Letter to the Editor
Clinical Microbiology

Ann Lab Med 2020;40:84-87
https://doi.org/10.3343/alm.2020.40.1.84
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Five Korean Cases of Respiratory Tract Infection by Filamentous Basidiomycetes

Jong-Mi Lee, M.D.[1], Eunhee Han, M.D.[1], Jayoung Kim, M.D.[2], Ji-Hyun Park, Ph.D.[3], Gi-Ho Sung, Ph.D.[3,4], Jong Hee Shin, M.D.[5], and Yeon-Joon Park, M.D.[1]

[1]Department of Laboratory Medicine, College of Medicine, Seoul St. Mary's Hospital, Catholic University of Korea, Seoul, Korea; [2]Department of Laboratory Medicine and [3]Institute for Healthcare and Life Sciences, International St. Mary's Hospital, College of Medicine, Catholic Kwandong University, Incheon, Korea; [4]Department of Microbiology, College of Medicine, Catholic Kwandong University, Gangneung, Korea; [5]Department of Laboratory Medicine, Chonnam National University School of Medicine, Gwangju, Korea

Dear Editor,

Invasive molds are the main cause of fungal diseases in immunocompromised patients; these diseases are diagnosed according to the guidelines of the European Organization for Research and Treatment of Cancer/Mycosis Study Group [1]. The mold species specified in these criteria are not clearly defined, but they usually include species with known pathogenic potential, such as *Aspergillus, Fusarium, Mucorales*, and *Scedosporium* spp. However, nonsporulating molds, especially basidiomycetes (BM), have been reported as emerging pathogens responsible for allergic and invasive diseases, most frequently involving the lungs [2]. As filamentous BM are white nonsporulating molds in culture, conventional identification is problematic. However, with recent advances in sequencing technologies, they have been reported as emerging pathogens [3]. Of the 218 global cases of human pathogenic BM, *Schizophyllum commune* is the most common (52%), followed by *Coprinopsis cinerea* (5.9%), *Emmia lacerata* (5%), and a few cases of *Irpex lacteus* [2].

Although BM are increasingly identified in clinical specimens, little is known about their clinical significance, and Korean cases are rarely reported. We describe five Korean cases of respiratory infections caused by BM among patients admitted to the Seoul St. Mary's Hospital, Seoul, Korea, between June 2016 and July 2017. They were three cases of respiratory tract infection caused by *C. cinerea, E. lacerate* (formerly *Ceriporia lacerate*), and *I. lacteus* and two cases of suspicious infection caused by *S. commune* and *P. spadiceum*. The Institutional Review Board of Seoul St. Mary's Hospital approved the study (approval no. KC19RESI0532) and waived the requirement to obtain informed consent from the patients because this is a retrospective study of clinical cases involving minimal risk to the patients.

The characteristics of the five cases and the fungal morphologies are presented in Table 1 and Fig. 1, respectively. They were all grown on Sabouraud dextrose agar after two weeks of incubation at 28°C under light. In all cases, the fungus was identified by sequencing the internal transcribed spacer (ITS)1/ITS2 and the 28S rRNA gene D1/D2 domains [4] using the following primer pairs: pITS1-F (5′-TCCGTAGGTGAACCTGCGG-3′) and pITS1-R (5′-GCTGCGTTCTTCATCGATGC-3′); pITS2-F (5′-GCATCGATGAAGGCAGC-3′) and pITS2-R (5′-TCCTCCGCTTATTATGC-3′); and D1/D2 regions-F (5′-GCATATCAATAAGCGGAAAAG-3′) and D1/D2 regions-R (5′-GGTCCGTGTTTCAAG ACGG-3). Then, the sequences were analyzed using Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/BLAST).

Antifungal susceptibility testing of the five isolates was retrospectively performed in triplicate according to the CLSI-M38

Received: January 21, 2019
Revision received: June 18, 2019
Accepted: August 7, 2019

Corresponding author: Yeon-Joon Park, M.D., Ph.D.
Department of Laboratory Medicine, College of Medicine, Seoul St. Mary's Hospital, Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea
Tel: +82-2-2258-1640, Fax: +82-2-2258-1719, E-mail: yjpk@catholic.ac.kr

ANNALS OF LABORATORY MEDICINE

Table 1. Characteristics and antifungal susceptibility test results of filamentous basidiomycetes isolated from patients with respiratory tract infection

