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Case Report

A case of otitis externa caused by *Schizophyllum commune*: An approach to antimicrobial stewardship using Gram staining of otorrhea in a medical clinic☆

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ABSTRACT

Recently, basidiomycete *Schizophyllum commune* has been reported as a cause of allergic bronchopulmonary mycosis. However, it is rare as a cause of otitis externa. We experienced a very rare case of otitis externa caused by *S. commune* in a 68-year-old man with a history of chronic otitis media. We performed Gram staining at the first consultation and follow-up treatment and found fungal cells on the smear and treated him with an appropriate antifungal drug. The results of identification and antifungal susceptibility testing obtained in cooperation with clinical microbiologists at other facilities was very important for future treatment planning decisions. Medical practitioners worldwide should introduce a Gram staining tool into their workflow and cooperate closely with clinical microbiologists to achieve antimicrobial stewardship.

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1. Introduction

Schizophyllum commune is one of the common basidiomycetes and is distributed on all continents. Recently, as diagnostic technology has advanced, the number of cases of allergic fungal rhinosinusitis and allergic bronchopulmonary mycosis due to *S. commune* has increased [1]. Besides, in the field of otolaryngology, although *S. commune* is often detected in patients with sinusitis, it rarely causes otitis externa, and currently, only one known case has been reported [2]. We report a case in which Gram staining was performed during a medical consultation at our clinic so that a very rare fungal disease could be diagnosed quickly and proper treatment could be administered.

2. Case report

A 68-year-old male farmer who had never cultivated mushrooms presented to our otolaryngology medical clinic in August 2017 with complaints of otorrhea and itching of the right ear canal of 10 days duration. Fig. 1 shows photos taken inside the ear canal at the second consultation and at recovery 2 months later. The patient had smoked 15 cigarettes per day for 45 years, drank one beer per day, and his activities of daily living were good. The patient had no particular family history. His past medical history was significant for right ear deafness and perforation of the tympanum after surgery for right otitis media cholesteatoma at the age of 36. The patient had acute exacerbation of chronic otitis media after the surgery, and he consulted our clinic for the first time in 2009, where was healed with conservative treatment. In September 2014 and August 2015, the patient underwent re-examination for exacerbations of acute chronic otitis media, and culture tests performed at those times detected clindamycin-resistant *Staphylococcus aureus* and *Enterobacter aerogenes*, respectively. However, he was cured with conservative treatment that did not use antibacterial drugs.

His general condition at presentation was good. He remembered that he had touched his ears with soiled fingers a few days before the onset of the otorrhea. Macroscopic findings of the ear canal at the first consultation showed otorrhea resembling a white bacterial mass in the ear canal and on the tympanic membrane (Fig. 1-a), and we found mold-like fungi using Gram stain microscopy of the otorrhea in our clinic. We suspected external ear infection related to chronic otitis media. Inflammatory signs were noted in the Gram

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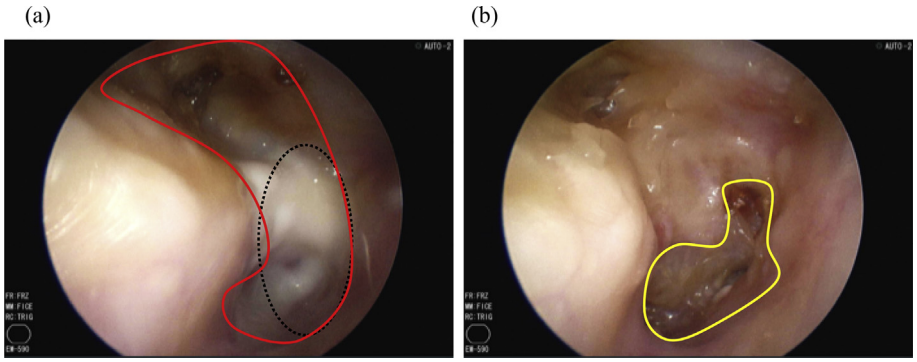

Fig. 1. Photos taken inside the ear canal at the second consultation and at recovery 2 months later. (a) Macroscopic findings of the ear canal at the second consultation showed otorrhea resembling a white bacterial mass after with clotrimazole cream coating (dotted line) and inflammation (red line) in the ear canal and on the tympanic membrane. (b) Two months later, we confirmed that the treatment was successful, and his symptoms had improved. Yellow line indicates a port of tympanic membrane perforation.

stain of this otorrhea specimen, and therefore we diagnosed him as having external otomycosis and immediately prescribed 1% clotrimazole cream (Bayer Yakuhin, Osaka, Japan) twice a day. Because we suspected a non-general bacteria and non-*Aspergillus* spp., we outsourced identification and antifungal susceptibility testing to a commercial laboratory and university laboratory. One week later, we confirmed both the disappearance of the mold-like fungi by Gram staining and that the treatment was successful and his symptoms had improved (Fig. 1-b). The patient has experienced no recurrence since then. In addition, no side effects were observed during treatment of the patient with clotrimazole cream.

