

# Changing Pattern of Candida Species in Vulvovaginal Candidiasis using Vitek 2

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Abstract: Vulvovaginal candidiasis (VVC) is a common fungal infection. VVC causes inflammation of vulva and vagina, characterized with erythematous vulva and vagina, with symptoms of pruritus and vaginal discharge. *Candida albicans* remains the cause of VVC in almost 80% cases. *Candida non-albicans* now are emerging threat as a cause of VVC, due to extensive usage of antifungal drugs. The importance of identifying *Candida* species within clinical samples is in order to provide information concerning the proper treatment for patients. Rapid identification of *Candida* species are essential in clinical laboratories. Vitek 2 is an automated microbiology identification system that evaluates an optical signal generated by biochemical reactions contained within a variety of identification cards. Vitek 2 could be used to identify *Candida sp.* to the species level. This microbiological system gives some advantages, it is faster than other diagnostic tools for VVC, it works automatically and accurately, and it practically do not need manual work so it also minimizes error. But for teaching hospital, it still need conventional method to study fungal morphology and to do Vitek 2 examination (from cornmeal-Tween 80 agar or CHROMagar Candida).

## 1 INTRODUCTION

*Candida* species are microorganisms which live normally in our body, the skin, mouth, gastrointestinal tract, and genitourinary tract, including vulva and vagina. It usually lives as benign commensals and produce no diseases. However, in women with some predisposition factors, such as vaginal douching usage, pregnancy, and immunosuppressed condition, the colony of *Candida sp.* will grow higher and causing VVC. This disease is usually a common problem on child-bearing age women and 5% will have recurrent infectious episodes (Vermitsky J, Self MJ, Chadwich SG, Trama JP, Adelson ME, Mordechai E, et al, 2008; Zeng J, Zong LL, Mao T, Huang YX, Xu ZM, 2011).

*Candida albicans* is responsible for infection in 80 to 90% of VVC cases, but now VVC due to *Candida non-albicans* have increased steadily over the latest decades (Srihartati E, Hoetomo MM, Ervianti E, 2006; Ervianti E, Sawitri, Murtiastutik D, Agusni RI., 2011; Jimoh O, Inabo HI, Yakubo SE, Ankuma SJ, Olayunka AT., 2016; Cassone A, 2016). The rise in VVC infections that more specifically caused by *Candida non-albicans* species, could be due to an increase in over-the-counter antifungal use.

Nowadays rapid identification of microorganisms that cause diseases are important in clinical laboratories. Better and faster diagnostic system for VVC would let physicians could be able to make therapeutic decisions based on species causative agents of VVC so enhance proper treatment for their patients. Physicians necessitate the rapid and accurate identification of yeasts to the species level by the clinical microbiology laboratory. Vitek 2 is an automated microbiology identification system that evaluates an optical signal generated by individual biochemical reactions contained within a variety of microbe identification cards. After inoculation with a standardized suspension of the unknown organism, each self-contained card is incubated and read by the instrument's internal optics. Vitek 2 provides a highly automated, objective yeast identification method with excellent performance. This system is useful for timely and accurate identification of significant yeast species in the clinical microbiology laboratory. Vitek 2 showed faster and better species identification result than other diagnostic systems such as conventional methods and nonculture methods because they can be time-consuming and manual-labour (Meurman O, Koskensalo A, Jalava-Rantakoko K, 2006; Rajkumari N, Mathur P, Xess I, Misra MC., 2014)

## 2 METHODS

This study was a cross-sectional descriptive study that identifying causes of VVC to the species level by using Vitek 2. Patients attending the Sexually Transmitted Infection (STI) Division Dermatovenereology Clinic of Dr Soetomo General Hospital Surabaya that suspected VVC were examined by the physician. The samples of this study were all VVC patients that fulfilled the inclusion criteria. The inclusion criteria were VVC patient, women age 15 years or more than 15 years, married or unmarried and willing to follow the research and signed the informed consent. The exclusion criteria were patients with negative culture result.

