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MALDI-TOF MS와 D2 rRNA 직접 염기서열분석으로 확인한 *Candida parapsilosis*와 *Trichosporon asahii*가 동시 분리된 진균혈증 1예

A Case of Fungemia with Co-isolation of *Candida parapsilosis* and *Trichosporon asahii* Confirmed by MALDI-TOF MS and D2 rRNA Sequencing

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Fungi are a major cause of human infections with diverse clinical manifestations. The incidence of fungal infections has increased over time, particularly in patients who have risk factors such as neutropenia, immune suppression, an intravascular catheter, parenteral nutrition, a prosthetic device, and prior broad spectrum antibiotic therapy. Here, we present an unusual case of co-infection by 2 distinct fungi, *Candida parapsilosis* and *Trichosporon asahii*, isolated from a patient who did not have any known risk factors initially, except active pulmonary tuberculosis. Despite the negative conversion of sputum acid-fast bacilli (AFB) culture test after treatment, clinical symptoms were refractory to therapy. The patient developed symptoms suggesting septic shock, and 2 distinct colonies were isolated from a blood specimen, which were identified as *C. parapsilosis* and *T. asahii* by MALDI-TOF and rRNA sequencing. Fever and hypotension were relieved after anti-fungal agent injection, and pulmonary lesions identified by imaging also improved.

Key Words: Fungemia, *Candida parapsilosis*, *Trichosporon asahii*, MALDI-TOF MS, D2 rRNA sequencing

INTRODUCTION

The incidence of fungal infections has markedly increased over the past few decades, particularly among immunocompromised patients and patients with serious general clinical status [1]. *Candida* species are currently a major cause of sepsis acquired in health care institutions [2] and the most common cause of blood stream fungal infections [3, 4]. Among *Candida* species, the incidence of *Candida parapsilosis* infection has rapidly increased [1]. This species is the second most common pathogen isolated from blood cultures of patients with candidemia in the USA [5]. Several studies of the worldwide incidence of *Candida* infections have reported that *C. parapsilosis* is a more common isolate than *Candida albicans* in some non-US regions [6]. *C. parapsilosis* is responsible for many clinical diseases such as fungemia [5, 7], endocarditis [8], meningitis [9], and peritonitis [10]. In addition to *Candida*, *Trichosporon* species are associated with diverse clinical symptoms, from mild to fatal [11]. *Trichosporon* species are widely distributed; they are sometimes present on the human body as

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microbiota [12] and occasionally cause opportunistic infections. Some species, such as *Trichosporon asahii, Trichosporon mucoides*, and *Trichosporon asteroides*, cause systemic infections [11, 12]. These uncommon opportunistic pathogens can infect patients who have the following risk factors: a vascular catheter, prior antibiotic therapy, immunosuppressive therapy, malignancy, being a transplant recipient, neutropenia, and parenteral nutrition for candidemia [13, 14], as well as hematologic disorders, immunosuppression, peritoneal dialysis, and solid tumors for trichosporonosis [15]. Infections by these pathogens can cause similar clinical symptoms, however each pathogen can develop resistance against a different antifungal agent [8, 16]. Therefore, accurate identification of the pathogen is essential for appropriate patient care. There are some reports of co-isolation of *C. parapsilosis* and *T. asahii* from the same patients in different locations and from different specimens [16], and co-isolation of other *Candida* and *Trichosporon* species from hair samples, which cause superficial infection [17]. Here, we report a case of simultaneous isolation of *C. parapsilosis* and *T. asahii* from a venous blood sample taken from a patient diagnosed with active pulmonary tuberculosis. To the best of our knowledge, this is the first report of the co-isolation of these two microorganisms from a patient blood sample.

CASE REPORT

A 57-year-old male visited the emergency room of Seoul National University Hospital, due to mild cough and dyspnea on exertion during the previous month, accompanied by weight loss of 10 kg over the last 2 years. The patient had no clinically significant history other than tuberculosis treated with medication 40 years prior and hemorrhoid for which he underwent surgery 5 years prior. Consolidations in both lungs were observed by chest radiography, and the presence of multiple enlarged lymph nodes with multiple cavitary consolidations in both lungs was confirmed by chest CT. Acid-fast bacilli (AFB) in sputum (grade 1+) were observed by Ziehl–Neelsen staining, and the presence of *Mycobacterium tuberculosis* without the rifampicin resistance gene was confirmed by molecular testing using Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA). *M. tuberculosis* was isolated from the sputum specimen taken after hospitalization, by culturing in Ogawa medium (grade 2+ by Ziehl–Neelsen staining). A blood culture test performed to investigate the possibility of bacterial co-infection was negative. The patient was treated with the first-line agents for tuberculosis, including isoniazid, rifampicin, ethambutol, and pyrazinamide. Despite continuous growth of AFB in a Mycobacteria Growth Indicator Tube (MGIT; BD Diagnostics, Sparks, MD, USA) and the presence of the microorganism in the patient's sputum confirmed by culture and staining, clinical symptoms improved. After 2 weeks of treatment, the patient was discharged from the hospital; he was given medication and ordered to visit ambulatory care for regular checks.

