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Etiology and Antifungal Sensitivity Test in Otomycosis Caused by *Candida* Sp.

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Abstract

Otomycosis is a common fungal infection of the external auditory meatus frequently diagnosed in otolaryngology outpatient clinics. Resistance to antifungals is currently a significant concern, with intrinsic and acquired resistance increasing among isolates that cause fungal infections. The purpose of this research was to identify *Candida* species causing otomycosis and determine the pattern of antifungal susceptibility among these *Candida* species. A prospective study was conducted in the Margono Soekarjo General Hospital and Department of Microbiology, Faculty of Medicine, Universitas Jenderal Soedirman Purwokerto, Indonesia, from April–September 2022. Forty-seven (47) clinical samples of otomycosis were collected from 41 patients and then isolated bedside on fungal culture media and was prepared on an object glass for direct microscopic examination of the specimens. Fungal identification was performed using 10% potassium hydroxide (KOH) to observe fungal elements. Samples were cultured on Saboraud dextrose agar (SDA) media with chloramphenicol and Czapek dox agar. The Germ Tube Test was used to identify *Candida* while yeast-specific identification and antifungal susceptibility assay using a rapid commercial kit was applied for specific identification of the fungus. Antifungal susceptibility patterns were obtained using the Integral System Yeast Plus (ISYP) media pack. *Candida* parapsilosis was the most prevalent *Candida* species discovered in this study, accounting for approximately 41.66%, which was followed by *Candida tropicalis* (25%) and *Candida krusei* (12.5%), whereas Candida albicans only accounted for 4.1% of the specimens. All Candida species were sensitive to flucitosine and ketoconazole, whereas the voriconazole sensitivity rate reached 96%. This study concludes that *Candida parapsilosis* is the most prevalent species of *Candida* in otomycosis, and that all Candida species are sensitive to Flucitosine, ketoconazole, and voriconazole.

Keywords: Antifungal sensitivity test, *candida*, otomycosis

Introduction

Otomycosis is a common infection of the external auditory meatus caused by a fungus that is frequently diagnosed in otolaryngology outpatient clinics. This disease is commonly encountered in nations with tropical and subtropical climates, with air humidity between 70 and 80 percent and air temperature between 15 and 30 degrees Celsius. The worldwide prevalence of Otitis Externa with otorrhea is roughly 9 to 30%.[1,2]

Warm and humid weather, external bacterial otitis, swimming, close or continuous contact with water; eczema, seborrhoeic dermatitis of the outer ear; external ear injury due to a foreign object; wearing headphones, anatomical disorders, diabetes mellitus, and immune deficiency are risk factors for otomycosis.[3]

Multiple types of fungi can cause otomycosis. *Aspergillus niger* and *Candida albicans* are the two fungi most associated with otomycosis.[4,5] Patients with otomycosis typically complain of itching, otalgia, a feeling of fullness in the ear, tinnitus, hearing loss, a sensation of congestion, and ear discharge.[6] Although otomycosis is not life-threatening, it can be frustrating for patients and physicians due to its long-term treatment requirements and high recurrence rate.[7]

Resistance to antifungals is currently a significant concern, with intrinsic and acquired resistance increasing among isolates causing

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fungal infections. Multiple reports of antifungal resistance among isolates from patients with fungal otitis.[8] Indonesia has a tropical climate, which may influence the incidence of otomycosis. However, a literature search reveals little about otomycosis regarding the type of fungus and antifungal sensitivity to the otomycosis-causing fungus. This study aims to identify Candida species and determine the pattern of antifungal susceptibility among Candida species that cause otomycosis.

Methods

A cross-sectional study was conducted in the Otorhinolaryngology Clinic at Margono Soekarjo Hospital and Department of Microbiology Faculty of Medicine Universitas Jenderal Soedirman Purwokerto, Indonesia, from April to September 2022. The study was approved by the Health Research Ethics Committee at the Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto (018/KEPK/PE/II/2022). The inclusion criteria for this study were patients with a clinical diagnosis of otomycosis, a minimum age of 17 years old, and written informed consent to participate. This study excluded patients with severe otitis externa and those who were uncooperative. Data were then tabulated for analyses using SPSS version 24.0 for the Windows program.

Clinical samples were collected by swabbing ear discharge, which ranged in color from white to black, using a sterile flexible mini-flocked swab (Copan, Italy). The sample was then isolated bedside on fungal culture media and in object glass for direct microscopic examination of specimens, then transferred to the Microbiology Laboratory Faculty of Medicine Universitas Jenderal Soedirman. Fungal identification was carried out using 10% KOH to observe fungal elements, including hyphae, spores, budding yeast, and yeast with pseudohyphae.

