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Case Report

A case of fungal otitis externa caused by coinfection of *Candida auris* and *Aspergillus flavus*

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ABSTRACT

Fungal otitis externa is a disease encountered occasionally and is caused mostly by *Aspergillus* or *Candida* spp. We report a woman with fungal otitis externa who also had typical findings in the external auditory canal. The results of a culture showed coinfection with *Candida auris* and *Aspergillus flavus*. Identification of both species was performed by sequencing analysis of the 26S rDNA (D1/D2) and β-tubulin regions. Additionally, the newly developed CHROMagar™ Candida Plus medium was a useful tool for the easy and rapid identification of *C. auris*. To the best of our knowledge, this is the first report of fungal otitis externa caused by coinfection with *C. auris* and *A. flavus*. This case showed good susceptibility to many antifungal drugs and fortunately had a good clinical course with 1% bifonazole cream, which was applied to the fungal coinfection. Notably, *C. auris* is a multidrug-resistant yeast-like fungus. The increase in drug-resistant fungi and co-infections caused by these pathogens can make the diagnosis and treatment more complex and difficult. To solve these problems, performing rapid and accurate identification and susceptibility testing using chromogenic medium and molecular biological analysis would be useful.

1. Introduction

Superficial and cutaneous fungal infection occasionally affects the skin and nails, including the external auditory canal. Kiakojuri et al. reported that, of 237 cases of fungal otitis externa, 74% were *Aspergillus* spp. and 26% were *Candida* spp [1]. To the best of our knowledge, there have been few reports of coinfection with *Aspergillus* spp. and *Candida* spp. in fungal otitis externa.

Candida auris is a global threat because it can colonize the skin, medical equipment, and hospital environment, causing nosocomial, bloodstream, and urinary tract infections [2]. *C. auris* has been identified on every continent and in 40 countries [3] after it was first isolated from the external auditory canal of a Japanese patient in 2009 [4]. There have not been many reports of fungal infections caused by *C. auris*, and it is not a major group of fungi for clinicians. One of the reasons for this lack of reports may be the difficulty of identification in the clinical laboratory.

We report a case of fungal otitis externa with details of the identification of the causative fungi as *C. auris* and *Aspergillus flavus* and of the clinical course. To the best of our knowledge, this is the first report describing fungal otitis externa caused by coinfection with *C. auris* and *A. flavus*.

2. Case report

A female patient in her 70s visited the Department of Otorhinolaryngology, Toho University Ohashi Medical Center to request a hearing aid owing to age-related hearing loss in her right ear. An examination of her ears showed that her right ear was normal, but her left ear was not. The left tympanic membrane was perforated, and white spores on crusts were observed in the external auditory canal. She had undergone surgery for chronic otitis media in her left ear as a child. She was deaf and had cavity problems after open cavity mastoidectomy. She was in good health and had no history of diabetes mellitus, other diseases, or

Abbreviations: CCP, CHROMagar™ Candida Plus; CDC, Centers for Disease Control and Prevention; MIC, minimum inhibitory concentration.

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medications that could lower her immune function. Recently, she had not suffered from any disease that requires the administration of antibiotics. Although she has a history of overseas travel, she has been staying domestically for the past nine years. Her most recent trip was to Guam, and prior to that, she had gone on sightseeing trips to Hawaii, Italy, and France. We considered that typical ear canal fungal infection was the most probable diagnosis. After a specimen was collected for fungal culture, the ear was cleaned with saline solution and 1% bifonazole cream was applied. In addition, we prescribed the same cream and instructed to apply it at home as well.

Culture specimens of otorrhea submitted to the clinical laboratory were incubated under aerobic conditions using 5% sheep blood agar and CHROMagar™ Candida media (Kanto Chemical Co., Inc., Tokyo, Japan). After incubation for 24 h at 35 °C, yeast-like fungal growth was observed on both media. After incubation for 48 h, the yeast-like fungi (TOH1990) showed a light pink color and slight growth of filamentous fungi (TOH1991) on CHROMagar Candida media. The isolated TOH1990 strain was identified as *Candida duobushaemulonii* sp. by VITEK® 2 compact (bioMerieux, Marcy l'Etoile, France) with a score of 98% (version 08.01), but was not identified by VITEK® MS (bioMerieux). When the TOH1990 strain was incubated in the recently developed CHROMagar™ Candida Plus (CCP) medium (Kanto Chemical Co., Inc., Tokyo, Japan) for 48 h, light blue circular colonies were observed (Fig. 1). In contrast, TOH1991, which was isolated at the same time, was observed under the microscope and identified as *Aspergillus* spp.