3. Microbiological analysis

Fig. 2 shows the rapid analysis results of Gram staining performed at the first consultation in our clinic. Bartholomew and Mittwer solution (Muto Pure Chemicals Corporation, Tokyo, Japan) was used as the Gram stain solution. The Gram stain findings revealed 4 + leukocytes (≥25/LPF), 1+ Gram-positive cocci suspected of being Staphylococci (<1/LPF), and 1+ mold-like fungi with septa (<1/LPF). The mycelium was not similar to that of *Aspergillus* spp. and did not show any obvious clamp connection. The general bacterial culture was negative.

The fungus culture was outsourced to a commercial laboratory. Fig. 3 shows the results of the fungal culture and lactophenol cotton blue staining. The fungus culture was cultured for 7 days under room air at 22–26 °C using Sabouraud agar (Nissui Pharmaceutical Corporation, Tokyo, Japan). As a result, we observed growth of a white, cottony, giant colony without conidiogenic structures on the Sabouraud agar. The culture was continued for 1 month, but fruiting body structures on the agar plate and obvious clamp connections were never observed microscopically.

However, we could obtain the results of identification and antifungal susceptibility testing from the university laboratory. The identification was determined by polymerase chain reaction (PCR) direct sequencing of the internal transcribed spacer (ITS) region of the small subunit ribosomal DNA gene [3]. The DNA was extracted using a MORA-EXTRACT kit (Kyokuto Pharmaceutical Industrial Corporation, Tokyo, Japan). The PCR was performed in a Thermal Cycler System 9700 (Life Technologies, Tokyo, Japan) using ITS1 primer (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′). We then performed the sequencing using an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA) after the amplicons were purified. The sequencing of the ITS region showed 100% identity (597/597) with *S. commune* GenBank accession number MH863418.1.

Table 1 shows the results of the antifungal susceptibility testing in this strain, which was performed using a slightly modified version of the CLSI-M38-A2 method [3]. The modifications included the use of a Dry Plate Eiken (Eiken, Tokyo, Japan) microplate and Roswell Park Memorial Institute (RPMI)-1640 medium with glutamine (Nacalai Tesque, Kyoto, Japan). In the imidazole group, the MIC of miconazole, which is similar to the clotrimazole administered in this case, was 1 μg/mL.

4. Discussion

We experienced a case of otitis externa caused by *S. commune* in a patient with a history of chronic otitis media, for which rapid

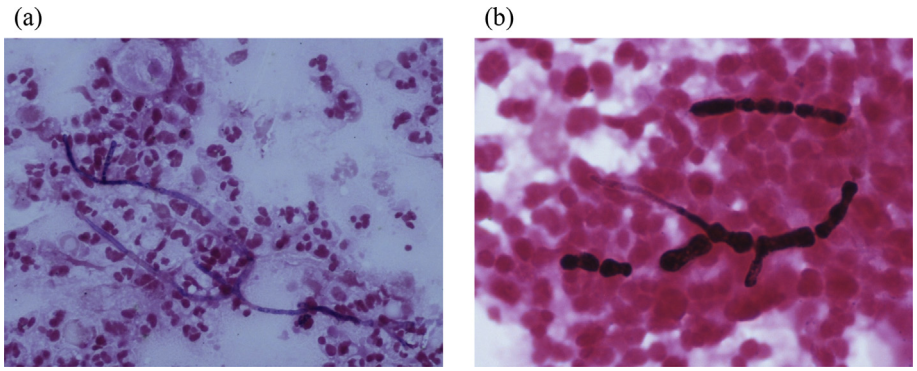

Fig. 2. Rapid analysis results of Gram staining performed at the first consultation in our clinic. The Bartholomew and Mittwer method was used. (a)×400 magnification, (b)×1000 magnification.

