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Oropharyngeal *Candida* species in individuals living with HIV in Semarang: Distribution and antifungal susceptibility patterns

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Abstract

Candidiasis is an infection caused by Candida species, which is the most common fungal infection. The prevalence of this infection increases with the rise in immunocompromised cases, such as HIV/AIDS. This study aims to determine microbiological data on Candida species infection and colonization, including types and susceptibility testing to fluconazole and voriconazole. This research is descriptive in nature. The study population consisted of people living with HIV who were screened by taking saliva samples, which showed the growth of Candida species. Identification of Candida species was performed using Gram staining, germ tube tests, and color identification in Hicrome Candida agar medium. Susceptibility testing was conducted using the Kirby-Bauer method for fluconazole and voriconazole. The diameter of the inhibition zone of Candida was measured and compared with CLSI (Clinical and Laboratory Standards Institute) standards. A total of 91 Candida species isolates were found. Candida albicans was the most frequently isolated species (75%), followed by Candida tropicalis (11%), Candida krusei (10%), and Candida glabrata (4%). The sensitivity of Candida albicans to fluconazole and voriconazole was 98.5%, while Candida tropicalis showed 100% sensitivity. Candida albicans remains the most prevalent isolate and is still sensitive to fluconazole and voriconazole.

Keywords: *Susceptibility testing; Candida sp; fluconazole; voriconazole*

INTRODUCTION

Candidiasis is an infection caused by *Candida* species, which is the most common fungal infection (Hellstein & Marek, 2019).The prevalence of this infection increases alongside the rise in immunocompromised cases, such as HIV-AIDS. Other risk factors for candidiasis include the use of broad-spectrum antibiotics, central venous catheters, blood transfusions, and total parenteral nutrition (Thomas-Rüddel et al., 2022), diabetes mellitus, hypertension, obesity, and care in intensive care units (Vinayagamoorthy et al., 2022).

The primary etiological agent of *Candida* infections is *Candida albicans*, which is a commensal flora in the mucosa and is often found in the oral cavity. This fungus can also be found in healthy individuals. Infections caused by *Candida albicans* are associated with immune status (Vila et al., 2020)**.** The adhesion of *Candida albicans* in the oral cavity is mediated by receptors. *Candida albicans* changes its morphology to an invasive form, known as pseudohyphae, after adhering to the host. The formation of pseudohyphae, aided by proteolytic enzymes, allows *Candida albicans* to spread (Chen et al., 2020; Lopes & Lionakis, 2022). An increase in infections caused by other *Candida* species, such as *Candida glabrata, Candida*

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krusei, *Candida tropicalis*, *Candida parapsilosis*, and *Candida auris*, has been reported in recent years(Lass-Flörl & Steixner, 2023; Vinayagamoorthy et al., 2022).

Oral candidiasis is diagnosed through anamnesis and physical examination of the oral cavity, including the mucosa, soft tissues, and teeth. Culture and sensitivity testing are essential to determine the species and susceptibility of each species to antifungal agents. Cultures are performed on Sabouraud dextrose agar. Oral hygiene and topical antifungals are usually sufficient for treating uncomplicated oral candidiasis (Hellstein & Marek, 2019). Nystatin and miconazole are the most commonly used topical antifungals. Both antifungals are highly effective but require prolonged use. Miconazole is more comfortable for patients but may interact with other medications. Other topical alternatives for oral candidiasis include amphotericin B or clotrimazole, although these topical medications may not always be available in the market. Oral fluconazole is effective in treating oral candidiasis that does not improve with topical treatment. Other systemic treatment alternatives include itraconazole, voriconazole, or posaconazole. Newer products include echinocandins (anidulafungin, caspofungin) and isavuconazole (Gómez-López, 2020).

