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Exploring Antibiotic Susceptibility in Otomycosis: Uncovering Mixed Infections of Fungal and Bacterial Origin in Indonesia

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ABSTRACT

Backgrounds: Otomycosis is a common fungal ear infection affecting people worldwide. Cases may be exacerbated by mixed fungal-bacterial infections, especially those involving antibiotic-resistant bacteria. Understanding the microbiological features and antibiotic susceptibility patterns of the pathogens involved is critical for treatment. This study aimed to investigate the prevalence of mixed fungal-bacterial infections in otomycosis cases in Indonesia, to identify the bacterial species involved, and to determine their antibiotic susceptibility patterns**.**
Materials & Methods: In this study, 47 ear swab specimens were collected from 41 clinically-diagnosed otomycosis cases from April to August 2022. The collected samples were processed by culture and microscopy to identify fungal and bacterial isolates. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method.
Findings: Fungal isolates alone were detected in 80.9% of the specimens, while 19.1% showed mixed fungal-bacterial growth. The most common fungi were Aspergillus (57.1%) and Candida (42.9%) species. Among bacterial isolates, *Staphylococcus aureus* was the most frequent (observed in 66.7% of mixed cases), followed by *Pseudomonas aeruginosa* (22.2%). Also, two of the six *S. aureus* isolates were methicillin-resistant (MRSA). Both *P. aeruginosa* isolates were extended-spectrum beta-lactamase (ESBL) producers.
Conclusion: Many otomycosis cases in this study demonstrated polymicrobial etiology. The emergence of antibiotic-resistant bacteria poses diagnostic and therapeutic challenges to healthcare systems.

Keywords: Otomycosis, Mixed infection, Bacterial, Fungal, Antibiotic sensitivity test

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Introduction

Otomycosis, a superficial fungal infection of the external ear canal, is a common otorhinolaryngologic condition with a global prevalence [1]. The disease frequently occurs in warm and humid climates and demonstrates fluctuating incidence and prevalence across different geographical regions. Estimated prevalence rates range from 9% in otitis externa cases to 30.4% in patients with otitis or inflammatory symptoms [2]. About 6% of otomycosis cases are caused by mixed bacterial-fungal infections, leading to an ongoing debate in the medical community about whether bacterial infections are always accompanied by fungal infections or could exist independently [3]. Additionally, some risk factors associated with otomycosis include prolonged exposure to water, use of hearing aids, and previous antibacterial therapy, which could further explain variations in the disease incidence across different populations. Understanding the epidemiological patterns of otomycosis is vital for effective prevention, diagnosis, and treatment strategies and requires ongoing research and surveillance across diverse environmental and socio-economic settings [4].

While otomycosis is often initiated by saprophytic fungi, a complex interplay between host and environmental factors contributes to its pathogenesis. *Aspergillus* and *Candida* species are prominently considered as the primary causes of otomycosis, although their distribution patterns are different. *A. tubingensis, A. niger, A. terreus, A. fumigatus,* and *C. albicans* are the most frequently isolated fungi from otomycosis cases [5-8]. Bacterial co-infections are uncommon in immunocompetent individuals, implying synergy between fungal and bacterial agents in the occurrence of otomycosis. The most common bacteria associated with otomycosis are *Staphylococcus aureus* and *Pseudomonas aeruginosa* [9].

Otomycosis is considered as a significant health challenge due to its potential complications and associated morbidity. The infection could lead to pain and itching in the ear canal, temporary hearing loss, and persistent discomfort. If left untreated or improperly managed, the disease could progress and lead to more severe complications such as chronic otitis externa, tympanic membrane perforation, and even involvement of the inner ear and adjacent structures. The presence of mixed infections with bacteria complicates the disease diagnosis and treatment and sometimes results in antibiotic resistance and treatment failure [5]. Furthermore, individuals with underlying health conditions like diabetes or immune impairment are at increased risk of severe complications. In some regions, mainly where humidity is high, the incidence of otomycosis may increase, leading to a higher burden on healthcare systems. Collectively, these factors contribute to the significant morbidity associated with otomycosis and underline the importance of timely diagnosis, appropriate treatment, and ongoing research to understand and effectively manage this multifaceted condition [7].