Patients were examined for VVC signs and symptoms. Samples were taken from vaginal swab, then underwent the direct microscopic examination (wet preparation, and Gram stain). Samples were tested for conventional methods and Vitek 2. Conventional methods consisted of Sabouraud dextrose agar (SDA) then Cornmeal-Tween 80 agar, carbohydrate fermentation test, and CHROMagar Candida (CAC). The first method is using SDA then Cornmeal-Tween 80 agar. This method showed structures of *Candida species* on microscope in 3 days (72 hours). The other method was carbohydrate fermentation test that consisted of 6 carbohydrates. The result of this test positive if there was changing color of broth to yellow and the tube inside the broth (Durham tube) filled with gas in 2-3 days (48– 72 hours). The third one was CHROMagar Candida that showed colonies in 1,5-2 days (36-48 hours). Color of colony showed the *Candida sp*. Overall all of this conventional method need 1,5-3 days (36-72 hours) to identify the species of *Candida*.

Colony from Cornmeal-Tween 80 agar or CAC were also checked by using Vitek 2. Four until six fresh colonies (16-24 hours age of colonies) and were taken and suspension with NaCl 0,85% in order to get standardized suspension (1.8-2 McF) were made. After inoculation with a standardized suspension of the unknown organism, each self-contained card is incubated and read by the instrument's internal optics. The reading result were compared to the baseline data of Vitek 2.

## 3 RESULTS

The most common age of patients is on young adult 15-24 age group. History of high frequency or recurrency of VVC is on 72% of patients. Clinical

examination showed edematous and erythematous vulva and vagina on all patients. There were variation result on direct microscopic examination from wet preparation and Gram stain. There were positive and negative result. There were no negative culture result.

Sabouraud dextrose agar then Cornmeal-Tween 80 agar examination showed specific characteristic of each species of *Candida*. Terminal vesicles (chlamydoconidia) with pseudohifa, blastoconidia looked like a flower, could be concluded as *Candida albicans*. Other samples with divaricated pseudohifa and oval blastoconidia, could be concluded as *Candida tropicalis*. Few samples with budding yeast like cell and no pseudohypha may reveal the species of *Candida glabrata*. The carbohydrate fermentation test showed positive result based on the changing color to yellow on 6 carbohydrates tested and the gas filled in the Durham tube. Positive result on dextrose and trehalose means that the sample grown was *Candida glabrata*. Positive result on dextrose, maltose, galactose and trehalose means that the sample was *Candida albicans*. Other positive result showed on dextrose, sucrose, maltose, galactose and trehalose means that the sample was *Candida tropicalis*, and one positive result showed on dextrose means *Candida parapsilosis* or could be other *Candida sp*, so it will need other conventional method, to get the final result. The last positive result on dextrose and sucrose only, this means could be other *Candida sp*, so that it will need other conventional method to support the definite result. CHROMagar Candida revealed the growth of *Candida sp* by its colony color. All samples revealed 1 type of colony color or 1 *Candida sp* in every sample, but only 4 samples showed 2 colony color (2 *Candida sp*). Colony color light green means the fungi was *Candida albicans* (if dark green means *C dubliniensis*). Purple colony color means the fungi was *Candida glabrata*. White colony color means *Candida parapsilosis*. Blue color means *Candida tropicalis*. This method should be completed with 2 other conventional methods (SDA then Cornmeal Tween 80 agar and carbohydrate test). The conventional methods from 25 sample showed 14 samples of *Candida albicans*, and others were *Candida non-albicans*. Five samples were *Candida glabrata*, 1 sample was *Candida parapsilosis* and 4 samples were grown with 2 *Candida sp* or infected by 2 *Candida sp*. Result of samples: 1 sample were combination *Candida albicans* with *Candida glabrata*, 1 sample were combination of *Candida albicans* with *Candida famata*, and 2 samples: each sample contained *Candida albicans* with *Candida tropicalis*.

Table 1: Comparison between Conventional methods and Vitek 2.

