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IDENTIFICATION OF CANDIDA SP. FUNGI IN THE ORAL CAVITY OF ACTIVE SMOKERS USING THE SWAB METHOD

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ABSTRACT
Active smoker is anyone who directly smokes cigarettes from a burning cigarette. The oral mucosa is the part that can be damaged by smoking. Candida infections usually develop on mucous membranes such as the mouth and are responsible for the appearance of disorders such as candidiasis in the oral cavity.Which has the potential to become fungal. Candida is a species that is often found in the oral cavity. This research aims to determine the presence of Candida.sp fungus in the oral cavity in Petoran village RT 02/RW09 Jebres Surakarta. This research was carried out in November 2023 - June 2024 using descriptive observational research with kuota sampling and 20 samples were taken of the Population of active smokers in Petoran village RT 02/09 Jeberas Surakarta. Samples were taken using the oral mucosa swab method, then cultured on PDA media and observed macroscopically and microscopically with an objective lens magnification of 40x. The data in this study were analyzed using a distribution approach to examine the spread of Candida sp. fungal infections among active smokers in Kampung Petoran. Out of the 20 samples collected, the identification results showed that 14 samples, or 70%, were infected with Candida sp., while the remaining 6 samples, or 30%, tested negative. This distribution of infections indicates that the majority of active smokers in the village were exposed to Candida sp. This analysis provides an initial overview of the prevalence of Candida sp. fungus among active smokers in the area.

Keywords: active smokers; candida sp.; swab method

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INTRODUCTION

The prevalence of active smokers in Indonesia continues to rise. Data from the 2023 Indonesia Health Survey (SKI) conducted by the Ministry of Health (Kemenkes) shows that the number of active smokers is estimated to reach 70 million people, with 7.4% of them being smokers aged 10-18 years. Children and adolescents represent the group with the most significant increase in the number of smokers. The 15-19 age group constitutes the largest percentage of smokers (56.5%), followed by the 10-14 age group (18.4%) (Kemenkes, 2024). The widespread habit of smoking has various impacts, one of which is causing oral cavity infections, beginning with changes in the composition of saliva and normal flora. The harmful effects of smoking are not only systemic but also contribute to the development of pathological conditions in the oral cavity. Teeth and soft tissues in the mouth are vulnerable to damage caused by smoking (Debby & Sayekti, 2022).

The oral mucosa is one of the areas that can be damaged by smoking. Continuous irritation from tobacco smoke causes thickening of the oral mucosal tissues. Before clinical symptoms

appear, irritation from tobacco smoke affects the epithelial cells of the mucosa, leading to increased activity. These symptoms become visible when cellular activity increases and the epithelium thickens, especially in the buccal mucosa (the mucosa facing the cheek) and the floor of the mouth. Changes in the oral mucosa appear as white patches, which may be caused by thickened epithelium saturated with saliva. Consequently, these changes can potentially foster the growth of fungi in the mouths of active smokers (Mulyati et al., 2019).

The normal flora of the human oral cavity includes various types of organisms such as eubacteria, archaea, protozoa, mycoplasma, and fungi (Muzurović et al., 2013). Fungi are among the organisms most frequently responsible for disorders in the oral cavity. The genus *Candida* is the most common cause. *Candida* infections usually develop on mucous membranes such as the mouth and are responsible for conditions like candidiasis in the oral cavity. Candidiasis is an opportunistic fungal infection commonly occurring in the oral cavity due to several predisposing factors, which cause *Candida* to become pathogenic (Rezeki & Rahmayanti, 2023).

Oral candidiasis cases can also occur in healthy individuals with reduced immune responses due to several factors such as endocrine disorders, malnutrition, dental prostheses, epithelial disturbances, high-carbohydrate diets, infants and the elderly, poor oral hygiene, and smokers (Mulyati et al., 2019). Candida is the most commonly found species in the oral cavity. This species has the characteristic of developing into both yeast and hyphae. The hyphal form plays an important role in causing disease by penetrating epithelial cells and causing tissue damage (Debby & Sayekti, 2022). Environmental factors can enhance the development of Candida from yeast into hyphae, which can be asymptomatic, such as the use of dental prostheses, saliva pH, interactions between Candida species, and smoking habits (Komariah & Sjam, 2012; Sophia & Suraini, 2023; Udayalaxmi & Shenoy, 2016).

Kampung Petoran is a densely populated settlement where most people work as construction laborers. The environment in Kampung Petoran is not conducive for teenagers, as many residents consume alcohol and smoke. Consequently, many teenagers in Kampung Petoran, particularly in RT 02/RW 09, smoke, and it is alarming that even elementary school children smoke, though they do so in secret to avoid detection by their parents. Currently, in Kampung Petoran, middle school (junior high school) students are allowed to smoke by their parents.

