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CASE REPORT/CAS CLINIQUE

A case of bilateral otomycosis associated with *Aspergillus flavus* and *A. terreus* in Taiwan

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Summary Otitis externa caused by fungi (otomycosis) occurs more commonly in tropical areas with high moisture than in temperate regions. Bilateral otomycosis is, however, rarely reported. In a case of bilateral otitis externa in a 56-year-old male patient in Taiwan, direct microscopic examination of the cerumen as well as isolation of strains indicated the presence of two *Aspergillus* species being different in each of both ears. The species were identified by DNA sequence comparisons and additional morphological confirmation of diagnostic characteristics as *Aspergillus flavus* and *Aspergillus terreus*. The rarely reported occurrence of two *Aspergillus* species in otitis of the same patient deserves attention in other cases of otomycosis, particularly with respect to potentially different resistances of different species against antifungals. Treatment with nystatin/neomycin was not successful, but with clotrimazole was effective.

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Introduction

Otitis externa is an inflammation of the external auditory channel and usually associated with infection by microorganisms, which in most cases belong to bacteria. In a study of more than 2000 persons with acute otitis externa in the USA, the most frequently isolated microorganisms belonged to the genera *Pseudomonas* and *Staphylococcus*, whereas only around 1% of the samples were fungi [1]. Otitis externa caused by fungi (otomycosis, excluding dermatophytosis of the ear here) occurs more commonly in tropical areas with high moisture, where otomycosis may make up to 47% of all cases of otitis externa [2]. Otomycosis needs particular care, since recent cases in Taiwan have been shown to become highly invasive and difficult to treat [3,4]. Fungi associated with otomycosis belong to the genera *Aspergillus* and *Candida*, with *Aspergillus* being represented by the species *Aspergillus alliaceus*, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus hollandicus*, *Aspergillus janus*, *Aspergillus luchuensis*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus terreus*, and *Aspergillus versicolor* [5–7]. *Aspergillus tubingensis* was isolated from cerumen [8]. Among these species, *Aspergillus niger* is the most frequently reported species [2,9]. In most publications, however, species identification did not include molecular markers and, according to present mycological standards, are largely doubtful. *Aspergillus* systematics has progressed rapidly within the last years and species identification has become a demanding task even for trained mycologists [10]. In *Aspergillus* species identification, internal transcribed spacer (ITS) sequences of the ribosomal *RNA* genes usually need to be complemented with sequences of protein genes, e.g. of the *beta-tubulin* gene [6,10,11]. Other genera have been recorded even more rarely, generally only by a single case for a particular species [12,13]. In a case of bilateral otitis externa in a patient in Taiwan, the fungal nature of the disease remained undiagnosed for several weeks.

Case report

Sampling

A 56-year-old otherwise healthy man has suffered from ear problem and received treatment with nystatin/neomycin cream (MYCOMB, Sinphar, Taiwan) for 2 months, but after a period of drying, the lesions were weeping, and the symptoms remained recurring. After mycological and medical diagnosis, topical treatment with a 1% clotrimazole cream (MYCOSTEN, Sinphar, Taiwan) twice per day began on 30. April 2015. The symptoms disappeared in November 2015.

A preliminary sample of cerumen separately from both ears in March 2015 transferred to corn meal agar (Fluka, with 0.2% chloramphenicol) yielded two species of *Aspergillus* differing by considerably different growth rates and color, with the slow-growing species forming whitish colonies and the quick-growing one producing yellowish colonies. Since a surface cleaning with ethanol prior to sampling was missing and samples were processed with a delay of several days, a second set of cerumen fragments were sampled after wiping the surface of both ears of the patient with ethanol on 11 April 2015 in Taipei, placed separately into autoclaved Eppendorf cups, and taken to the laboratory. Three samples from the left ear were divided each into three subsamples and placed onto one 1.3% malt extract agar plate (MEA; Fluka, with 0.2% chloramphenicol); one sample from the right ear was divided into three subsamples which were placed onto one MEA plate on 15 April 2015. For direct microscopic examination, one additional subsample each from the left and the right ear was placed the same day as for cultivation into a drop of a mixture of lactic acid and polyvinyl alcohol with cotton blue [14], covered with a cover glass and heated until boiling. For morphological and molecular reproducibility of the identification, dried cultures were deposited as vouchers at National Museum of Natural Science, Taichung, Taiwan (TNM).

For nucDNA extraction, PCR, sequencing and sequence editing, the methods described in Yeh and Kirschner (2014) were used [15]. Additionally, the primer pair TUB2Fd/TUB4Rd was used for amplification of the *beta-tubulin* gene [16]. Related DNA sequences of ITS and the *beta-tubulin* gene were searched using the BLAST function of GenBank.

Microscopy of cerumen

In direct microscopical investigation, hyphal structures and subglobose conidia were found in the cerumen samples from both ears. In the sample from the right ear, hyphal structures similar to degenerated *Aspergilllus* conidiophores (Fig. 1F) and small (up to 3 μm) conidia were found. The sample from the right ear contained conspicuously branched hyphae and different kinds of conidia (Fig. 1C and E). Some conidia were pale brown and rough and measured approximately 5–7 μm. Other conidia were smaller (3 μm and smaller), colorless and without clearly visible surface ornament.

Identification of fungal species

Two different species of *Aspergillus* were obtained from the samples of the left and right ear, respectively. Each of the three subsamples yielded the same *Aspergillus* species, namely *A. flavus* from the three samples from the left ear (representative isolate R. Kirschner 4188) and *A. terreus* (R. Kirschner 4187) from the right ear (Fig. 1A and B). Both species differed considerably by the quick growth and yellowish colonies of *A. flavus* and the slow growth and whitish colonies of *A. terreus*. The smaller conidia found in the cerumen from the left ear conform to the conidium size of *A. terreus* and the larger ones from the right ear to *A. flavus*. The species were identified by ITS and *beta-tubulin* gene sequence comparisons using BLAST and additional morphological confirmation of diagnostic characteristics. ITS sequences were deposited in GenBank (KT778598 for *A. flavus* and KT778597 for *A. terreus*) and *beta-tubulin* gene sequences in the DNA Database of Japan (LC085664 for *A. flavus* and LC085663 for *A. terreus*). Since certain species of *Aspergillus* species cannot be identified by ITS sequences alone [10], *beta-tubulin* gene sequences and diagnostic morphological markers were additionally considered [6,17]. Using ITS sequences, however, resolution is sufficient for *A. flavus*/*Aspergillus oryzae* based on 100% sequence similarity (591/591 bp each for several strains of *A. flavus* and *A. oryzae*), whereas similarities with sequences