		Coprinopsis cinerea	*Emmia lacerata*	*Irpex lacteus*	*Schizophyllum commune*	*Porostereum spadiceum*
Age (yr)/Sex		22/Male	38/Female	64/Female	66/Female	89/Male
Underlying conditions		Acute leukemia	Acute leukemia	Pulmonary tuberculosis, rheumatoid arthritis	Multiple myeloma Stage III	Prostate cancer
WBC count /neutrophil/ eosinophil (%)		0.4×10^9/L/ 68%/ 0%	8.4×10^9/L/ 95%/ 0%	14.9×10^9/L/ 72.8%/1.3%	9.0×10^9/L/ 88%/ 0%	6.8×10^9/L/81.6%/2.0%
Diagnosis		Pneumonia	Pneumonia	Pneumonia	Multi-organ failure	Pneumonia
Respiratory symptom		Cough	Occasional hemoptysis	Cough, sputum, dyspnea	Dyspnea	Dyspnea
Chest CT finding		Nodular consolidation with halo Small ill-defined nodular opacities	Atelectasis with pneumonic infiltration	Micronodules and cavities	Left pleural effusion	Both pleural effusion
Co-infection		Not detected	*Escherichia coli* in urine	*Pseudomonas aeruginosa* in sputum	*Candida tropicalis* in urine	*Escherichia coli* in urine *Clostridioides difficile* in stool
Respiratory virus		Rhinovirus detected	Rhinovirus detected	Not detected	Not detected	Not detected
Specimen type		Bronchial washing	Sputum	Bronchial washing	Sputum	Bronchial washing
Treatment		Voriconazole → Posaconazole	Itraconazole	Voriconazole	Antibiotics	Antibiotics
Outcome		Recovered	Recovered	Recovered	Death	Death
GenBank accession no		ITS2 region; MF987832 D1D2 region; MF987831	ITS2 region; MF987826 D1D2 region; MF987825	ITS2 region; MF987830 D1D2 region; MF987829	ITS2 region; MF987828 D1D2 region; MF987827	ITS2 region; MF987824 D1D2 region; MF987823
Antifungal susceptibility test*	Flucytosine	NA	NA	NA	2 (MIC, μg/mL)	<0.12 (MIC, μg/mL)
	Amphotericin B	NA	NA	NA	0.125 (MIC, μg/mL)	0.25 (MIC, μg/mL)
	Voriconazole	NA	NA	NA	0.06–0.125 (MIC, μg/mL)	0.125–0.25 (MIC, μg/mL)
	Itraconazole	NA	NA	NA	0.125–0.25 (MIC, μg/mL)	0.25–0.5 (MIC, μg/mL)
	Ketoconazole	NA	NA	NA	0.06–0.125 (MIC, μg/mL)	0.25–0.5 (MIC, μg/mL)
	Micafungin	NA	NA	NA	>16 (MEC, μg/mL)	>16 (MEC, μg/mL)
	Caspofungin	NA	NA	NA	>16 (MEC, μg/mL)	>16 (MEC, μg/mL)
	Anidulafungin	NA	NA	NA	>16 (MEC, μg/mL)	8 (MEC, μg/mL)

*The results of antifungal agents showing variable MICs in triplicate tests are presented as a range.
Galactomannan was not tested in *Porostereum spadiceum*; galactomannan was not detected in the other four cases.
Abbreviations: NA, not available; MIC, minimum inhibitory concentration; MEC, minimum effective concentration; WBC, white blood cell; CT, computed tomography.