In the study by (Sophia & Suraini, 2023), the identification of Candida sp. was conducted, where 20 samples were cultured for 2x24 hours and subjected to macroscopic and microscopic tests. *Candida* sp. was found in 13 samples, representing 65%, while the remaining 7 samples were non-*Candida* sp., representing 35%. In the study by Debby and Sayekti (2022), it was reported that among 6 smoker respondents (30%), Candida sp. was found, whereas no Candida sp. growth was observed in 12 other respondents (70%). Among non-smoker respondents, *Candida* sp. was found in 2 respondents (10%), while no Candida sp. growth was found in 18 other respondents (90%). Smoking not only causes systemic effects but also leads to pathological conditions in the oral cavity, teeth, and soft tissues (Mulyati et al., 2019). Studies suggest that smoking impacts saliva production, which plays a vital role in maintaining oral health. Nicotine consumption is connected to both structural and functional changes in the salivary glands, causing a decrease in the release of key antimicrobial proteins such as lactoferrin (Lac) and lysozyme (Lys). Prolonged smoking is linked to hyposalivation, a condition marked by reduced saliva output, potentially leading to various oral health complications. The heat produced by smoking can also influence vascularization in the mouth. Smoking introduces numerous harmful substances that can

cause inflammation and changes in blood vessels, possibly hindering blood circulation and the overall health of oral tissues. This may weaken the immune response, making the mouth more vulnerable to infections and other oral diseases (Khan et al., 2010; Mori et al., 2023; Sever et al., 2023; Zięba et al., 2023a, 2023b).

Saliva plays a vital role in oral homeostasis. It is essential for lubrication, and protection against viruses, bacteria, and fungi. The thinning of the oral mucosal epithelium and reduced saliva secretion make it easier for *Candida* sp. to colonize the oral cavity and invade the mucosa, leading to oral candidiasis. Even in non-smokers, *Candida* sp. can be found, as Candida naturally exists in saliva. However, in active smokers, Candida can become opportunistic. Therefore, these conditions can potentially promote the growth of fungi in the mouths of active smokers (Rezeki & Rahmayanti, 2023). Based on the above explanation, it is necessary to conduct research on the identification of Candida fungi using oral mucosa swabs in active smokers in Kampung Petoran RT 02/RW 09, Jebres, Surakarta.

METHOD

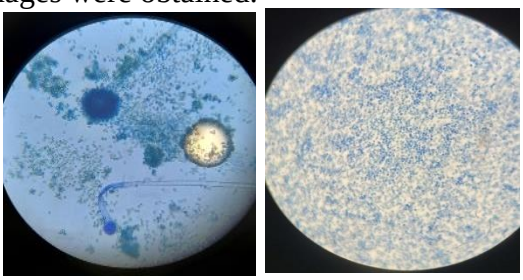
The type of research used is descriptive observational research conducted at the Parasitology Laboratory of the National School of Health Sciences from November 2023 to June 2024. The sampling technique used in this study is quota sampling, with a total of 20 swab samples collected in the field. The data source obtained is primary data in the form of the results of the identification of *Candida* sp. The data in this study were analyzed using a distribution approach to examine the spread of *Candida* sp. fungal infections among active smokers. This study began with providing informed consent to the respondents as proof of their agreement to participate in the research. Additionally, a questionnaire regarding smoking habits was also completed. After the informed consent and questionnaire were filled out, sample collection preparation was conducted, including sterilized cotton swabs, 0.9% NaCl, a spirit burner, and an ice box. Next, the spirit burner was lit with a match, and the sterilized cotton swab was dipped into the 0.9% NaCl solution correctly so that the swab wouldn't be too wet during sampling. The sample was taken during the daytime. For sample collection, the respondent sat comfortably, the spirit burner was lit, and then the sterilized cotton swab was evenly swabbed. The swab was then placed into a NaCl tube, broken off so that the lid could be closed, labeled, and then placed in the ice box. The collected mucosal swab samples were taken to the laboratory for inoculation on PDA media.