One class of antifungal agents to be discussed is the azole group. Azole antifungals can be divided into two subclasses: imidazoles and triazoles. Imidazoles contain a heterocyclic ring with two nitrogen atoms, while triazole groups consist of three nitrogen atoms. Ketoconazole is the only imidazole that can be applied systemically. Fluconazole and itraconazole, along with the newer broad-spectrum antifungal voriconazole, posaconazole, and isavuconazole, are triazoles. The mechanism of action of azole antifungals inhibits 14-α-demethylase by binding to its heme group. This enzyme is necessary for the conversion of lanosterol to ergosterol. The lack of ergosterol in the fungal cell membrane and the accumulation of toxic precursors contribute to the fungistatic effect of azole drugs. Fluconazole is a triazole that consists of a phenyl ring substituted with two fluorine atoms at positions 2 and 4 and two azole rings. Unlike other azoles, this azole has a high solubility in water. Various *Candida* species are susceptible to fluconazole (Bellmann & Smuszkiewicz, 2017). *Candida albicans* remains the most common species causing infections, and fluconazole plays a crucial role in antifungal prophylaxis. Another azole group is voriconazole, which has a chemical structure similar to fluconazole but a broader antifungal spectrum (Bellmann & Smuszkiewicz, 2017). Studies have shown that fluconazole and voriconazole are effective against *Candida albicans*(Partha et al., 2022). Other studies indicate that fluconazole and voriconazole demonstrate similar effectiveness against *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* in post-radiotherapy patients suffering from candidiasis (Golestannejad et al., 2022; Jørgensen *et al*., 2014). Some resistance to antifungal agents has been reported in various studies. Resistance to antifungal agents leads to increased morbidity and mortality among patients(Khanina et al., 2021; Trevijano-Contador et al., 2022; Vinayagamoorthy et al., 2022). This study aims to determine microbiological data on *Candida* species infections and colonization, including types and their sensitivity to fluconazole and voriconazole.

METHOD

This is a descriptive study conducted at the Microbiology Laboratory of Sultan Agung Islamic Hospital in Semarang. The study population consisted of people living with HIV who were screened by taking saliva samples that showed the growth of *Candida* species. Saliva was collected in sterile containers, then centrifuged and cultured on Sabouraud Dextrose agar. *Candida* was identified using Gram staining, germ tube tests, and color identification in Hicrome *Candida* agar. Identification of *Candida* species on Hicrome agar was performed according to the identification manual provided by the manufacturer. *Candida albicans* appears light green, *Candida glabrata* appears cream to white, *Candida krusei* appears pink-purple, and

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Candida tropicalis appears blue. Colonies were then subjected to susceptibility testing using the Kirby-Bauer method against fluconazole and voriconazole. The medium used for susceptibility testing was Mueller-Hinton Agar supplemented with Dextrose and Methylene Blue. The diameter of the inhibition zone of *Candida* was measured using calipers and then matched with CLSI standards to determine whether it was resistant, intermediate, or sensitive. *Candida albicans* and *Candida tropicalis* are considered sensitive to fluconazole with an inhibition zone diameter of ≥ 17 mm, and resistant with ≤ 13 mm. *Candida krusei* and *Candida glabrata* were not tested for fluconazole susceptibility due to their intrinsic resistance to fluconazole. *Candida albicans* and *Candida tropicalis* are considered sensitive to voriconazole with an inhibition zone diameter of ≥ 17 mm, and resistant with ≤ 14 mm. *Candida krusei* is sensitive to voriconazole with an inhibition zone diameter of ≥ 15 mm, and resistant with ≤ 12 mm. *Candida glabrata* was not tested for voriconazole susceptibility due to its intrinsic resistance.

RESULTS AND DISCUSSION

This study aimed to collect epidemiological data on the colonization of Candida species in the oral cavities of HIV-infected patients. The analysis of 91 positive samples showed growth on Sabouraud Dextrose Agar, followed by Gram staining, germ tube tests, and identification on Hicrome Agar *Candida*.

