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Species Distribution and Antifungal Susceptibility of *Candida* spp. responsible for Pulmonary Candidiasis

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Abstract. Fungal infection can occur in almost all parts of the human body, including the respiratory system. One group of fungi causing opportunistic infections in the lungs also known as pulmonary candidiasis is *Candida* spp. This study aimed to determine species diversity and antifungal sensitivity of *Candida* sp. causing pulmonary candidiasis from sputum specimen isolates in the Zainoel Abidin Hospital (ZAH). The sputum specimens were from inpatients and outpatients during a period of January 2019 to January 2021. Identification of *Candida* spp. and the antifungal sensitivity were carried out using culture and Gram Staining as well as the *VITEK® 2 Compact*. The results of this study indicated that there were six species of *Candida* sp. obtained from the study period. Of total 73 isolates, the highest percentage of species was *Candida albicans* (56.16%). The percentage of pulmonary candidiasis based on age was dominated by the early-late age category 46-65 years (50.68%) and based on sex was dominated by men (71.23%). Antifungal susceptibility assays revealed that the *Candida* species remained highly susceptible to the antifungals (amphotericin B, caspofungin, flucytosine, fluconazole, micafungin, and voriconazole), i.e., greater than 91 percent. Overall, the results of this study indicated that pulmonary candidiasis were predominantly related to *C. albicans* and C. tropicalis infections in ZAH and the sensitivity of antifungal drugs remained empirically and definitively effective. This research might be important as a part of infection prevention and control strategies, as well as the administration of empirical antifungals to combat *Candida*-mediated lung infections.

Key words: antifungal, antifungal susceptibility, *Candida* spp., pulmonary candidiasis, sputum specimen

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INTRODUCTION

Fungal infections remain a major problem due to their significantly increasing rate of occurrences ranging from superficial topical infections to the serious systemic infections, including pulmonary infections. One of the main causative agents for fungal mediated-pulmonary infection is *Candida* spp., a group of opportunistic fungal species causing pulmonary candidiasis predominantly affecting individuals who have disrupted immune status (Pendleton et al., 2018). *Candida* sp. normally exists as microbiota especially in the digestive tracts, vagina, urethra, oral cavity, mucous membranes, and skin. *Candida* sp. consists of more than 150 species with *Candida albicans* as the most predominant species causing both acute and sub-acute fungal pneumonia-like infections in humans (Barkauskas & Perfect, 2009). In the last two decades, pulmonary fungal infections tend to increase rapidly in line with the increasing number of patients with immunosuppressive conditions due to HIV and tuberculosis (Yahaya et al., 2014).

Long-term antibiotic and long-term steroid administration in patients with suppressive immune systems disrupting the balance of normal flora in the airways are believed to trigger the growth of *Candida* sp. (Fajri et al., 2018). A variety of antifungal medications are commonly used to treat Candidal infections including polyene, imidazole, triazole, and echinocandins in oropharyngeal candidiasis. The occurrence of antifungal resistance in *Candida* sp. resulting in limited antifungal options for the fungal pathogens. Hence, information of the causative species and their sensitivities are essential as a basis for selecting the appropriate antifungal for the patients. Therefore, the objective of the study was to determine species diversity and antifungal sensitivity of *Candida* spp. isolates causing pulmonary candidiasis from sputum specimens in the Zainoel Abidin Hospital (ZAH), Banda Aceh Indonesia. This is the first study to look into the spread of *Candida* spp. that cause pulmonary candidiasis in the ZAH, as well as their antifungal sensitivity. This research is essential for determining the benefits of infection prevention and control measures, as well as the provision of empirical antifungals to treat Candida-related pulmonary infections in hospital and community settings.

METHODS

Fungal sample collection and observation

The fungal pathogens were isolated from the sputum of inpatients and outpatients of the ZAH Banda Aceh, Indonesia during a period of January 2019 to January 2021. A volume of 3-5 mL sputum sample was checked for its quality with macroscopic appearances that was evenly purulent white-yellow-greenish thick. The samples were subjected to direct microscopic observation with Gram staining.