Dual fungal-bacterial infections pose therapeutic challenges due to misdiagnosis, antibiotic overuse, and emerging drug resistance. Studies have revealed increased prevalence rates of methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *P. aeruginosa* among otomycosis patients [10, 11]. This drug resistance underscores the need to characterize bacterial patterns and antibiotic susceptibility profiles, especially in resource-limited settings. However, the current literature lacks comprehensive data on mixed otomycosis infections and antibiograms in many regions of the world,

including Indonesia.

Objectives: This study aimed to determine the prevalence of bacterial co-infections in otomycosis patients, identify bacterial species, and assess their antibiotic sensitivity patterns. The findings are expected to expand the limited available evidence on complex otomycosis cases and contribute to appropriate antibiotic stewardship and implementation of prevention strategies in Indonesia and similar environments.

Materials and Methods

Study design and setting: This cross-sectional study analyzed the clinical specimens of otomycosis patients visiting an otorhinolaryngology outpatient clinic at a tertiary care hospital in Purwokerto, Indonesia from April to August 2022.

Participants: The study population comprised 41 otomycosis patients aged above 17 years. Inclusion criteria were clinical diagnosis of otomycosis by an ENT (ear, nose, throat) surgeon with inspection of signs revealing inflammation and debris in the ear canal. Exclusion criteria included severe otitis externa and non-cooperation in sample collection.

Sample size: The sample size was determined using 95% confidence level and 10% margin of error based on clinical records of otomycosis cases in the past year. The minimum sample size calculated was 41 patients.

Specimen collection: Ear discharge specimens were collected by an ENT specialist using sterile flocked swabs. Bilateral samples were obtained from patients with otomycosis in their both ears, resulting in 47 swab specimens from 41 cases. The collected samples were transported in Amies transport medium to the microbiology laboratory for analysis.

Microbiological testing: Direct microscopy was performed by Gram staining and 10% KOH mount. Specimens were inoculated on two types of agar: Sabouraud dextrose agar and Czapek dox agar. Sabouraud dextrose agar plates were incubated at 25 °C, while Czapek dox agar plates were incubated at 37 °C. Both sets of plates were incubated for up to 4 weeks. Bacterial culture involved inoculation on HiCrome UTI agar and incubation at 37 °C for 24 hours. Identification of isolates was done using standard microbiological techniques [12].

Antibiotic susceptibility testing: Antibiotic sensitivity was determined by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2022 [13]. Inhibition zone diameters were interpreted as sensitive or resistant based on the breakpoint criteria in CLSI M100, 2022 [13].

Statistical analysis: Data were recorded and analyzed using Microsoft Excel. Descriptive statistics were presented as frequency and percentage.

Findings

This study analyzed 47 clinical specimens of 41 clinically-diagnosed otomycosis patients. The clinical and demographic characteristics of otomycosis cases are shown in Table 1. Most patients were 26-35 years old (29.3%, 12 of 41). The most common clinical symptom was itchiness, experienced by 90.2% of patients. Deafness or hearing impairment was also common, reported in 87.8% of cases. In summary, pruritus, deafness, otorrhea, tinnitus, clogged ear, otalgia, and dizziness were the most common clinical features experienced by otomycosis patients. Vertigo was the rarest symptom.

Table 2 details the distribution of infection among 41 otomycosis patients. Regarding distribution, most otomycosis cases were unilateral, that is, the infection occurred in only one ear. Specifically, in 53.6% (n=22)

of patients, only the right ear was infected, while in 36.7% (n=15), only the left ear was infected. Bilateral infections affecting both ears were less common, accounting for 9.7% (n=4) of cases. As shown in Table 2, unilateral otomycosis was more common in this sample, and the right ear was more frequently involved than the left ear.

Table 3 compares the types of infections in 47 specimens. As shown in this table, the vast majority of specimens (80.9%, 38 of 47) were exclusively diagnosed with fungal infections. On the other hand, cases with mixed infections involving both fungal and bacterial pathogens were relatively rare, accounting for only 19.1% (9 of 47) of cases. This significant disparity highlights the predominance of fungal infections in the studied sample.

Table 4 shows the distribution of fungal isolates recovered from 47 specimens of otomycosis cases. Out of 56 fungal isolates, 42.9% (n=24) were *Candida* species, among which *C. parapsilosis* was the most common (n=10, 17.9%).

Aspergillus species accounted for 57.1% (n=32) of all fungal isolates, among which *A. flavus* was predominant (26.8%, n=15).

