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**IDENTIFICATION OF CANDIDA SPECIES IN DIABETIC PATIENTS  
WITH COATED TONGUE**

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**Abstract**

**Background:** Coated tongue is a condition in which the dorsum of the tongue is covered by a white layer containing food debris or microorganisms such as *Candida* species (*Candida* spp). Factor that can influence coated tongue including a condition such as Diabetes mellitus which often reduce the production of saliva and initiate the increase of *Candida* spp in number. **Objective:** to determine various species of *Candida* spp. in the saliva of Diabetes mellitus patients with coated tongue as a preliminary condition to detect oral candidiasis. **Methods:** Coated tongue examination was performed by evaluating the lesion visually or using tongue scraper. Salivary collection was performed using oral rinse concentrate method. Saliva samples were inoculated in Chromogenic Agar to identify *Candida* spp. by examining the colour and the form of colony. **Results:** Eight samples (88.8%) were identified as *C. albicans* while *C. parapsilosis* was presented in 5 samples (66.6%) and *C. tropicalis* in 2 samples (22.2%). Overall, *C. albicans* is the most common *Candida* spp. that found in oral cavity. *Candida* spp. as normal flora in the oral cavity could be pathogen if the body's immune system decreases. In this study, there was a slight difference between *Candida* spp. found in the saliva of Diabetes mellitus patients compared to the control group. **Conclusions:** *Candida* spp. that identified in Diabetes mellitus patients with coated tongue are *C.albicans*, *C. glabrata*, and *C. tropicalis*.

**Keywords:** *Candida* spp., saliva, Diabetes mellitus, coated tongue

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**INTRODUCTION**

Coated tongue is a condition in which the dorsum of the tongue is covered by a yellowish or brownish white layer containing food residue or microorganism.<sup>1</sup> It is often found in elderly age because salivary glands function is decreased due to degenerative conditions and others factors including systemic condition, drugs, poor oral hygiene, allergies, and vitamin deficiency.<sup>2</sup> Diabetes mellitus is one of many other diseases that can cause coated tongue.<sup>3</sup> Previous study has revealed that the highest prevalence of oral lesions in people with diabetes mellitus is coated tongue.<sup>4</sup>

Patients of diabetes mellitus show an alteration in salivary glands that cause the decrease of salivary flow rates and the increase of salivary

viscosity.<sup>5</sup> These conditions reduce saliva ability in cleaning the oral cavity and also in providing antibacterial activity. The dorsum of the tongue becomes more retentive upon food debris, exfoliated mucosal cells, and microorganisms.<sup>3</sup> Impaired salivary gland function can predispose to a fungal infection induced by various of *Candida* spp.<sup>6</sup>

*Candida* spp. as a normal flora has a high percentage in the oral cavity. It is about 30-50% of the total microorganisms. In healthy individuals, *Candida* exists harmlessly in mucus membranes such as ears, eyes, gastrointestinal tract, mouth, nose, reproductive organs, sinuses, skin, stool and vagina, etc. The cell wall component of *Candida* spp. such as mannose, C3d receptors, mannoprotein and saccharins help it to penetrate the surrounding tissue.<sup>6</sup>

The spores of *Candida* are the harmless form of this commensal dimorphic fungus that may transform into an invasive and pathogenic pseudohyphae. The transition of *Candida* from a harmless commensal to a pathogenic organism is complex and is related to subtle environmental changes that lead to the expression of innumerable virulence factors.<sup>7</sup> *Candida spp.* can be pathogenic in immunocompromised patients. If it continues, it will cause *Candida spp.* to induce fungal infections called candidiasis.<sup>5,8</sup>

The purpose of this study is to determine various species of *Candida spp.* that found in the saliva of diabetes mellitus patients with coated tongue. The reason for using saliva is because of its higher sensitivity and capability to identify *Candida spp.* in oral cavity. Identification of the control group is conducted in healthy people with the presence coated tongue. The presence of the control group is subjected to see differences in *Candida spp.* between normal subjects and diabetes mellitus patients.<sup>9</sup>