Conventional Methods	Vitek 2
All samples revealed growth of <i>Candida sp</i> to the species level: <i>C albicans</i> (the highest), <i>C glabrata</i> , <i>C tropicalis</i> , <i>C parapsilosis</i> , <i>C famata</i>	All samples revealed growth of <i>Candida sp</i> to the species level: <i>C albicans</i> (the highest), <i>C glabrata</i> , <i>C tropicalis</i> , <i>C parapsilosis</i> , <i>C famata</i>
Time-consuming (36-72 hours)	Faster (18 hours)
Laborious	Automatic machine, minimizing error

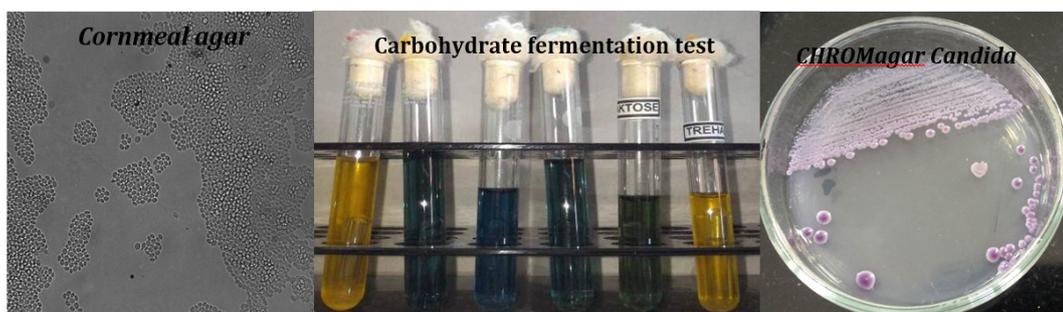


Figure 1: Conventional methods (SDA then *Cornmeal agar*, Carbohydrate fermentation test, *CHROMagar Candida*).

The image shows a laboratory report from BBLK SURABAYA. The report includes patient information, test details, and a biochemical profile. The 'Selected Organism' is identified as *Candida glabrata* with a 99% probability and excellent confidence. The 'Biochemical Details' table shows various test results for the organism.

Biochemical Details																	
3	LysA	-	4	MLTa	-	5	LeuA	+	7	ARG	-	10	ERYa	-	12	GLYLa	-
13	TyA	-	14	DNAG	-	15	ADPa	-	18	AMPa	-	19	SCALa	-	20	GENa	-
21	dGLUa	+	23	LACa	-	24	MAAdGa	-	29	dCELa	-	27	GGT	-	28	dMALa	-
29	dRFAa	-	30	NAGA1	-	32	dMNEa	+	33	dMELa	-	34	dMLZa	-	38	SBELa	-
39	RRFAa	-	40	XLTa	-	42	dSORa	-	44	SACa	-	45	URE	-	46	AGLU	-
47	STURa	-	48	dTRCa	+	49	NDPa	-	51	dRPa	-	52	dCETa	-	53	ESC	-
54	dLTA	(+)	55	dXYLa	-	56	LATa	-	58	ACEa	+	59	dITa	-	60	dRTa	-
61	dPRDa	+	62	dKCa	-	63	NAGa	-	64	dGNTa	-						

Figure 2: Result sheet of Vitek 2.

In Vitek 2, 25 of samples showed result of *Candida sp* 100% same as conventional methods. Fourteen samples were *Candida albicans*, and others were *Candida non-albicans*. Five samples were *Candida glabrata*, 1 sample was *Candida parapsilosis* and 4 samples were grown with 2 *Candida sp* or infected by 2 *Candida sp*. Result of 4 samples: 1 sample were combination *Candida albicans* with *Candida glabrata*, 1 sample were combination of *Candida albicans* with *Candida famata*, and 2 samples: each sample contained *Candida albicans* with *Candida tropicalis*. From table 1 showed that the result of species from 25 samples of Vitek 2 was 100% the

same as in conventional methods. Vitek 2 has some advantages that it is faster and works automatically.