We utilized the extracted DNA to conduct a polymerase chain reaction (PCR) of the 26S D1/D2 region for the TOH1990 strain and the β-tubulin region for the TOH1991 strain, following previously described procedures [5,6]. The sequence data were analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The DNA sequence of the amplicon of TOH1990 was identical (100%: 535/535) to that of *C. auris* (GenBank accession no, CPO43535.1). In addition, the DNA sequence of the amplicon of TOH1991 was identical (99.8%: 488/489) to that of *A. flavus* (GenBank accession no, MT942057.1). We classified the Clade using Colony PCR, a technique recently reported by Aswathy et al. [7]. The results revealed that the TOH1990 strain was classified as Clade II (East Asian type) of *C. auris*.

The antifungal susceptibility of TOH1990 was investigated using yeast-like fungi DP "*Eiken*" (Eiken Chemical, Co., Ltd., Tochigi, Japan).

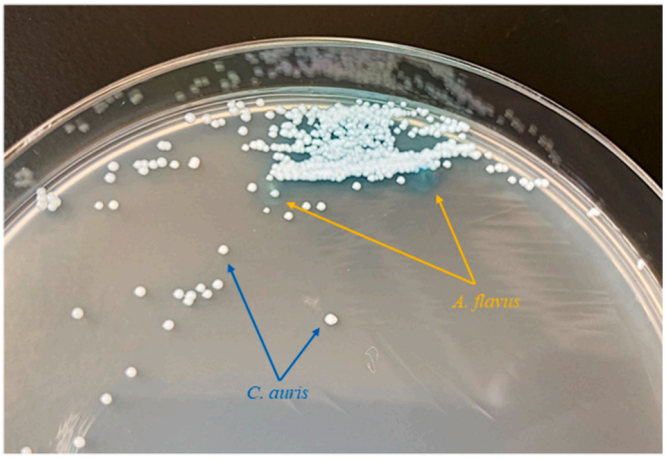

Fig. 1. *Candida auris* and *Aspergillus flavus* on CHROMagar™ Candida Plus medium at 48 h of incubation.
The morphological characteristics of the two types of colonies are different in shape and color. *C. auris* colonies are circular, light blue, and matte in appearance with no gloss. *A. flavus* colonies are flat, suede-like, and olive in color, with lighter tints on the periphery. Growth of both colonies is good at 48 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Yeast-like fungi DP "*Eiken*" is a 96-well plate coated with antimicrobial agents developed for assessing the antimicrobial susceptibility of yeast-like fungi and is performed by the microdilution method. This method follows the Clinical and Laboratory Standards Institute M27-A3 document. To determine drug susceptibility, we applied the conservative breakpoints developed by the Centers for Disease Control and Prevention (CDC) for other *Candida* spp [8,9]. The minimum inhibitory concentrations (MICs) of the TOH1990 strain showed fluconazole (1 mg/L), voriconazole (0.015 mg/L), amphotericin B (0.5 mg/L), micafungin (0.25 mg/L), and caspofungin (0.25 mg/L). The MICs were low for all of the antifungal agents measured (Table 1).

After 2 weeks of treatment, the findings in the left external auditory canal were normal. During this period, the causative fungus was identified and drug susceptibility testing was performed, which showed that azoles were effective. However, treatment with bifonazole was continued because a subclinical fungal infection in the external auditory canal was considered likely. Three weeks later, treatment was terminated because there was no finding of recurrence in the patient's left ear.

3. Discussion

We report a common case of fungal otitis externa, but multiple fungi, *C. auris* and *A. flavus,* were detected. Cases of superficial skin infections, especially otitis externa, in which multiple fungal pathogens have been detected are relatively rare. Gawaz et al. reported that 6% of all superficial mycoses had been caused by mixed infections and that this proportion is increasing [10]. They also reported that dermatophyte and yeast co-infections on the skin other than the hands and feet occurred in only 1.6% of superficial mycoses.