#### MATERIALS AND METHODS

This study is an observational descriptive study with a cross-sectional approach.<sup>10</sup> Samples were collected from controlled diabetes mellitus patients in elderly age at Prolanis (Program Pengelolaan Penyakit Kronis) at PT Puskesmas Ujung Berung, Bandung. The study groups consisted of 9 patients diagnosed with diabetes mellitus and 9 healthy people as the control group. The number of samples in diabetes mellitus group were determined using Slovin formula with 10% error rate where a result of 80 people was obtained as the total population.<sup>10</sup>

This study has received ethical permission from the Medical Health Ethics Committee, Faculty of Medicine, Padjadjaran University number 1174/UN6.C.10/PN/2017 and registration number: 0217111269. Informed consent was obtained from the participants at the beginning of the study.

Before the assessment of coated tongue, oral cavity examination was done thoroughly. Coated tongue examination was performed by evaluating the lesion visually or using tongue scraper with a direct scrapping motion from posterior to anterior. If there is no erythema formed on the tongue of the patient, the lesion is diagnosed as coated tongue.<sup>11</sup> Referring to Miyazaki classification, the assessment of the coated tongue are scored based on four distinctive number according to the distribution area (Table 1).<sup>12</sup>

Table 1. Coated Tongue classification based on distribution area<sup>(12)</sup>

Score	Distribution Area
0	Not visible
1	< 1/3 of the tongue surface is covered
2	< 2/3 of the tongue surface is covered
3	> 2/3 of the tongue surface is covered

Saliva collection was performed using Oral Rinse Concentrate method based on Tooyama and Raju study. Patient was instructed to rinse with 10 ml of sterile phosphate buffer saline for 1 minute. A total of 7-10 ml solution subsequently collected into sterile container and stored in a refrigerant containing ice cube.<sup>7,9</sup> Collected samples were then taken to the Microbiology Laboratory, Faculty of Dentistry, Universitas Padjadjaran.

Identification of *Candida spp.* was determined by using Chromogenic Agar. The solution was centrifuged under 4000 rpm at 4°C for 20 minutes.<sup>9,13</sup> Pellet substance was left in the centrifugation tube and 1 ml of sterile distilled water was added into the tube.<sup>9</sup> A total of 0.1 ml solution was placed in the middle part of Chromogenic Agar.<sup>14</sup> Solution was spreaded using inoculating loop or spreader to the entire surface of agar. After 48 hours incubation at 37°C temperature, the type of *Candida spp.* was identified through color differences and colonies produce in Chromogenic Agar. (Table 2 and Figure 1)

Table 2. Overview of *Candida* Species that grow in Chromogenic Agar.<sup>15</sup>

Species	Colony Characteristic	
	Colour	Morphology
<i>C.albicans</i>	Bright green, bluish green (Tosca)	Medium size, smooth, convex, creamy
<i>C.dublinsiensis</i>	Bluish green darker	Medium size, smooth, convex, creamy
<i>C.tropicalis</i>	Dark blue	Medium, smooth, convex, creamy
<i>C.krusei</i>	Purplish pink with pale edges	Medium-large, flat, rough
<i>C.glabrata</i>	Pink with dark purple, dark purple, light brown or dark center with pale edges	Small-medium, smooth, convex, creamy
<i>C.parapsilosis</i>	Pale pink, brown with pale edges	Small-medium, smooth