#### 4 DISCUSSION

The most common age of patients was an age of young adult 15-24 group from total of 25 patients. It could be explained that the 15-24 age group is child-bearing age, which is high of estrogen hormone, allowing increase growth of *Candida sp*. History of high frequency or recurrency of VVC was on 72% of subjects. It means that a lot of women with VVC were often not going to the doctor seeking for treatment,

because maybe they think this disease is not life-threatening and would heal itself, and it could be because of the predisposition factors that still exist on them (Khairnar R, Khairnar A., 2017; Fidel PL, Cutright J, Steele C., 2000; Kalia N, Singh J, Sharma S, Kamboj SS, Arora H, Kaur M., 2015). Clinical examination showed edematous and erythematous vulva and vagina on all patients. This is concordance that clinical signs of VVC are edematous and erythematous vulva and vagina. Direct microscopic result from wet preparation and Gram stain showed positive and also negative result, but there were no negative colony culture result. This showed that direct microscopic examination is only an additional tool supporting the examination. The negative result of direct microscopic examination did not exclude the diagnosis of VVC, but signs of clinical examination establish the diagnosis (Kundu RV, Garg A., 2013; Sobel JD, 2008).

There are 3 conventional methods that support each other. SDA then Cornmeal Tween 80 agar showed specific characteristic of each species of *Candida*. The carbohydrate fermentation test showed positive result based on the changing color to yellow on 6 carbohydrate tested and the gas filled in the Durham tube. CAC revealed the growth of *Candida sp* by its colony color. The conventional methods from 25 samples showed 14 samples of *Candida albicans*, and others were *Candida non-albicans*, 5 samples were *Candida glabrata*. One sample were *Candida parapsilosis* and 4 samples were grown with 2 *Candida sp* or infected by 2 *Candida sp*. Result of samples: 1 sample were combination *Candida albicans* with *Candida glabrata*, 1 sample were combination of *Candida albicans* with *Candida famata*, and 2 samples: each sample contained *Candida albicans* with *Candida tropicalis* (Suyoso S. Mucosal candidiasis. In: Bramono K, Suyoso S, Indriatmi W, Ramali LM, Widaty S, Ervianti E, editors., 2013; Larone DH, 2011)

In Vitek 2 from 25 samples showed result of species identification *Candida sp* 100%, the same as conventional methods. The highest number species were on 14 samples (*Candida albicans*). The conventional methods need about 36-72 hours to identify the species, but Vitek 2 only needs 18 hours. Vitek 2 has more advantages than conventional methods. Vitek 2 immediately yielded the result of species by the machine itself, Vitek 2 has faster time than conventional methods to identify to the species level and can work automatically so it does not need manual labour, and it minimizes error (Mona et al., 2015; Esmat MM, Mohamed T, Abdelrahman AH, 2005).

## 5 CONCLUSION

The best method of identification of the species is using the combination of this 3 conventional methods. Conventional identification methods are still considered to be the reference standard for the identification of yeast isolates and also for education purposes, but are laborious and time-consuming. Beside that, conventional methods can show structures of *Candida sp* clearly that this structures could not be seen using Vitek 2. Conventional methods are important and useful for learning fungi especially in teaching hospital, Dr Soetomo Surabaya Hospital. A fast and accurate technique for yeast identification is very important for microbiological laboratories. According to the results found in the present study, the Vitek 2 system (from cornmeal-Tween 80 agar or CAC) identified most clinically important *Candida sp* reliably within 18 hours, and appears to be an excellent alternative identification method for performing fungal diagnostics.

The result showed the most common yeast causing VVC was *Candida albicans* (56%), but there were increasing of *Candida non-albicans* that cause VVC. Most *Candida non-albicans* are usually causing antifungal resistance. It is therefore important that there should be increased awareness among physicians on the rising prevalence of *Candida non-albicans*, due to the reduced susceptibility to azoles. Prior identification to the species level on VVC is essential to ensure early diagnosis of *Candida non-albicans* infection and in order to give proper treatment.

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