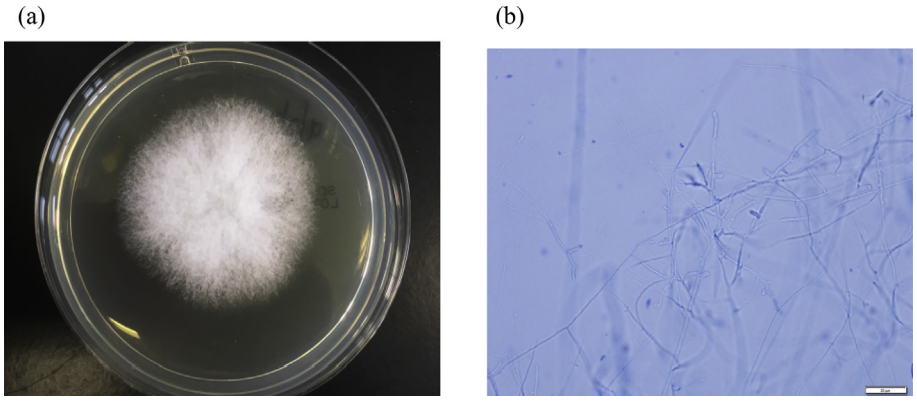

Fig. 3. Giant fungal colony cultured on Sabouraud agar and microscopic findings by lactophenol cotton blue stain in this case. (a) The fungus was cultured for 7 days under room air at 22–26 °C using Sabouraud agar. (b) Photomicrograph at × 400 magnification.

Gram staining in the medical clinic was extremely useful in the diagnosis. *S. commune* is widely distributed in various environments, especially in decaying vegetation [4]. It is the most frequent filamentous basidiomycetes fungus causing allergic sinusitis and bronchopulmonary mycosis in humans [5]. According to a previous global study, mycosis caused by *S. commune* have been reported with increasing frequency in the last few years, and almost half (46%) of all detections in the world are reported to be in Japan [1]. Moreover, among 71 reported cases of *S. commune*, 67 (94%) were cases of bronchopulmonary disease and sinusitis, mycosis of the orbit and brain were rarely reported, and its detection in otitis externa was reported in only a single report [2]. Of note, the present patient was a non-immunocompromised host. In this case, Gram staining showed infiltration of inflammatory cells, and only *S. commune* was detected. In addition, the patient was cured by the antifungal drug clotrimazole cream. Therefore, we diagnosed otitis externa caused by *S. commune*. We supposed that the underlying disease in this case was chronic otitis media with tympanic membrane perforation and that its internal structure was weak. Therefore, we considered there to be a trivial chance of bacteria colonizing in the leachate and causing inflammation. However, it is unknown whether the patient touching his ear with soiled fingers was directly linked to the otitis externa.

The identification of *S. commune* by phenotype is very difficult because it does not show distinctive colonies and morphology [6–8], especially in its monokaryotic isolate [8]. Therefore, DNA sequencing is required [8,9]. In our case also, it was difficult to identify *S. commune* phenotypically, but Gram staining performed at the first consultation in the medical clinic was very useful in helping to determine an initial therapeutic agent.

Table 1
Results of antifungal susceptibility testing of Schizophyllum commune in this case.

Antibiotic	MIC (μg/mL)
Micafungin	4
Caspofungin	2
Fluconazole	8
Miconazole	1
Itraconazole	0.03
Voriconazole	0.06
5-Fluorocytosine	8
Amphotericin B	0.06

There is currently no standard treatment for *S. commune* mycosis. The results of antifungal susceptibility testing for itraconazole, voriconazole, and amphotericin B were in concordance with those of other studies [7,10–13], and the MICs were low. In addition, in the imidazole group, the MIC for miconazole, which is similar to the clotrimazole administered in this patient, was also slightly low. In judging the appropriateness of this treatment, we confirmed the disappearance of the bacteria by Gram staining and the subsequent treatment success. Therefore, in cases of *S. commune* mycosis, we recommend the workflow used in this patient. The results of identification and susceptibility testing are very important in determining antifungal agents. Therefore, medical practitioners worldwide should introduce a Gram staining tool into their workflow and should cooperate closely with clinical microbiologists to achieve antimicrobial stewardship.

In conclusion, we experienced a very rare case of otitis externa caused by *S. commune*, which could be diagnosed quickly by Gram staining and proper treatment could be administered.

Conflicts of interest

None.

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