The fungal identification began by planting the collected swabs onto PDA media using a streaking method. Afterward, the media was wrapped with plastic wrap. The streaked media was incubated in an incubator at 37°C for 2 days. Colonies that grew on the PDA media were collected using an ose loop, selecting yeast-shaped colonies suspected to be of the Candida genus, and one PDA medium without inoculum served as the control. Macroscopic identification was performed by directly observing the colonies growing on the PDA media with the naked eye. During macroscopic examination, attention was paid to colony color, shape, and type. The characteristics of the *Candida* colonies were recorded. Microscopic identification was performed by taking a colony, placing it on an LPCB (Lactophenol Cotton Blue) drop, and covering it with a coverslip. The prepared slide was then observed under a microscope at 10× and 40× magnifications. The observed fungal structures were noted, particularly the pseudohyphae and blastospores.

RESULTS

Among the 20 active smokers in Kampung Petoran 02/09 Jebres Surakarta, it was found that 14 individuals were infected with *Candida* sp., while 6 individuals were not infected. Based

on macroscopic observations followed by microscopic examination using a 40x objective lens, the following images were obtained.



(a) (b)

Figure 1. Microscopic results of fungal colonies on PDA media (a) Morphology of *Aspergillus sp*., (b) Morphology of *Candida* sp. (Personal Documentation, 2024)

The identification results of Candida sp. and the questionnaire responses are presented sequentially in Tables 1 and 2 as follows:

Table 1.
Identification results of *Candida* sp. for each respondent

Sample No	Fungal identification	Result
1	*Candida sp*	Positive
2	*Candida sp*	Positive
3	*Candida sp*	Positive
4	-	Negative
5	*Candida sp*	Positive
6	*Candida sp*	Positive
7	*Aspergilus sp*	Positive
8	*Candida sp*	Positive
9	*Candida sp*	Positive
10	*Candida sp*	Positive
11	-	Negative
12	-	Negative
13	*Candida sp*	Positive
14	*Candida sp*	Positive
15	*Candida sp*	Positive
16	*Candida sp*	Positive
17	*Candida sp*	Positive
18	-	Negative
19	-	Negative
20	*Candida sp*	Positive

Table 2.
Questionnaire on the profile of active smoker respondents

No	Question	Answer	
		Yes	No
1.	I always maintain oral hygiene	20	0
2.	I smoke at least 5 cigarettes a day	18	2
3.	I smoke every day	19	1
4.	I have been smoking for more than 3 years	17	3
5.	I have been smoking for less than 3 years	2	18
6.	I always smoke after eating	20	0
7.	I have shared a cigarette with a friend	13	7
8.	I immediately buy cigarettes when I run out of them	17	3

DISCUSSION

Table 1, it is observed that out of 20 respondents, 14 were infected with *Candida* sp., 1 was infected with *Aspergillus*, and 5 were not infected with any fungus. This outcome can be attributed to several factors, as indicated in Table 2. Despite all respondents claiming to maintain oral hygiene (question 1), a significant number still showed infection. The results of this study do not align with Mersi&Lailiqonita (2021), which states that Smoking does not contribute to the growth of oral Candida colonies. Adopting good oral hygiene practices may help regulate the proliferation of these colonies. This could be due to nicotine in cigarettes disrupting the oral microbial balance and creating an environment conducive to *Candida* sp. growth. The high number of daily smokers (question 3) also contributes to mucosal damage, as chemicals in cigarettes can cause infection and damage to the oral mucosa, making it more susceptible to infections. Furthermore, the impact of smoking on the oral microbiome can lead to an increased risk of infections, as cigarette smoke tends to inhibit beneficial bacterial species while promoting the proliferation of opportunistic pathogens, thereby facilitating conditions favorable for infections like candidosis (Thomas et al., 2014; Wu et al., 2016). Moreover, the relationship between smoking and the development of oral candidosis is underscored by findings that indicate cigarette smoke exposure can significantly disrupt the delicate balance of the oral microbiome, creating an environment where opportunistic pathogens such as Candida can thrive, leading to the high infection rates observed in the study population (Patil et al., 2015; Thomas et al., 2014). The prevalence of oral fungal infections, particularly *Candida* sp., among the respondents despite their reported maintenance of oral hygiene, suggests that additional factors, such as smoking, may be playing a significant role in the etiology of these infections. The existing body of research has consistently shown that chronic tobacco use is associated with an increased carriage of pathogenic organisms and a disruption of microbial diversity within the oral cavity (Davis et al., 2014; Thomas et al., 2014).