The percentages listed for organisms in Table 4 represent their proportion out of

Table 1) Clinical characteristics of otomycosis cases (n=41)

Clinical Characteristics	Number of Cases	Percentage
Age (years)		
17 – 25	6	14.6
26 – 35	12	29.3
36 – 45	8	19.5
46 – 55	6	14.6
56 – 65	7	17.1
>65	2	4.9
Clinical symptoms		
Itchiness	37	90.2
Deafness	36	87.8
Discharge	29	70.7
Tinnitus	25	61
Clogged	34	82.9
Otalgia	23	56.1
Dizziness	18	43.9
Vertigo	2	4.9

Table 2) Distribution of otomycosis infection among 41 patients

Parameter of Otomycosis	Number of Cases	Percentage (%)
Distribution		
Right	22	53.6
Left	15	36.7
Unilateral	37	90.3
Bilateral	4	9.7

Table 3) Type of otomycosis infection among 47 specimens

Parameter of Otomycosis	Number of Cases	Percentage (%)
Microorganisms		
Fungal	38	80.9
Mixed infection	9	19.1

Table 4) Fungal isolates recovered from 47 clinical specimens of otomycosis cases (n=56)

Microorganisms	N	%
Candida spp.	24	42.9
Candida albicans	1	1.8
Candida tropicalis	6	10.7
Candida parapsilosis	10	17.9
Candida krusei	3	5.4
Candida kefyr	2	3.6
Candida utilis	1	1.8
Candida glabrata	1	1.8
Aspergillus spp.	32	57.1
Aspergillus flavus	15	26.8
Aspergillus fumigatus	3	5.4
Aspergillus niger	9	16.1
Aspergillus terreus	1	1.8
Aspergillus candidus	1	1.8
Aspergillus glaucus	1	1.8
Aspergillus oryzae	1	1.8
Aspergillus tamarii	1	1.8

the total fungal isolates (n=56). In summary, *Candida* and *Aspergillus* species were the primary fungal pathogens in this otomycosis sample, with *C. parapsilosis* and *A. flavus* being the predominant isolates.

Table 5 details the microbial isolates identified in 47 clinical specimens of otomycosis patients with mixed fungal-bacterial infections. Overall, nine (19.1%) cases with mixed infection were found. The predominant bacterial co-isolate was *S. aureus* found in six out of nine cases with mixed infection (66.7%), followed by *P. aeruginosa*, present in two cases (22.2%). Another bacterial isolate recovered from mixed cases was *Enterococcus faecalis* found in one sample (11.1%, 1 of 9 cases). Some cases showed multiple fungal isolates coinciding with bacterial growth. For example, one case had *A. flavus* and *A. fumigatus* along with *S. aureus*, while another case demonstrated *C. tropicalis* and *A. niger* along with *P. aeruginosa*. As shown in Table 5, *A. flavus* was the most common fungal pathogen detected in mixed otomycosis infections, while *S. aureus* was the most common accompanying bacterial isolate. These data provide insights into the diverse microbial interactions underlying these complex otomycosis cases.

Table 6 summarizes the antibiotic susceptibility patterns of bacterial isolates recovered from otomycosis patients with mixed fungal-bacterial infections. A total of six *S. aureus* isolates were tested. *S. aureus* displayed high susceptibility to gentamicin (100%), moderate susceptibility to cefoxitin, ciprofloxacin, levofloxacin, and ofloxacin (66.7% each), but low susceptibility to clindamycin (50%). Also, two *P. aeruginosa*

Table 5) Mixed infection isolates detected in 47 specimens of otomycosis cases

Fungal Isolates	Number (%) of Specimens Positive for Fungi (n=47)	Associated Bacterial Isolates	N	%
Unifungal infection				
Aspergillus flavus	14 (29.8)	*Enterococcus faecalis*	1	11.1
Aspergillus niger	8 (17.0)	*Staphylococcus aureus*	2	22.2
Aspergillus terreus	1 (2.1)	*Staphylococcus aureus*	1	11.1
Aspergillus oryzae	1 (2.1)	*Pseudomonas aeruginosa*	1	11.1
Aspergillus tamarii	1 (2.1)	*Staphylococcus aureus*	1	11.1
Candida tropicalis	5 (10.6)	*Staphylococcus aureus*	1	11.1
Mixed fungal infection				
Aspergillus flavus, Aspergillus fumigatus	1 (2.1)	*Staphylococcus aureus*	1	11.1
Candida tropicalis Aspergillus niger	1 (2.1)	*Pseudomonas aeruginosa*	1	11.1