The samples were grown in an aerobic environment for four weeks on SDA media supplemented with chloramphenicol (Himedia, Mumbai, India) and Czapek dox agar (Himedia). Cultures are checked every 3-5 days. Fungal cultures must be incubated for at least 30 days before being judged negatively. Identification was carried out based on colony morphology and microscopy with Lactophenol cotton blue (LPCB). Candida was identified by a germ tube test using colonies inserted in serum, incubating for 1-2 hours at 37°C, and observing the presence of germination without restriction in *Candida albicans.*

Yeast-specific identification and antifungal susceptibility assay using rapid commercial kit Specific identification and four antifungal susceptibilities patterns were obtained using Integral System Yeast Plus (ISYP) commercial equipment (Liofilchem, Italy), according to the manufacturer's instructions. Briefly, Candida conidial suspension was prepared from 24h new culture on Sabouraud dextrose agar (Merck, Germany) at 35°C. The cell density was adjusted to 0.5 McFarland standard and inoculated to 12 microwells containing dried sugar. Thirteen wells had chromogenic substrate; then, the suspension was subjected to thedilute and released into subjected antifungal wells. The following antifungal concentrations were utilized at the end of the experiment: Nystatin (1.25 g/mL), Amphotericin B (2.0 g/mL); 5-Fluorocytosine (16.0 g/mL); Econazole (2.0 g/mL), Ketoconazole (0.5 g/mL), Clotrimazole (1.0 g/mL), Miconazole (2.0 At the time, the 24th well was used as a gauge. The wells, except well thirteen, were covered with a drop of Vaseline oil. The microplates were incubated at 36±1 °C for 48 hours, and the color shift was read. Sugar assimilation patterns were analyzed, a code was generated, and the corresponding species was located using this method. The *Candida albicans* ATCC 24433, *C. glabrata* CBS 138, and *C. krusei* ATCC 6258 were quality controls.

Results

This study collected 47 samples of otomycosis from 41 patients, of whom 6 (12.76%) had a bilateral infection. Patients with male sex were more common among the 47 samples (51.1% versus 48.9% for females). The age ranges 36 to 40 years and 56 to 60 years account for 14.9% of the population. This study also determined that the right ear is more susceptible to otomycosis than the left (59.6%: 40.4%), according to Table 1.

In the present study, a total of 47 specimens were obtained from 41 patients diagnosed with otomycosis, comprising 35 cases of unilateral and 6 cases of bilateral involvement. Among the 56 isolates cultured, 38 were identified as single infections, while 18 exhibited mixed infections.

Table 2 contains descriptions pertaining to the number and type of candida species. *Candida parapsilosis* was the most prevalent Candida species discovered in this study, accounting for

Table 1 Characteristics of Patients with Otomycosis

Characteristics	Number	Percentage
Sex		
Men	24	51.1
Women	23	48.9
Age (in years)		
16–20	5	10.6
21–25	1	2.1
26–30	9	19.1
31–35	5	10.6
36–40	7	14.9
41–45	2	4.3
46–50	5	10.6
51–55	2	4.3
56–60	7	14.9
61–65	2	4.3
65–70	1	2.1
71–75	1	2.1
Site of ear		
Right ear	28	59.6
Left ear	19	40.4
Unilateral	35	74.3
Bilateral	6	12.7

approximately 41,66%, followed by *Candida tropicalis* at 25% and *Candida krusei* at 12.5%, whereas *Candida albicans* accounted for only 4.1%. (Table 2).

Table 2 Species of Candida in Patients with Otomycosis

Species	Number (n=56)	Percentage
Aspergillus spp	32	53.1
Candida spp	24	42.9
Candida parapsilosis	10	10/24 (41.66)
Candida tropicalis	6	6/24 (25.00)
Candida krusei	3	3/24 (12.5)
Candida kefyr	2	2/24 (8.33)
Candida glabrata	1	1/24 (4.1)
Candida utilis	1	1/24 (4.1)
Candida albicans	1	1/24 (4.1)

Table 3 Antifungal Susceptibility Among Candida Strains

Species	Antifungal Agent	Percentage of Sensitivity
Candida spp.	Nyst 1.25 µg/mL	42
	Ampho 2 µg/mL	38
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	25
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	33
	Micon 2 µg/mL	29
	Itra 1 µg/mL	54
	Vori 2 µg/mL	96
	Fluco 64 µg/mL	79
Candida parapsilosis	Nyst 1.25 µg/mL	10
	Ampho 2 µg/mL	30
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	0
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	0
	Micon 2 µg/mL	0
	Itra 1 µg/mL	50
	Vori 2 µg/mL	90
	Fluco 64 µg/mL	90
Candida tropicalis	Nyst 1.25 µg/mL	67
	Ampho 2 µg/mL	17
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	33
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	33
	Micon 2 µg/mL	33
	Itra 1 µg/mL	33
	Vori 2 µg/mL	100
	Fluco 64 µg/mL	33
Candida krusei	Nyst 1.25 µg/mL	67
	Ampho 2 µg/mL	67
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	67