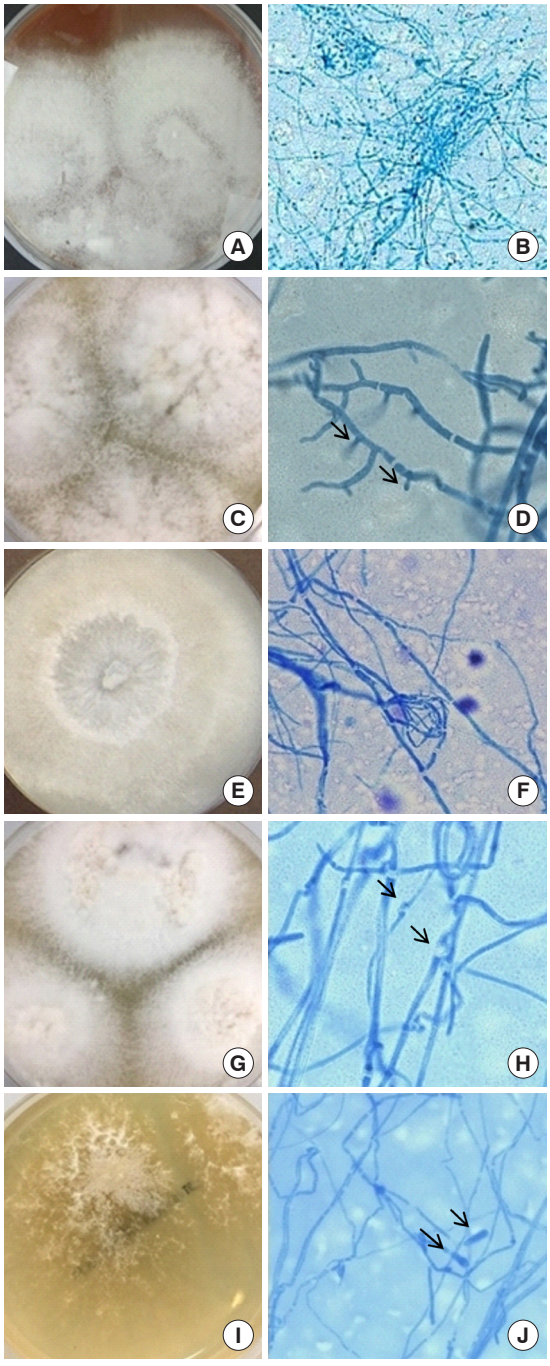

Fig. 1. White mold growth in five cases of basidiomycetes inoculated on Sabouraud dextrose agar (SDA) following incubation at 28°C under light for two weeks; lactophenol cotton blue mount of the mold slide culture on SDA at a magnification of ×400. (A) White colony and (B) hyaline hyphae with clusters of rectangular arthroconidia of *Coprinopsis cinerea*; (C) white colony and (D) spicules (arrows) of *Emmia lacerata*; (E) white colony of *Irpex lacteus*; (F) *I. lacteus* slide showing only hyaline hyphae; (G) white colony and (H) clamp connections (arrows) of *Schizophyllum commune*; (I) white colony of *Porostereum spadiceum*; (J) *P. spadiceum* showing enlarged hyphae (arrows).

guidelines with a few modifications [5]. The isolates were cultured on potato dextrose agar for five days at 28°C and then shifted to 37°C incubation for five days for sporulation. The final inoculum of the homogenized fungal hyphae was adjusted to a density of 2.5–5.0×10^4 hyphal fragments/mL by adjusting the optical density at 530 nm to 0.13–0.18 using a spectrophotometer (VERSAmax microplate reader, Molecular Devices LLC, CA, USA). The microtiter plate was incubated at 35°C for 72 hours. Results were obtained only for *S. commune* and *P. spadiceum*. They showed low minimum inhibitory concentrations (MICs) for flucytosine, amphotericin B (AMB), voriconazole (VRC), itraconazole, and ketoconazole and high minimum effective concentrations for micafungin, caspofungin, and anidulafungin. For the remaining three isolates, we could not determine the MICs.

C. cinerea is normally found in compost and sewage; however, it rarely causes pulmonary infections, endophthalmitis, endocarditis, and chronic sinusitis [2]. A previous study reported an isolate susceptible to voriconazole and posaconazole but resistant to AMB, caspofungin, and micafungin [6]. Our patient also recovered from fungal pneumonia after treatment with voriconazole and posaconazole.

E. lacerate, an agent of white rot on wood, has recently been reported as a human pathogen with low MICs for azoles but high MICs for echinocandins [7, 8]. To our knowledge, this is the first clinical report of *E. lacerate* in Korea, and the patient recovered from fungal pneumonia after itraconazole treatment.

Another wood-decaying fungus, *I. lacteus*, has been rarely reported. A patient from Austria presenting with a pulmonary abscess was cured following treatment with voriconazole and AMB [9]. Our patient also showed improved chest computed tomography (CT) findings after treatment with voriconazole.

S. commune mainly causes sinusitis, allergic bronchopulmonary disease, fungal ball, and asthma. One Korean patient, who had a sino-orbital infection, was successfully cured using voriconazole and AMB [10], which is in line with our susceptibility test results.