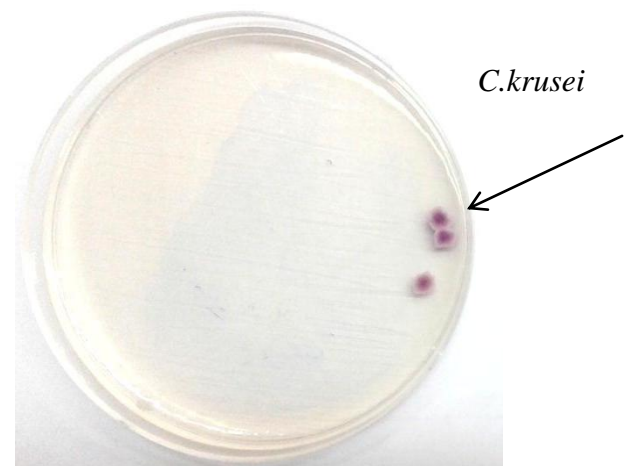
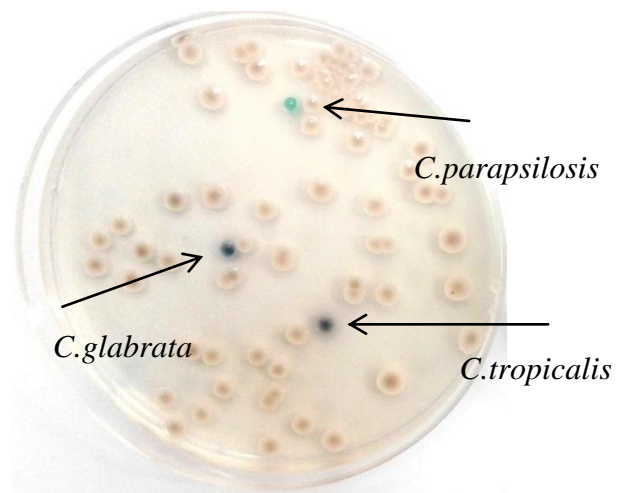
Table 3. Distribution of Coated Tongue in Study Group

Study Groups	Frequency	Percentage
Diabetes mellitus Group		
Score 0	0	0,0%
Score 1	2	22,2%
Score 2	6	66,7%
Score 3	1	11,1%
Control Group		
Score 0	0	0,0%
Score 1	2	22,2%
Score 2	7	77,8%
Score 3	0	0,0%

**RESULTS**

Coated tongue distribution among the samples was generally assessed in score 2. In patients with DM, 6 samples (66.7%) were presented with coated tongue which is classified in score 2, followed by score 1 and score 3. In the control group, 7 samples (77.8%) were depicted in score 2 and followed by the identification in score 1 (Table 3).

There were 6 species that identified after the incubation. In Diabetes mellitus patients, 8 (88.8%) were identified as *C.albicans* while *C. parapsilosis* was found in 5 patients (66.6%) and *C.tropicalis* in 2 patients (22.2%). In the control group, 4 (44.4%) were identified as *C. albicans*. *C. dublinsiensis* and *C. glabrata* were found in 2 subjects (22.2%) while *C. krusei* and *C. parapsilosis* were found in only 1 subject (11.1%). Overall, *C.albicans* was the most common strain to be found in both Diabetes mellitus patients and the control group. The results of the identification are presented in table 4 and 5.