The culture-based method utilized Potato Dextrose Agar (PDA) media, which is commonly used due to its carbohydrate content. PDA supports the growth of Candida better than alternative media, owing to its simplicity and efficacy in supporting a wide range of fungi, making it a suitable choice for assessing fungal proliferation in various biological samples (Black, 2020; Nurdin, 2020; Qurrohman et al., 2022). Aspergillus growth in samples could be due to less sterility during sample collection, as the swabs were taken some distance from the spirit burner, allowing Aspergillus contamination. The microscopic investigation of fungal species has long been a fundamental tool in the field of mycology, providing researchers with the ability to delve deeply into the intricate structural features of these fascinating organisms. In the present study, we employed the LPCB staining technique, a versatile reagent that enhances the visibility of fungal structures and preserves their morphological integrity. Our microscopic examination revealed the distinctive characteristics of two commonly encountered fungal species, Aspergillus sp. and Candida sp. Aspergillus sp. displayed the hallmark features of this genus, including the presence of vesicles adorned with sterigmata and rough, round conidia arranged in a fan-like pattern, along with septate hyphae. In contrast, Candida sp. exhibited the characteristic blastospores, a key diagnostic feature of this yeast-like fungus. The LPCB staining method has proven to be a valuable tool in the investigation of fungal species, as it not only highlights the unique structural elements of these microorganisms but also preserves their cellular integrity (Byadarahally Raju & Rajappa, 2011; Jacobsen et al., 2012; Jiang et al., 2021; Saigal et al., 2011). The study findings indicate that despite respondents smoking and claiming to maintain oral hygiene, many still share cigarettes or smoke with others. Therefore, practicing proper oral hygiene, reducing

smoking, and avoiding sharing cigarettes are crucial to prevent various diseases, especially fungal infections.

CONCLUSION

Based on the examination of *Candida* sp. in the oral cavity of active smokers, it was found that out of 20 respondents, 14 individuals (70%) were infected with *Candida* sp., while 6 individuals (30%) were not infected with *Candida* sp.

REFERENCES

Black, W. D. (2020). A comparison of several media types and basic techniques used to assess outdoor airborne fungi in Melbourne, Australia. *PLoS ONE*, *15*(12 December), 1–25. https://doi.org/10.1371/journal.pone.0238901

Byadarahally Raju, S., & Rajappa, S. (2011). Isolation and Identification of Candida from the Oral Cavity . *ISRN Dentistry*, *2011*(Table 2), 1–7. https://doi.org/10.5402/2011/487921

Davis, S. E., Hopke, A., Minkin, S. C., Montedonico, A. E., Wheeler, R. T., & Reynolds, T. B. (2014). Masking of β(1-3)-glucan in the cell wall of Candida albicans from detection by innate immune cells depends on phosphatidylserine. *Infection and Immunity*, *82*(10), 4405–4413. https://doi.org/10.1128/IAI.01612-14

Debby, R., & Sayekti, F. D. J. (2022). Hubungan Merokok Dengan Jumlah Koloni. *Meditory*, *10*(6), 119–127.

Jacobsen, I. D., Wilson, D., Wächtler, B., Brunke, S., Naglik, J. R., & Hube, B. (2012). Candida albicans dimorphism as a therapeutic target. *Expert Review of Anti-Infective Therapy*, *10*(1), 85–93. https://doi.org/10.1586/eri.11.152

Jiang, L., Li, X., Gu, R., & Mu, D. (2021). Rapid Detection of Aspergillus fumigatus Using Multiple Cross Displacement Amplification Combined With Nanoparticles-Based Lateral Flow. *Frontiers in Cellular and Infection Microbiology*, *11*(April), 1–9. https://doi.org/10.3389/fcimb.2021.622402

Kemenkes. (2024). *Perokok Aktif di Indonesia Tembus 70 Juta Orang, Mayoritas Anak Muda*.

Khan, G. J., Javed, M., & Ishaq, M. (2010). *354-358-1-Pb*. *8*(2), 221–224.

Komariah, & Sjam, R. (2012). Majalah Kedokteran FK UKI 2012 Vol XXVIII No.1 Januari - Maret Tinjauan Pustaka Kolonisasi. *Majalah Kedokteran FK UKI*, *XXVIII*(1), 39–47.

Mersil, S., & Lailiqonita, N. (2021). Does Smoking Cause Oral Candida Colonies Growth? *Moestopo International Review on Social, Humanities, and Sciences*, *1*(1), 1–6. https://doi.org/10.32509/mirshus.v1i1.4

Mori, Y., Tanaka, M., Kozai, H., Aoyama, Y., Shigeno, Y., Hotta, K., Aoike, M., Kawamura, H., Tsurudome, M., & Ito, M. (2023). Effects of Heat-Not-Burn Cigarette Smoking on the Secretion of Saliva and Its Innate Immune System Components. *Healthcare (Switzerland)*, *11*(1), 1–9. https://doi.org/10.3390/healthcare11010132