Table 6) Antibiotic susceptibility patterns of bacterial isolates recovered from the study group

Microorganism	*Antibiotics												
	AMP	FOX	CIP	LEV	OFX	SXT	CN	DA	CTX	CRO	CAZ	AK	MEM
Staphylococcus aureus (n=6)	-	66,7	66,7	66,7	66,7	66,7	100	50	-	-	-	-	-
Pseudomonas aeruginosa (n=2)		-	100	100	100	-	100	-	0	0	0	100	100
Enterococcus faecalis (n=1)	100	-	100	100	100	0	0	0	-	-	-	-	-

*AMP: ampicillin, FOX: cefoxitin, CIP: ciprofloxacin, LEV: levofloxacin, OFX: ofloxacin, SXT: trimethoprim sulfamethoksazole, CN: gentamicin, DA: clindamycine, CTX: cefotaxime, CRO: ceftriaxone, CAZ: ceftazidime, AK: amikacin, MEM: meropenem
Number: sensitivity
- : not tested

isolates were analyzed. *P. aeruginosa* showed high susceptibility to ciprofloxacin, levofloxacin, ofloxacin, gentamicin, amikacin, and meropenem (100% each) but no susceptibility to cefotaxime, ceftriaxone, and ceftazidime (0%). In addition, one *E. faecalis* isolate was evaluated. *E. faecalis* showed high susceptibility to ampicillin, ciprofloxacin, levofloxacin, and ofloxacin (100% each) but no susceptibility to trimethoprim sulfamethoxazole, gentamicin, and clindamycin (0%). Two of the six *S. aureus* isolates were methicillin-resistant (MRSA). Both *P. aeruginosa* isolates were extended-spectrum beta-lactamase (ESBL) producers. In summary, *S. aureus* displayed high susceptibility to gentamicin and variable sensitivity to fluoroquinolones. *P. aeruginosa* was primarily sensitive to fluoroquinolones, aminoglycosides, and carbapenems. *E. faecalis* was susceptible to ampicillin and fluoroquinolones but resistant to other antibiotic classes.

Discussion

This study found that 19.1% of clinical specimens of otomycosis patients in Indonesia demonstrated bacterial co-Table infections, consistent with the documented range of 6-38% in prior reports [9, 14]. The higher isolation rates of *S. aureus* (66.7%) and *P. aeruginosa* (22.2%) align with the results of previous studies identifying these bacteria as predominant bacterial pathogens in otomycosis [15]. Intriguingly, fungal isolates alone were recovered from 80.9% of specimens, reflecting the primary fungal etiology of most otomycosis infections. *Aspergillus* species accounted for over half of fungal isolates, followed by *Candida* spp. This profile mirrors global data on the prevalence of fungal species in otomycosis.

In this study, 90.3% of otomycosis cases were unilateral, consistent with the unilateral rates of 72-93% reported in previous studies [12, 16]. The higher incidence rate of infection in the right ear compared to the left ear contradicts some studies results [14, 17, 18] but aligns with suspected moisture and anatomical factors [19]. Further research should be done to examine laterality differences.

The age distribution mirrors the peaks described in younger adults [4, 17, 20], likely related to occupational and recreational habits. Fungal isolates alone were obtained from 80.9% of specimens, consistent with the well-established primary fungal etiology

of otomycosis [5, 21]. *Aspergillus* and *Candida* species were predominant, mirroring global data [1, 6]. However, the distribution patterns of these pathogens could vary across different regions [20, 22, 23], emphasizing the need for localized epidemiological studies. The predominant clinical symptoms identified in this study, including itchiness, deafness, otorrhea, and otalgia, align with the previously described classic otomycosis presentations [24-26]. Pruritus, in particular, was experienced by over 90% of patients, consistent with the literature citing pruritus as a hallmark otomycosis symptom [24, 27]. Tinnitus and a blocked ear sensation were also common complaints, likely resulting from inflammation, edema, and debris accumulation in the infected ear canal [28, 29]. While vertigo was rare in the study sample, prior studies have reported that vertigo is a rare finding [24, 27]. The broad consistency of clinical features with initial evidence lends further credence to the otomycosis diagnosis. However, some variation is expected due to differences in setting, climate, microbiological features, and host factors. Ongoing documentation of signs and symptoms in diverse regions could illuminate the spectrum of clinical symptoms associated with this common ear infection.