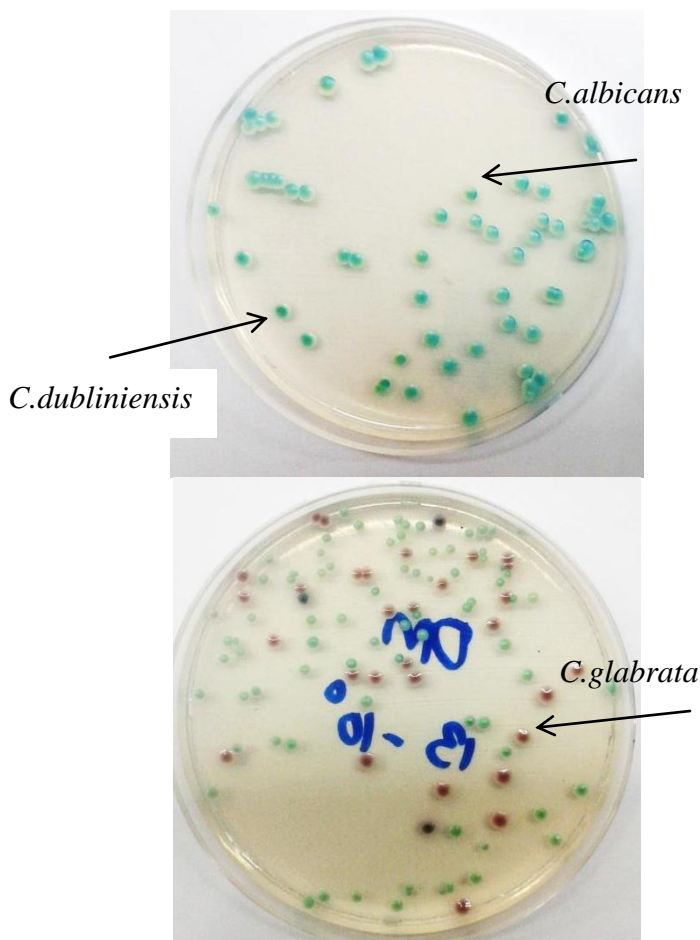


Figure 1. *Candida spp.* colonies in Chromogenic Media Agar

Tabel 4. Identification of *Candida spp.* in Diabetes mellitus Group

Candida species	Study Samples (n = 9)									Percentage
	1	2	3	4	5	6	7	8	9	
<i>C.albicans</i>	+	-	+	+	+	+	+	+	+	88,8%
<i>C.dubliniensis</i>	-	-	-	-	-	-	-	-	-	0,0%
<i>C.krusei</i>	-	-	-	-	-	-	-	-	-	0,0%
<i>C.tropicalis</i>	-	-	-	-	-	-	+	-	+	22,2%
<i>C.glabrata</i>	-	+	+	+	+	+	-	-	+	66,6%
<i>C.parapsilosis</i>	-	-	-	-	-	-	-	-	-	0,0%

Table 5. Identification of *Candida spp.* in control group

Candida species	Study Samples (n=9)									Percentage
	1	2	3	4	5	6	7	8	9	
<i>C.albicans</i>	+	-	-	+	-	+	+	-	-	44,4%
<i>C.dubliniensis</i>	+	-	-	+	-	-	-	-	-	22,2%
<i>C.krusei</i>	-	+	-	-	-	-	-	-	-	11,1%
<i>C.tropicalis</i>	-	-	-	+	-	-	-	-	-	11,1%
<i>C.glabrata</i>	-	-	-	+	-	-	-	+	-	22,2%
<i>C.parapsilosis</i>	-	-	-	+	-	-	-	-	-	11,1%

## DISCUSSION

The comparison of male and female samples ratio obtained a result of 1: 2. The majority of the samples were categorized in 45 to 54 and 55 to 64 year-age group. Patients who were subjected for this study includes those who suffered from Diabetes mellitus (DM) type 2. Indonesian Ministry of Health's Data and Information Center (2014) states that 90% of Diabetes mellitus patients in Indonesia were diagnosed with DM type 2. It was dominated by female with the ratio 1:1,4.<sup>16</sup> The National Diabetes Statistic in America showed that the majority of Diabetes mellitus patients were in the age group of 45-64 which is in accordance with the current study.<sup>17</sup>

The distribution of coated tongue among samples are mostly identified in score 2, signifying that the layer covered 2/3 surface of the tongue. One sample from DM patients group was scored 3 referring to the layer coverage on 3/3 surface of the tongue. Previous studies showed that the most common score for coated tongue observed among elder aged 45 to 90 years were score 2 and 3.<sup>18</sup> Another studies in elderly people also showed that according to Miyazaki index, the majority of patients were identified in score 2 and the rest were presented in score 1.<sup>2</sup> Diabetes mellitus affect the salivary glands thus decreasing the production of saliva. The viscosity of saliva is increase and salivary secretion contains glucose more than normal. As the result, tongue surface is more susceptible for the retention and the proliferation of microorganisms such as *Candida spp.*. Coated tongue was formed by the

accumulation of food debris and the proliferation of microorganisms.<sup>19,20</sup>