Candidal culture and antifungal susceptibility

The clinical samples were also inoculated to plates containing Sabouraud dextrose agar (Merck, Germany), blood agar (Merck, Germany), MacConkey agar (Merck, Germany), and chocolate blood agar (Merck, Germany). The plates were then incubated for 24 hours at 37 ºC before they were identified morphologically through macroscopic and microscopic observation. Moreover, additional identification as well as antifungal sensitivity profiles were performed using VITEK® 2 Compact (Biomeriux, Lyon, France). A pure *Candida* colony recovered from the clinical samples was suspended in NaCl 0.45% equivalent to 1.8-2.2 McFarland Standard solution before the suspension was inoculated into cassettes of YST (yeast) and AST (antimicrobial susceptibility testing) for identification and anti-fungal susceptibility, respectively following the manufacturer's instruction.

Data analysis and ethical clearance

The species distribution of *Candida* sp. was analyzed based on types of clinical specimens, gender and age. The research has been approved for ethical clearance from the Ethical Clearance Committee for Health Research, Faculty of Medicine, Universitas Syiah Kuala with registration number 326/EA/FK-RSUDZA/2020.

RESULTS AND DISCUSSION

During a period of January 2019 to January 2021, there were 73 isolates of *Candida* spp. obtained from a total of 1.707 clinical samples, i.e., sputum and endotracheal tube (ETT) mucus suspected for pulmonary infections (Table 1). Table 1 summarizes *Candida* spp. that remain to be the main fungal etiological agents causing pulmonary candidal infections. *Candida* spp. cause pulmonary infections four times higher than other fungal pathogens causing the same infections. However, bacteria are still considered the major pathogens for pulmonary infections in this study.

Table 1. Prevalence of pulmonary candidiasis during a period of January 2019 to January 2021 at Zainoel Abidin Hospital Aceh Indonesia based on clinical specimens and etiological agents

Types of Clinical Specimens	Etiological Agents				Total
	Nonpathogenic bacteria	Bacteria	Other Fungi	*Candida* spp.	
Sputum	539 (40.16%)	735 (54.77%)	12 (0.89%)	56 (4.17%)	1,342 (100%)
Endotracheal Tube Mucus	50 (13.70%)	290 (79.45%)	8 (2.19%)	17 (4.66%)	365 (100%)
Total	589 (34.50%)	1,025 (60.05%)	20 (1.17%)	73 (4.28%)	1,707 (100%)

The isolates of *Candida* spp. were able to grow on the blood, chocolate, and Sabouraud dextrose agar but unable to grow on MacConkey agar as the initial characteristics of yeast cells. The isolates on the agar showed morphological features of small, round, raised, and creamy colonies (Figure 1a). Based on microscopic observation, the yeast had positive gram staining with rice-like shaped cells in both direct microscopic observation from sputum samples as well from pure colonies grown on the agar media (Figure 1c).

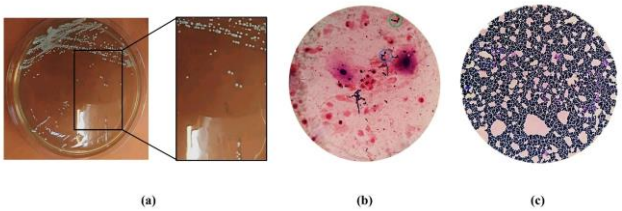

Figure 1. The morphological features of *Candida* sp. recovered from sputum specimens at the Zainoel Abidin hospital in Banda Aceh, Indonesia. (a) colonies grown on Sabouraud dextrose agar after incubation at 37°C for 24 hr; (b) positive Gram staining from direct observation of sputum with 1,000X magnification exhibiting Candidal cells with pseudohyphae (blue circle) and budding (green circle); (c) positive Gram staining from pure culture of *Candida* spp with 1,000X magnification.

In addition to residing as human microflora, *Candida* sp. is able to switch from a harmless commensal to pathogens by expressing a set of virulence factors leading to candidiasis depending on the host immune status (Calderone & Clancy, 2011). The switching might be indicated by changing the formation from unicellular yeast cells to filamentous hyphal form (Singh et al., 2015). In this research, the occurrence of Candida-related pulmonary infections was observed by both culturing and direct microscopy approaches. The typical morphological characteristics of *Candida* spp. isolates with small round creamy colonies and appeared as dark blue after Gram-staining were shown in the current research (Byadarahally Raju & Rajappa, 2011). Also, the pseudohyphae form of the *Candida* sp. was found during the study whose structure is commonly formed at specific environmental fungal conditions subsequently affecting a delay in both cell-cycle progression and extension of the apical growth period of the fungi (Bravo Ruiz et al., 2020).