Additionally, co-infections with *C. auris* and *Aspergillus* spp., which have recently required attention as drug-resistant Candida, have not been reported to date. The prevalence of *C. auris* is increasing worldwide [11], but it remains rare in Japan [12]. The detection of this pathogen in an older but healthy woman suggests that it may have already spread in Japanese society and caused community-acquired infections. Fungal co-infections can occur in patients with normal immune function.

One reason why *C. auris* has not been easily detected previously is that it may have been overlooked in the biochemical characterization used in many clinical microbiology laboratories. *C. auris* is misidentified by commercially available biochemical characterization tests (https://www.cdc.gov/fungal/diseases/candidiasis/recommendations.html). Therefore, being aware of the species of bacteria that can be misidentified as *C. auris* by automated equipment in a facility is important. We strongly suspected *C. auris* because the name of *C. duobushaemulonii* appeared in our first VITEK® 2 result, the specimen showed otorrhea, and the MIC of amphotericin B was as high as 0.5 mg/L. Therefore, we performed sequencing analysis targeting the 26S rDNA D1/D2 region. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and sequencing of the 26S D1/D2 region are necessary for the accurate identification of *C. auris* [13]. However, these methods are problematic owing to the costs and time required. The newly developed

Table 1
Antibiotic susceptibility profile of the *Candida auris* TOH1990 strain.

Antifungal agent	Breakpoint	MIC, mg/L	Interpretation
Amphotericin B[a]	2	0.5	S
Flucytosine	128	0.12	–
Miconazole	–	0.12	–
Fluconazole[a]	32	1	S
Itraconazole	–	0.03	–
Voriconazole	2	0.015	–
Micafungin[a]	4	0.03	S
Caspofungin[a]	2	0.25	S

Abbreviations: MIC, minimum inhibitory concentration; S, susceptible; CDC, Centers for Disease Control and Prevention.
[a] Breakpoints are those recommended by the CDC [6].

CCP medium showed light blue colonies, which is different from other *Candida* species, and is useful for identifying *C. auris*. Mulet Bayona (2020) reported that the sensitivity and specificity of CCP for *C. auris* were both 100% [14]. CCP medium is a simple and cost-effective method for the detection and identification of *C. auris* at the species level. Multiplex polymerase chain reaction and loop-mediated isothermal amplification methods can also be used to identify *C. auris,* which may enable more clinical laboratories to accurately identify it [15,16]. These methods will enable more detailed monitoring and surveillance of the prevalence and drug resistance in each country and region in the future. The TOH1990 strain was classified as Clade II, which is primarily isolated in Japan and Korea. The antifungal susceptibility test results were relatively positive and consistent with the Clade II characteristics. As there was no recent travel history overseas, it suggests that this pathogen is potentially endemic in Japan.

In this case, a clinical response to the bifonazole cream was observed. However, *C. auris* has been reported to be less susceptible to azoles, polyenes, and echinocandins [11,17]. *Aspergillus* spp. have also been reported to be resistant to azole drugs [18], and care should be taken in the selection of antifungal drugs.

This case was clinically similar to known cases and had no specific features. Therefore, identifying the causative fungus or multiple infections was difficult until the results of culture tests were available. Infections caused by resistant fungus or multiple fungi can reduce treatment responsiveness and worsen the prognosis. Coinfection may have been overlooked in facilities where fungal culture tests were not available. Therefore, fungal culture tests at the time of the first visit are important.

In conclusion, taking into consideration the characteristics of each fungus, we speculate that co-infections, such as those with *C. auris* and *A. flavus*, may complicate treatment. The emergence of resistant fungi is expected to increase in the future. Therefore, sufficient clinical samples should be collected before starting treatment, and treatment based on information about the causative fungi and drug sensitivity should be performed. Consequently, the establishment of a simple and accurate method of fungal identification and rapid drug susceptibility testing is required.

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Authorship statement

YI and NI organized and coordinated the study. YI and NI wrote a draft of the manuscript. YI, NI, NK, and MY designed the study. NI, SY, and MY performed clinical data analysis. NK and MO were responsible for analysis of the isolates. All authors contributed to writing of the final manuscript. All authors contributed to the management or administration of the study. All authors met the ICMJE authorship criteria.

Declaration of competing interest

All the authors declare the absence of any dual or conflicting interest.

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