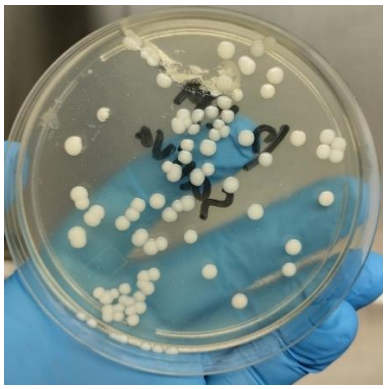

Figure 1. Colonies of *Candida* species on Sabouraud Dextrose Agar

The colonies of *Candida* species on Sabouraud Dextrose Agar appeared white and round. These colonies were incubated under aerobic conditions at a temperature of 37°C. The resulting colonies were subsequently subjected to Gram staining to assess their microscopic morphology and staining characteristics. Additionally, a germ tube test was performed by suspending a colony in 0.5 ml of serum, followed by incubation under aerobic conditions at 37°C for 2 hours. This test was conducted to determine whether *Candida* species produced buds, which is crucial for differentiating *Candida albicans* from *Candida non-albicans* species. The results of the incubation to observe bud formation are illustrated in Figure 2.

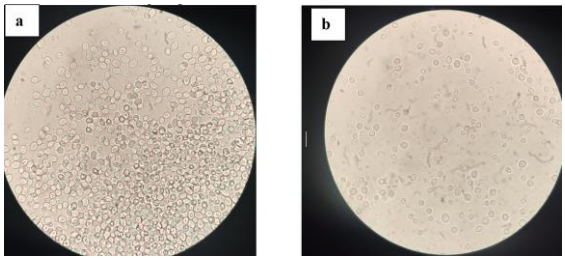

Figure 2. *Germ tube* negative (a) and *germ tube* positive (b)

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Gram staining of the saliva samples was conducted to examine the morphology of the yeast. Some samples also showed growth of *Staphylococcus* species. Sabouraud Dextrose Agar was supplemented with chloramphenicol, and the pH was adjusted to an acidic level to prevent bacterial growth. Gram staining was performed to eliminate colonies that were contaminants in the medium.

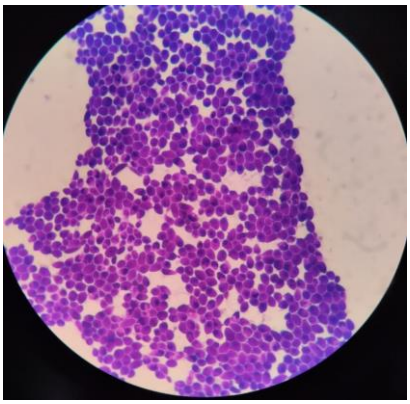

Figure 3. The colonies of *Candida* species in Gram staining

The yeast morphology in Gram staining appeared round or oval, larger than bacteria. The Gram staining of *Candida* species was Gram-positive.

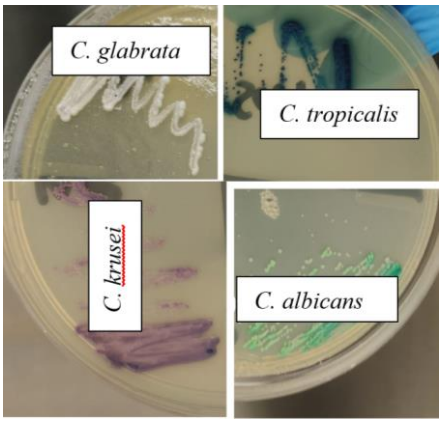

Figure 4. Identification of *Candida* sp using Hicrome Agar

After performing Gram staining and the germ tube test, the *Candida* colonies were subsequently cultured on Hicrome Agar. *Candida albicans* exhibited a green coloration, *Candida tropicalis* appeared blue, *Candida glabrata* was cream-white, and *Candida krusei* displayed a pink-purple. *Candida albicans* was the most frequently isolated species (75%), followed by *Candida tropicalis* (11%), *Candida krusei* (10%), and *Candida glabrata* (4%).