In this study, 80.9% of cases demonstrated purely fungal growth, consistent with the established primary fungal etiology of otomycosis [12]. *Aspergillus* species were predominant isolates, particularly *A. flavus*, followed by *Candida* spp., with *C. parapsilosis* being the most common species. These findings are consistent with the results of previous studies indicating that *Aspergillus* and *Candida* genera are typically the causes of most otomycosis cases [5]. However, the distribution patterns of these species could be different across different regions. For example, *A. tubingensis* is predominant in western China, while *A. terreus* is more common in southeast China [17,23]. The need for localized epidemiological data is highlighted due to the variability in fungal patterns, which could help in empirical antifungal selection. Continuous surveillance should be done to monitor emerging epidemiological trends.

In this study, 19.1% of cases demonstrated bacterial co-infections, consistent with reported mixed infection rates [9, 14]. *S. aureus* and *P. aeruginosa* were the primary isolates, aligning with the described bacterial predominance [15]. Co-localization of these bacteria with *Aspergillus* spp. may indicate synergistic colonization.

Worryingly, MRSA and ESBL-producing strains were identified among the isolates, concurring with increased antibiotic resistance [1, 10]. Ongoing surveillance of resistance phenotypes is critical to optimize treatment. Bacterial infection may be involved as an underlying or predisposing factor in otomycosis pathogenesis, facilitated by microtrauma, moisture, and prior antibiotic use [30, 31]. Further studies should be done to explore the interplay and sequence of fungal-bacterial co-infections. In this study, *S. aureus* isolates displayed susceptibility to gentamycin but variable sensitivity to other antibiotics like fluoroquinolones. Worryingly, 33% of *S. aureus* isolates were MRSA. Both *P. aeruginosa* isolates showed susceptibility to fluoroquinolones, aminoglycosides, and carbapenems, and both of them were ESBL-producers. The identification of resistance phenotypes in this study agrees with the literature on escalating antibiotic resistance [10, 11, 32, 33]. These findings demonstrate the need for responsible antibiotic stewardship guided by local susceptibility profiles rather than empirical broad-spectrum antibiotic use, which could exacerbate antibiotic resistance. For

example, gentamycin could be considered as a suitable first-line empirical antibiotic therapy for *S. aureus* infections based on observed sensitivities to this antibiotic. Identifying local resistance mechanisms and novel treatment approaches are also critical to counter the diminishing efficacy of conventional antibiotics [34]. Continuous surveillance should be done to track susceptibility patterns over time to support optimal empiric therapy recommendations. Hospitals should periodically compile recent antibiogram data to tailor otomycosis treatment guidelines.

Conclusion

In conclusion, this study revealed a 19.1% prevalence of bacterial co-infections among clinical specimens of otomycosis patients in Indonesia. *S. aureus* and *P. aeruginosa* were the most common bacterial isolates. The emergence of MRSA and ESBL-producing strains demonstrates increasing antibiotic resistance. *S. aureus* showed susceptibility to gentamycin, while *P. aeruginosa* was primarily sensitive to fluoroquinolones, aminoglycosides, and carbapenems.

These findings highlight the need for ongoing microbiological surveillance, particularly on resistance phenotypes, to optimize antibiotic therapy and stewardship. Regional antibiogram data are essential to guide clinical practice guidelines and limit empirical broad-spectrum antibiotic use. Further research should be done to explore local resistance mechanisms and novel therapeutic approaches to prevent further development of multidrug resistance.

This study provides valuable insights into complex otomycosis infections in Indonesia and emphasizes the importance of tailored diagnostic and treatment strategies based on local susceptibility patterns. The use of these susceptibility patterns may involve the use of localized antibiogram data to improve clinical practice guidelines regarding empirical selection of optimal antibiotic type and dosage. Continued efforts are necessary to monitor evolving microbiological trends and curb antibiotic resistance through antimicrobial stewardship and infection control programs.

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Ethical permissions: This study was approved by the Faculty of Medicine's Ethics Committee at Jenderal Soedirman University with reference number 018/KEPK/PE/II/2022.

Conflicts of interests: The authors declare that they have no conflict of interest.

Authors' contributions: NK aided in conceptualizing the study, supervising the writing of this publication, and doing the statistical analysis. ABD contributed to study conceptualization and specimen collection. NK, RANH, and APKNW contributed to sample collection and data analysis. NK and LR contributed to paper drafting, revision, and final approval. The paper was written collaboratively; all authors read and approved the final draft. All authors discussed the results and commented on the manuscript.

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