Mulyati, M., Nurdiani, C. U., & Safitri, W. (2019). Identifikasi Jamur Candida Sp .Pada Rongga Mulut Perokok Aktif Di Rw 09 Komplek Koperasi Curug Cimanggis Depok. *Anakes : Jurnal Ilmiah Analis Kesehatan*, *5*(1), 88–99. https://doi.org/10.37012/anakes.v5i1.335

Muzurović, S., Hukić, M., Babajić, E., & Smajić, R. (2013). The relationship between cigarette smoking and oral colonization with Candida species in healthy adult subjects. *Medicinski Glasnik : Official Publication of the Medical Association of Zenica-Doboj Canton, Bosnia and Herzegovina*, *10*(2), 397–399.

Nurdin, E. (2020). Comparison growth of Candida albicans in alternative media and semi-synthetic on fisheries cerumen in bastiong karance Ternate City. *International Journal of Applied Biology*, *4*(1), 115–124. https://doi.org/10.20956/ijab.v4i1.10333

Patil, S., Rao, R. S., Majumdar, B., & Anil, S. (2015). Clinical appearance of oral Candida infection and therapeutic strategies. *Frontiers in Microbiology*, *6*(DEC), 1–10. https://doi.org/10.3389/fmicb.2015.01391

Qurrohman, M. T., Sayekti, F., & Haryatmi, D. (2022). Combination of Corn, Cassava and Yellow Sweet Potato as Media Growth of Candida Albicans. *Indonesian Journal of Global Health Research*, *4*(3), 553.

Rezeki, S., & Rahmayanti, F. (2023). PERAWATAN KANDIDIASIS ORAL PADA PASIEN HIV/AIDS (Studi Pustaka). *Cakradonya Dental Journal*, *13*(1), 39–47. https://doi.org/10.24815/cdj.v13i1.20913

Saigal, S., Bhargava, A., Mehra, S., & Dakwala, F. (2011). Identification of Candida albicans by using different culture medias and its association in potentially malignant and malignant lesions. *Contemporary Clinical Dentistry*, *2*(3), 188. https://doi.org/10.4103/0976-237x.86454

Sever, E., Božac, E., Saltović, E., Simonić-Kocijan, S., Brumini, M., & Glažar, I. (2023). Impact of the Tobacco Heating System and Cigarette Smoking on the Oral Cavity: A Pilot Study. *Dentistry Journal*, *11*(11). https://doi.org/10.3390/dj11110251

Sophia, A., & Suraini. (2023). Analisa Jamur Candida albicans Pada Swab Mukosa Mulut Perokok Aktif di Lubuk Buaya. *Jurnal Biologi Makassar*, *8*, 31–38.

Thomas, A. M., Gleber-Netto, F. O., Fernandes, G. R., Amorim, M., Barbosa, L. F., Francisco, A. L. N., Guerra De Andrade, A., Setubal, J. C., Kowalski, L. P., Nunes, D. N., & Dias-Neto, E. (2014). Alcohol and tobacco consumption affects bacterial richness in oral cavity mucosa biofilms. *BMC Microbiology*, *14*(1), 1–12. https://doi.org/10.1186/s12866-014-0250-2

Udayalaxmi, J., & Shenoy, N. (2016). Comparison between biofilm production, phospholipase and haemolytic activity of different species of candida isolated from dental caries lesions in children. *Journal of Clinical and Diagnostic Research*, *10*(4), DC21–DC23. https://doi.org/10.7860/JCDR/2016/17019.7643

Wu, J., Peters, B. A., Dominianni, C., Zhang, Y., Pei, Z., Yang, L., Ma, Y., Purdue, M. P., Jacobs, E. J., Gapstur, S. M., Li, H., Alekseyenko, A. V., Hayes, R. B., & Ahn, J. (2016). Cigarette smoking and the oral microbiome in a large study of American adults. *ISME Journal*, *10*(10), 2435–2446. https://doi.org/10.1038/ismej.2016.37

Zięba, S., Błachnio-Zabielska, A., Maciejczyk, Pogodzińska, K., Szuta, M., Lo Giudice, G., Lo Giudice, R., & Zalewska, A. (2023a). Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products. *Medical Science Monitor*, *30*.

https://doi.org/10.12659/MSM.942507

Zięba, S., Błachnio-Zabielska, A., Maciejczyk, Pogodzińska, K., Szuta, M., Lo Giudice, G., Lo Giudice, R., & Zalewska, A. (2023b). Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products. *Medical Science Monitor*, *30*(November). https://doi.org/10.12659/MSM.942507.