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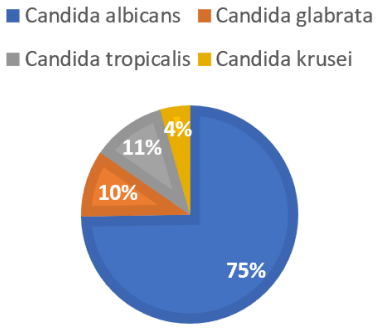

Figure 5. Distribution of *Candida* sp among immunocompromised patients

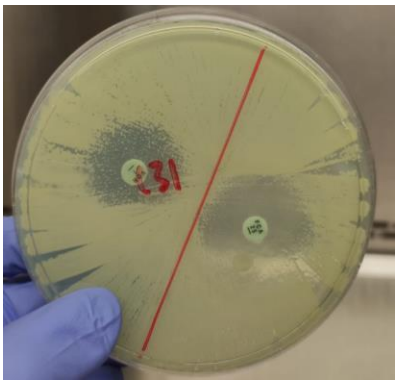

Figure 6. Susceptibility Test of *Candida* sp

Antifungal susceptibility test performed by disk diffusion method revealed the sensitivity of *Candida albicans* to fluconazole and voriconazole was 98.5%, while *Candida tropicalis* showed 100% sensitivity

Tabel 1. Susceptibility of Candida species to antifungal drugs

Candida spp	n	% Sensitivity Fluconazole	% Sensitivity Voriconazole
Candida albicans	68	98,5	98,5
Candida tropicalis	10	100	100
Candida krusei	4	IR	100
Candida glabrata	9	IR	IR

IR: Intrinsic Resistance

Oropharyngeal candidiasis (OPC) is an opportunistic fungal infection that affects the oral mucosa. *Candida albicans* is the most common causative agent of OPC. Non-albicans Candida species have also been reported to be responsible for OPC (Aboualigalehdari et al., 2020). Similar to this study, *Candida albicans* is the most isolated in HIV patients. *Candida albicans* is a ubiquitous commensal yeast in healthy individuals and a significant opportunistic pathogen in immunocompromised patients (Erfaninejad et al., 2024). However, the importance of non-albicans Candida species (e.g, *C.glabrata, C.tropicalis, C.krusei, C.dubliniensis, C.parapsilosis, C. guilliermondii, and C.kefyr*) is increasing over time (Keyvanfar et al., 2024). Among people living with HIV/AIDS, oral pharyngeal candidiasis (OPC) is one of the most common indicators

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of HIV infection (Erfaninejad *et al.*, 2024). The first-line treatment for OPC in HIV patients is fluconazole. Overall, the resistance pattern to antifungal agents in HIV/AIDS individuals undergoing OPC is changing, leading to increasingly serious medical concerns. Recurrent infections necessitate the extensive consumption of antifungal agents by those living with HIV/AIDS. Our findings revealed that Candida isolates were sensitive to azoles, ranging from 98,5 to 100%. This result is different from previous research, which found the prevalence of azole resistance in Candida isolates: fluconazole 56,7%, voriconazole 43%. The high prevalence of azole resistance may be attributed to cross-resistance to fluconazole, which is routinely administered to HIV-positive patients with clinical manifestations of OPC without testing for antifungal sensitivity. Thus, the increased proportion of resistant Candida spp may be caused by prolonged azole administration (Keyvanfar et al., 2024).

CONCLUSION

Candida albicans is the most frequently isolated species found in this study, followed by *Candida tropicalis*, *Candida krusei*, and *Candida glabrata*. The sensitivity of *Candida albicans* remains intact against fluconazole and voriconazole, although resistant strains of Candida albicans have been identified against these antifungal agents. Candida non-albicans species remain sensitive to fluconazole and voriconazole, except for *Candida glabrata* and *Candida krusei* due to their intrinsic resistance.

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