P. spadiceum has also been isolated from respiratory specimens; however, its pathogenicity is yet to be established [2]. A study on antifungal susceptibility test using two isolates of *P. spadiceum* showed low MICs for AMB, voriconazole, and itraconazole [3], similar to our results.

As BM have been reported as human pathogens only recently and most of these reports mainly detail isolate characteristics, there are few clues to aid in the diagnosis of infection caused by these emerging pathogens. Of our five patients, three showed supportive chest CT findings, such as consolidation, nodular

https://doi.org/10.3343/alm.2020.40.1.84

opacity, air-fluid level, and cavitation, while the other two patients showed only pleural effusions that were insufficient to support fungal pneumonia. However, we hypothesized that these two cases were fungal infections because the patients were immunocompromised, and the respiratory tract specimens were negative for acid-fast bacilli, aerobic pathogens, and respiratory viruses. In addition, *S. commune* is the most common BM that causes respiratory tract infections in immunocompromised patients. *P. spadiceum* was isolated from a bronchial wash specimen. Of note, the three patients treated with appropriate antifungal agents survived, while the other two patients who received only antibiotics died.

To our knowledge, this is the first report on respiratory infections caused by BMs in Korea. Although there was insufficient clinical evidence for *S. commune* and *P. spadiceum* infections, it is still important to identify these rare pathogens using molecular techniques to accumulate data. These efforts will help us better understand these infections and develop future diagnostic and therapeutic guidelines.

Author Contributions

All authors contributed equally to this study.

Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Research Funding

This work was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Republic of Korea (HI16C0443), and the Korea Medical Fungal Pathogen Resource Bank (KMFRB) under National Culture Collection for Pathogens (NCCP) of the Korea Center for Disease Control and Prevention (SPRB-2018-02).

ORCID

Jong-Mi Lee https://orcid.org/0000-0003-2890-5285
Eunhee Han https://orcid.org/0000-0001-6519-1539
Jayoung Kim https://orcid.org/0000-0003-2977-1813
Ji-Hyun Park https://orcid.org/0000-0002-3002-6856
Gi-Ho Sung https://orcid.org/0000-0002-1861-5543
Jong Hee Shin https://orcid.org/0000-0001-9593-476X
Yeon-Joon Park https://orcid.org/0000-0003-2182-5821

REFERENCES

1. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008;46:1813-21.
2. Chowdhary A, Kathuria S, Agarwal K, Meis JF. Recognizing filamentous basidiomycetes as agents of human disease: a review. Med Mycol 2014; 52:782-97.
3. Singh PK, Kathuria S, Agarwal K, Gaur SN, Meis JF, Chowdhary A. Clinical significance and molecular characterization of nonsporulating molds isolated from the respiratory tracts of bronchopulmonary mycosis patients with special reference to basidiomycetes. J Clin Microbiol 2013; 51:3331-7.
4. CLSI. Interpretive criteria for identification of bacteria and fungi by DNA target sequencing. CLSI MM18-A. Wayne, PA: Clinical and Laboratory Standards Institute. 2007.
5. CLSI. Reference method for broth dilution antimicrobial susceptibility testing of filamentous fungi. Approved standard 3rd ed. CLSI M38. Wayne, PA: Clinical and Laboratory Standards Institute. 2017.
6. Abuali MM, Posada R, Del Toro G, Roman E, Ramani R, Chaturvedi S, et al. *Rhizomucor variabilis* var. *regularior* and *Hormographiella aspergillata* infections in a leukemic bone marrow transplant recipient with refractory neutropenia. J Clin Microbiol 2009;47:4176-9.
7. Chowdhary A, Agarwal K, Kathuria S, Singh PK, Roy P, Gaur SN, et al. Clinical significance of filamentous basidiomycetes illustrated by isolates of the novel opportunist *Ceriporia lacerata* from the human respiratory tract. J Clin Microbiol 2013;51:585-90.
8. Brandt ME. Filamentous basidiomycetes in the clinical laboratory. Curr Fungal Infect Rep 2013;7:219-23.
9. Buzina W, Lass-Flörl C, Kropshofer G, Freund MC, Marth E. The polypore mushroom *Irpex lacteus*, a new causative agent of fungal infections. J Clin Microbiol 2005;43:2009-11.
10. Sa HS, Ko KS, Woo KI, Peck KR, Kim YD. A case of sino-orbital infection caused by the *Schizophyllum commune*. Diagn Microbiol Infect Dis 2012;73:376-7.