint of the patients of VVC is 1 month until 9 months. From this, we can deduct that there might be a recurrent VVC that was ignored by the patientand causing delayed proper treatment.

Clinical examination revealed edematous and erythematous vulva and vagina on all 25 patients. This means clinical sign of VVC is the first and definite diagnosis of VVC withthe direct microscopic examination was not the only parameter to diagnose VVC (Sobel, 2008).

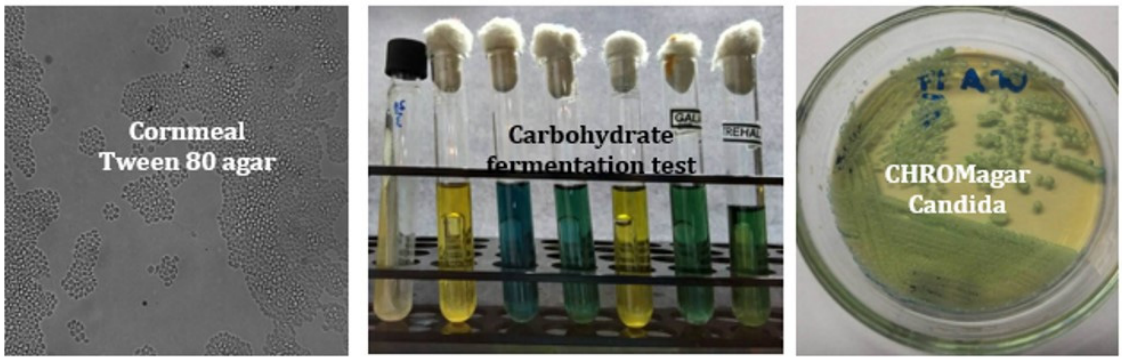

Figure 1: Conventional methods (SDA then Cornmeal Tween 80 agar, carbohydrate fermentation test, and CHROM agar Candida).

Cornmeal Tween 80 agar showed the specific morphology of every *Candida*. One sample with budding yeast-like cell with no pseudohyfa indicates the presence of*Candida glabrata*. However, there was also a sample that showed yeast cell only and, no pseudohyfa.This could not conclude the presence of *Candida glabrata* and additional examination using other conventional method was needed to identify the species (Golia et al., 2013; Suyoso, 2013).

The carbohydrate fermentation test shown positive result based on the changing color of broth to yellow and the gas formationin the Durham tube. This test, however, is not reliable when used without other additional method. For example, positive result of dextrose and trehalose indicates presence of *Candida glabrata*, while positive result of dextrose and maltose, galactose and trehalose indicates presence of *Candida albicans*. Therefore, a dubious positive result of only dextrose and maltose could not accurately indicate the presence of *Candida albicans*.CAC will be useful for the additional examination (Devi et al., 2014; Larone, 2011).

CAC revealed specific color for eachcolony.There was a sample which color was pink to purple, thus almost similar to *Candida glabrata*. The carbohydrate fermentation test could be used to differentiate dubious results from this examination in order to get the final diagnosis (Faraz et al., 2016; Suyoso, 2013; Vijaya et al., 2011).

5 CONCLUSION

There were increasing of non-*albicans Candida sp* in the agents causing VVC. Non-*albicans Candida sp* is an emerging threat fungi due to its antifungal resistance. Identification of causative agent of VVC down to the species level is important to give proper treatment to the VVC patients. There are 3 conventional methods that can be used to identify *Candida sp* to the species level. *Cornmeal agar* showed the morphology of *Candida sp*. Carbohydrate fermentation test revealed positive result if there is brothcolor transformation to yellow and Durham tube inside filled with gas. CHROM agar Candida showed *Candida sp* by its colony color. These 3 methods are still recommended for identification of *Candida sp* to the species level and should be performed simultaneously to support each other's data to get the final definite species identification result.

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