*Candida spp.* is a normal flora found in the digestive tract, mucous membranes of the oral cavity, vagina, urethra, and skin. Pathogenic properties of *Candida spp.* will appear when the body's resistance decreases. Its ability to invade host tissue is supported by the virulence factors found in the components of *Candida spp.* cell wall.<sup>21,22</sup> Adhesion occurs when fungal cell produce adhesin which help the morphogenesis of *Candida spp.* to transform the spore into hyphae. Enzymes produce from cell walls are associated with lipid, protein, and polysaccharides components and can damage the physical defenses of the host. Biofilm formation is supported by the generation of hyphae which is influenced by cell wall components such as polysaccharides (glucan and chitin) and lipids (ergosterol and phospholipomannan).<sup>21,23</sup> Phenotypic switching involves specific genes and supports *Candida spp.* to adapt in host environment.<sup>23</sup> Hyphae can promote biofilm formation, destroy macrophages, invade host tissue and obtain the required nutrients.<sup>21</sup>

In this study, *Candida spp.* among DM patients and control group have similar species namely *C.albicans*, *C.glabrata*, and *C.tropicalis*. There were three types of *Candida spp.* that identified in control group but not in Diabetes mellitus patients namely *C.krusei*, *C.dubliniensis*, and *C.parapsilosis*. Previous studies that examined controlled diabetes mellitus patients reported the identification of *C.albicans*, *C.tropicalis*, *C.dubliniensis*, and *C.parapsilosis*.<sup>24</sup>

*C.albicans* was the most common species that can be found in both sample groups. Previous study with samples of controlled DM patients who had denture stomatitis in their oral cavities presented *C.albicans* as the dominating strain among other species such as *C.glabrata* and *C.tropicalis*.<sup>25</sup> The reason for the high number of *Candida spp.* in DM patient is the decrease in salivary pH. Dehghan *et al* described that collagenolytic enzyme that produced by *C.albicans* can reduce human dentinal collagen and dentinal structure. This enzyme activity will be optimal at pH 3.5 to 4.0. *C. albicans* produces lactic acid as the result of carbohydrates fermentation resulting in the reduction of hydroxyapatite structure in the teeth, which can be the reason for low salivary pH level in DM patients<sup>22</sup>.

The other species that identified in this study were *C.glabrata*, *C.tropicalis*, *C.dubliniensis*, *C.krusei*, and *C. parapsilosis*. Previous study reported that *C. glabrata* was identified among DM patients as their samples. The oral isolates of *C. glabrata* produced strong biofilm. It might be caused by the higher aggressivity of *C.glabrata* in

producing a great quantity of biofilm matrices and in increasing the chitin concentrations in the cell walls. In their study, they also found *C.tropicalis* among the samples of diabetic patient.<sup>26</sup> *C. tropicalis* is the most virulent strain of *non-Candida albicans* species. This is due to its ability to adhere on epithelial cells in vitro and produce sufficient proteinase.<sup>27</sup>

Other study detected *C.dubliniensis* which has been isolated from both diabetic and non-diabetic patients. Interestingly, the authors did not found *C. dubliniensis* among *Candida spp.* isolate from diabetic patients. It may be related to identification techniques problems, since *C.dubliniensis* and *C.albicans* have similar phenotypic characteristics.<sup>26</sup> *C.dubliniensis* is associated with oral lesions in HIV patients. It also shows low virulence that vascular or systemic infections due to this species are rarely found. Celia *et al.* isolated *C. krusei* in their samples of diabetic patient.<sup>27</sup> Those finding is different from current study which identified *C.krusei* in the samples of controlled group.<sup>26</sup> *C. krusei* infection usually occurs in patients with chronic disease and rarely causes Candidemia. *C. parapsilosis* have adhesion ability and it can form biofilms on the surface of the instruments for a treatment. If the blood vessels are exposed, it may initiate fungal infection via the instruments.<sup>26</sup>

In this study, *Candida spp.* identified in Diabetes mellitus patients were *C. albicans*, *C. glabrata* and *C. tropicalis*. *C. albicans* is the most common species that can be found in both samples. Various precautions such as cleaning the tongue with tongue scraper and eating fibre-rich foods can reduce the formation of coated tongue in patients.

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