There were six species of a total 73 *Candida* spp. isolates identified in the present study, namely *C. albicans, C. tropicalis, C. famata, C. lusitaniae, C. ciferrii,* and *C. dubliniensis* (Table 2). *C. albicans* and *Candida tropicalis* were the two most predominant fungal species causing pulmonary candidiasis approaching 57% and 32%, respectively. Interestingly, all candidal species were detected and identified in sputum samples, whereas there were only three candidal species found in the ETT samples.

Table 2. Distribution of *Candida* spp. causing pulmonary candidiasis based on clinical specimens during a period of January 2019 to January 2021 at Zainoel Abidin Hospital Aceh Indonesia

Types of Clinical Specimens	Candidal Species						Total
	C. tropicalis	*C. albicans*	*C. lusitaniae*	*C. famata*	*C. ciferrii*	*C. dubliniensis*	
Sputum	13 (23.21%)	35 (62.50%)	1 (1.79%)	4 (7.14%)	2 (3.57%)	1 (1.79%)	56 (76.71%)
ETT	10 (58.82%)	6 (35.29%)	1 (5.88%)	-	-	-	17 (23.29%)
Total	23 (31.51%)	41(56.16%)	2 (2.74%)	4 (5.48%)	2 (2.74%)	1 (1.37%)	73 (100%)

Among microbial pathogens, *Candida* is the least common of causative agents for pulmonary infections in the current study. This aligns with previous study indicating that the most prevalent pathogens causing pulmonary infections, such as pneumonia, was dominated by Gram-negative bacteria (79.5%) whereas *Candida* spp. contributed to 1.7% of 117 clinical specimens (Widyaningsih & Buntaran, 2016). Interestingly, in the present study, there were a number of non-pathogenic bacteria detected in the sputum and ETT samples. These non-pathogenic bacteria are associated with the normal flora which are commonly occurred during sample collections.

In terms of species distribution, *Candida albicans* followed by oleh *Candida tropicalis* were two most predominant Candida species causing pulmonary infections in the present study. Previous investigations found that these two candida species are the major pathogens causing candidiasis in general (Suhartono et al., 2020) as well as pulmonary candidiasis including tuberculosis in particular (Hadadi-Fishani et al., 2020). High prevalence of *C. albicans* causing pulmonary infections might be associated with their adaptability by means of phenotypic switching and attachment ability to the host cells during invasion. Candidal attachment which is the most crucial step of the fungal infections is facilitated by adhesins allowing the fungal pathogens adhere to the host cells (Martin et al., 2021).

Table 3 Summarizes that pulmonary candidiasis prevalently occurs in the elderly men (46-65 years). Additionally, males were twice more prevalent to suffer pulmonary candidiasis than females accounting for 52 (71.23%) and 21(28.77%) isolates, respectively.

Table 3. Distribution of pulmonary candidiasis based on age and gender during a period of January 2019 to January 2021 at Zainoel Abidin Hospital Aceh Indonesia

Age Groups (years)	Sex (%)		Total (%)
	Males	Females	
0-5	2 (2.74%)	-	2 (2.74%)
6-11	1 (1.37%)	-	1 (1.37%)
12-25	4 (5.48%)	3 (4.11%)	7 (9.59%)
26-45	6 (8.22%)	5 (6.85%)	11 (15.07%)
46-65	27 (36.99%)	10 (13.70%)	37 (50.68%)
>65	12 (16.44%)	3 (4.11%)	15 (20.55%)
Total	52 (71.23%)	21(28.77)	73 (100%)

In the current study, elderly patients (46-65 years or older) and men are prone to suffer from pulmonary candidiasis. Aging is presumably correlated with declining lung physiology and immune status along with other complicated diseases including suffering from comorbidities such as diabetes mellitus, chronic pulmonary diseases, and cardiovascular diseases as well as multiple organ failure (Terraneo et al., 2016). The most common chronic pulmonary disease in geriatric patients is pneumonia due to decreasing lung compliance as well as immunological status changes along with disorders on temperature regulatory response and various cardiopulmonary as consequences of aging process (Putri & Hasan, 2014). It is believed that there is a progressive decline of the total lymphocyte and absolute number of T and B cells among age groups from infants to adults (Valiathan et al., 2016).