Table 3 (continued)

	Candida Strains	Percentage of Sensitivity
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	67
	Micon 2 µg/mL	67
	Itra 1 µg/mL	67
	Vori 2 µg/mL	100
	Fluco 64 µg/mL	100
Candida kefyr	Nyst 1.25 µg/mL	50
	Ampho 2 µg/mL	50
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	50
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	100
	Micon 2 µg/mL	50
	Itra 1 µg/mL	100
	Vori 2 µg/mL	100
	Fluco 64 µg/mL	100
Candida albicans	Nyst 1.25 µg/mL	100
	Ampho 2 µg/mL	100
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	0
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	100
	Micon 2 µg/mL	100
	Itra 1 µg/mL	100
	Vori 2 µg/mL	100
	Fluco 64 µg/mL	100
Candida glabrata	Nyst 1.25 µg/mL	100
	Ampho 2 µg/mL	100
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	100
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	100
	Micon 2 µg/mL	100
	Itra 1 µg/mL	100
	Vori 2 µg/mL	100
	Fluco 64 µg/mL	100

Table 3 Antifungal Susceptibility Among Candida Strains

Species	Antifungal Agent	Percentage of Sensitivity
Candida utilis	Nyst 1.25 µg/mL	0
	Ampho 2 µg/mL	0
	Flucy 16 µg/mL	100
	Econ 2 µg/mL	0
	Keto 0.5 µg/mL	100
	Clotri 1 µg/mL	0
	Micon 2 µg/mL	0
	Itra 1 µg/mL	0
	Vori 2 µg/mL	100
	Fluco 64 µg/mL	100

*Spp: Specieses; Nyst: Nystatine; Ampho: Ampotericine B; Flucy: Flucytosine; Econ: Econazole; Keto: Ketoconazole; Clotri: Clotrimazole; Micon: Miconazole; Itra: Itraconazole; Vori: Voriconazole; Fluco: Fluconazole

Table 3 lists the in vitro susceptibility profile of 24 *Candida spp.* Isolates against ten antifungal agents, respectively. In the context of this study, it has been observed that Candida spp. displays complete sensitivity to two antifungal agents, specifically Flucytosine and Ketoconazole. Ten strains of Candida parapsilosis demonstrated resistance to Econazole, Clotrimazole, and Miconazole, while displaying elevated sensitivity towards Ketoconazole and Flucytosine. Conversely, Candida tropicalis exhibited notable sensitivity to Ketoconazole, Flucytosine, and Voriconazole.

Discussion

Otomycosis is prevalent in regions with high heat and humidity levels. The current samples were collected in the city of Purwokerto in the province of Central Java, where the climate is typically warm and humid.

The incidence of otomycosis varies geographically and is primarily influenced by environmental conditions. This pathology may account for up to 25% of infectious otitis in some tropical and subtropical regions, where it can reach high prevalence rates.[9] All ages are susceptible, although we have observed a disproportionate number of cases in patients aged 26 to 30. Distribution by gender is meaningless. In contrast to other series, there

was a slight preponderance of men in ours (51.1%; 49.9%). Some authors report a higher rate of male involvement. Saki et al.[3] and Navaneethan et al.[10] said that females are more susceptible to otomycosis than males.

Otomycosis is typically a unilateral disease, with bilateral involvement being infrequent. Compared to the left side, the right side was more prevalent in our study (59.6% vs. 40.4%). Similar to the study by Aremu et al.[11] and Pandey et al.[12], bilateral involvement was observed in only 12.7 percent of cases. Most people are right-handed and tend to self-clean their right ear more frequently with unsterile objects.

Candida parapsilosis, Candida tropicalis, Candida krusei, Candida kefyr, Candida glabrata, Candida utilis, and *Candida albicans* were identified in this study. *Candida parapsilosis* was the most commonly observed species in *Candida spp.* Culture examination, followed by *Candida tropicalis*. *Candida albicans* were discovered in only one culture. Our findings are consistent with those of Kim[13], who discovered that *Candida parapsilosis* was most prevalent in *Candida spp.* cultures. In contrast, Otašević et al.[14] and Gharaghani et al.[15] discovered that *Candida albicans* were the most abundant Candida species in adult patients with otomycosis. The results of this study differ from those of a study conducted by Kiakojuri et al.[8], which found the highest prevalence of *Candida orthopsilosis* in cultures of *Candida spp*. Fungi causing otomycosis vary geographically. The prevalence of *Candida parapsilosis* in our region may be attributable to environmental factors or a strong preference of this species for colonizing the external auditory canal.[13]