Regarding to the gender, men are prone to suffer pulmonary candidiasis owing to risk factors such as cigarette smoking habits and more air polluted exposure than women. Heavy smokers and outdoor activities tend to cause irritation of their respiratory tract leading to mucus secretion facilitating the growth of fungi including *Candida* spp. as well as increase the risk of developing lung disease and cause bronchitis and pneumonia (Elfidasari et al., 2013). Additionally, exposure to smoke and air-polluted environment might reduce personal immune status (Soysa & Ellepola, 2005) in addition to enhancing the candidal virulence factors by secreting histolytic enzymes and adhesion (Baboni et al., 2009).

In terms of antimicrobial susceptibility, the assay test showed that the sensitivity of the fungal pathogens across the *Candida* species against the antifungals (amphotericin B, caspofungin, flucytosine, fluconazole, micafungin, and voriconazole) was still high, i.e., more than 91%, meaning that the antifungals were still effective to treat almost all *Candida* spp. causing pulmonary infections in this study (Table 4). One species, however, i.e., *Candida ciferrii*, exhibited only 50% susceptibility against amphotericin B and voriconazole.

Table 4. Antifungal sensitivity (%) of *Candida* spp. responsible for pulmonary candidiasis during a period of January 2019 to January 2021 at Zainoel Abidin Hospital Aceh Indonesia

Species	(n)	Percentage of Antifungals Sensitivity					
		ApB	CAS	FCT	FLU	MCF	VRC
Candida albicans	41	95	98	98	95	98	98
Candida tropicalis	23	96	100	100	91	100	100
Candida famata	4	100	100	100	100	100	100
Candida lusitaniae	2	100	100	100	100	100	100
Candida ciferrii	2	50	100	100	100	100	50
Candida dubliniensis	1	100	100	100	100	100	100

Note: ApB (Amphotericin B), CAS (Caspofungin), FCT (Flucytosine), FLU (Fluconazole), MCF (Micafungin), and VRC (Voriconazole)

In the antifungal susceptibility tests, the sensitivity percentage of *Candida* spp. responsible for pulmonary candidiasis in the Zainoel Abidin Hospital in Aceh, Indonesia generally remained high indicating that the fungal pathogens are still susceptible to the current antifungals, i.e. amphotericin B, caspofungin, flucytosine, fluconazole, micafungin, and voriconazole. This finding is corroborated with previous study demonstrating that *Candida* spp. was still susceptible to fluconazole (Reza et al., 2017) or amphotericin B (Chiu et al., 2006). In the ZAH, fluconazole is still the primary prescribed drug to treat pulmonary candidiasis. One species (*Candida ciferrii*), however, exhibited 50% susceptibility against amphotericin B

and voriconazole. This finding aligns with previous research signifying the antifungal resistance of *C. ciferrii* to first-line drugs including amphotericin B and fluconazole (Agın et al., 2011). Although most of the candidal pathogens found in the present study remained susceptible, it is essential to monitor and evaluate the efficacy of antifungal administration for candidal infections regularly since other research reported the increasing trend of resistance of the fungal pathogen due to biofilm-mediated infection (Sahal & Bilkay, 2018) and the expression of various virulent factors (Zuza-Alves et al., 2017, Silva et al., 2012).

CONCLUSION

Overall, six species of a total 73 *Candida* spp. isolates related to pulmonary candidiasis were identified in the present study, namely *C. albicans, C. tropicalis, C. famata, C. lusitaniae, C. ciferrii,* and *C. dubliniensis* with the first-two fungal species were most predominantly detected. Additionally, this study indicates also that pulmonary candidiasis in the Zainoel Abidin Hospital (ZAH) is still rare and the sensitivity of antifungal drugs remained empirically and definitively effective.

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