The sensitivity of Candida spp. to Flucytosine and Ketoconazole was found to be 100 percent, with Voriconazole achieving a sensitivity of 96 percent. These results contrast with those reported by Kiakojuri et al., who observed good sensitivity of Candida spp. to Nystatin, Clotrimazole, and Fluconazole in otomycosis patients. Additionally, the current study's findings slightly differ from those of Otasevic et al., who reported excellent sensitivity of Candida parapsilosis to Amphotericin B, Itraconazole, and Voriconazole in an antifungal sensitivity test. In contrast, our study showed good sensitivity of Candida parapsilosis to Flucytosine, Ketoconazole, Voriconazole, and Fluconazole.

The arsenal used to treat Candida infections consists of a variety of chemical classes. The efficacy of azoles, polyenes, echinocandins, nucleoside analogs, and allylamines varies according to the type and site of infection as well as the sensitivity of the Candida species. The most popular class of antifungal medications used to treat and prevent Candida infections are azoles. An essential enzyme in the manufacture of ergosterol, 14alpha demethylase encoded by Erg11p gene, is the target of azoles. Azoles successfully reduce the cell's ergosterol levels by binding to Erg11p. Its inhibition is caused by the bond between the free nitrogen atom of the azole ring and the iron atom of the enzyme's heme group. The buildup and metabolism of 14alpha methylated sterol species result in the creation of hazardous chemicals that cannot effectively replace ergosterol. Aside from this, azoles are also accountable for raising the quantities of reactive oxygen species (ROS). The generation of poisonous sterols and elevated ROS levels both prevent the infected fungus from growing.[16,17,18]

Polyenes medicines are fungicidal and target ergosterol in the plasma membrane. They create pores when they bind to ergosterol. Monovalent ions (K+, Na+, H+, and Cl) quickly leak out of pores, which results in the death of fungus cells. Nystatin and amphotericin B are examples of polyene medicines.[17,18]

The incidence of fungal infections has increased dramatically during the past three decades, concurrently with growing acquired and intrinsic resistance to antifungal medications. Nevertheless, antifungal resistance must be evaluated independently for each antifungal class and fungus genus. Mechanisms of antifungal resistance to azole-class medicines can be caused by efflux mediated by multidrug transporters, decreased affinity in Erg11p due to mutations, overexpression of ERG11, and modifications in the ergosterol biosynthesis pathway. While the mechanism of resistance to polyene antifungal medicines is the absence of ergosterol (loss of function mutation in ERG3 or ERG6) and a decrease in ergosterol content in cells, ergosterol deficiency is the cause of polyene antifungal drug resistance.[18]

Candida albicans resistance to azole antifungals has been the subject of the most research. The existence of point mutations in ERG11 has been identified as a resistance mechanism in this species. Previous research has identified amino acid substitutions that decrease fluconazole susceptibility and noted that a number of these crucial allelic variations cluster in three "hotspot" regions within the Erg11p gene. The mechanism is identical to that of Candida parapsilosis; however, in Candida parapsilosis, there is a rise in the production of

major facilitator superfamily transporters, which reduces intracellular accumulation of azoles.[15]

Otomycosis is a common disease encountered in outpatient ENT care. Patients with superficial infections and chronic colonization should be treated with intensive debridement, cleansing, and topical antifungal medications. Systemic antifungal therapy should not be prescribed except for malignant invasive (acute or chronic) otitis externa complicated by mastoiditis, meningitis, or both. Most patients respond favorably to topical treatment. The benefits of topical antifungals include local application, rapid achievement of the desired drug concentration on the skin's surface after application, a higher antifungal engagement at the affected site, lack of systemic side effects and complications due to limited systemic absorption, and very low incidence of drug interaction.[19]

Ketoconazole, Flucytosine, and Voriconazole demonstrated excellent sensitivity in this study, but only Ketoconazole was listed in the National Formulary. It is available in both tablet and ointment form.[20] Because systemic treatment lengthens the drug route, is absorbed, and is distributed throughout the body, the effectiveness of therapy at the site of infection is diminished, making otomycosis challenging to treat and frustrating for both doctors and patients.[7,21] At the same time, ointments have the disadvantage of being unable to be applied in difficult-to-reach areas.[19] The limitations of the study including only using a single culture medium to detect fungal growth and no molecular analysis (e.g. DNA sequencing) to definitively confirm Candida species identification.

In conclusion, *Candida parapsilosis* is the most prevalent otomycotic Candida pathogen complex, accounting for 41.66% of the isolates. Based on the susceptibility test, it has been determined that the otomycosis agents found in our hospital are sensitive to Ketoconazole, Flucytosine, and Voriconazole.

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