He revisited the hospital for a follow-up 2 weeks later with sustained clinical symptoms, including cough, sputum, and dyspnea, despite regular medication. A chest radiography showed increased opacity and extent of pulmonary lesions, suggesting disease progression, and the clinicians advised hospitalization because of possible treatment failure. In addition to the first-line agents, prothionamide, cycloserine, para-aminosalicylate, and streptomycin were added to the treatment. Moxifloxacin and amoxicillin/clavulanate were also administered to control possible unproven bacterial co-infection. Pulmonary lesions worsened gradually as determined by radiography and follow-up CT-imaging, despite treatment using an alternative regimen for tuberculosis even after streptomycin was replaced with amikacin on hospital day (HD) 9. Administration of metronidazole and vancomycin was initiated to treat recurrent mild diarrhea, which occurred after hospitalization. All the anti-tuberculosis drugs and antibiotics used were ineffective for the control of respiratory symptoms, and linezolid, which was used to replace vancomycin, was also ineffective.

Tests were performed to identify the possible cause of the poor therapeutic response, such as drug resistance, inappropriate drug dosing, or impaired immunity. The *M. tuberculosis* isolates did not show any resistance to any of the first-line drugs, and molecular testing for the *NAT2* gene encoding the enzyme N-acetyltransferase 2 to evaluate isoniazid metabolism revealed it to be wild type. The patient did not show any evidence of metabolic syndromes causing immunosuppression, and the results of human immunodeficiency virus (HIV) antigen/antibody assay and HIV viral load quantitative test were negative. The interferon-gamma release assay test appeared positive, and Mantoux test showed 20 mm induration, indicating normal T cell immunity.

The extent of disease was increased as confirmed by imaging, and the patient's clinical status deteriorated continuously. From HD 22, methylprednisolone infusion was initiated to treat the

progressing pulmonary lesions. At HD 25, high fever and hypotension with systolic blood pressure <90 mmHg appeared, and the possibility of septic shock was considered. However, the source of systemic infection could not be identified. Sputum collected at HD 3 was the last specimen that revealed positive results from *M. tuberculosis* culture; specimens from HD 7 and later showed negative results in MGIT and Ogawa media, except for occasional ordinary bacterial contamination. Intermittent positive results were identified only by smear staining. Serial blood culture tests for evaluation of co-infection with other infectious agents consistently showed no growth. Parenteral nutrition support was supplied to the patient through a peripheral intravascular catheter from HD 30 to treat ongoing weight loss because of diarrhea and decreased oral intake because of anorexia. The clinical status showed no significant changes, methylprednisolone infusion was tapered, and blood culture tests showed negative results until HD 42. From HD 43, episodic events of high fever and hypotension arose intermittently, and prednisolone infusion was initiated.

Microbial growth was detected for the first time in a blood culture test from a specimen taken at HD 51, during which 2 distinct colonies grew on blood agar plates. Cream-colored, smooth colonies (Fig. 1) and cream-colored, moist, wrinkled colonies (Fig. 2) were isolated on Sabouraud Dextrose Agar by sub-isolation. Gram staining revealed that the 2 distinct colonies consisted of yeast-like cells. After selective cultivation of cells from each colony, an identification test was performed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Bremen, Germany) and the 2 isolates were identified as *Candida parapsilosis* (score, 1.854) and *Trichosporon*

Fig. 1. (A) Colonial morphology of *Candida parapsilosis* isolates with cream-colored, smooth colonies. (B) Magnification of a portion of the image shown in (A).

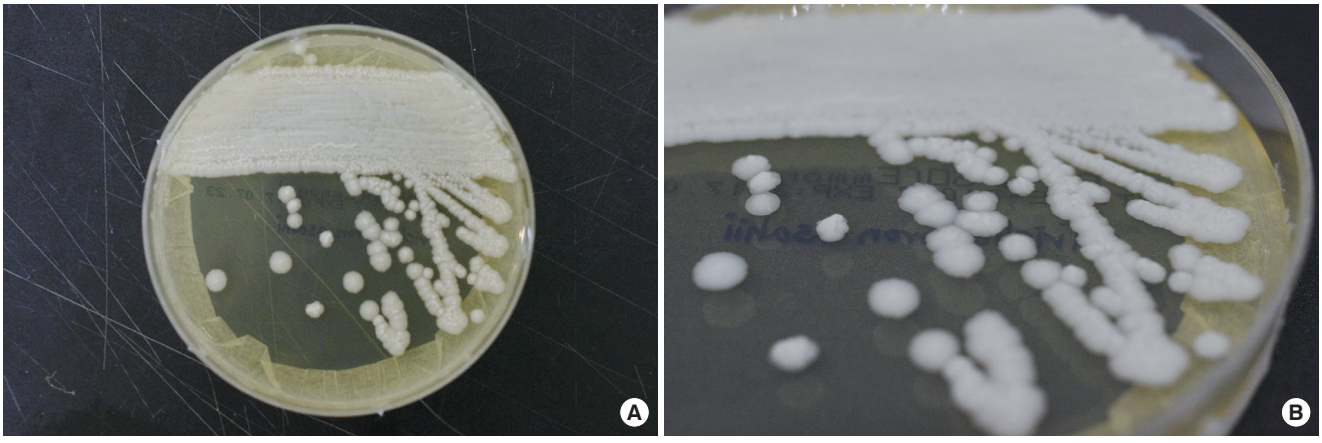

Fig. 2. (A) Colonial morphology of *Trichosporon asahii* isolates with cream-colored, moist, wrinkled colonies. (B) Magnification of a portion of the image shown in (A).

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asahii (score, 1.723), indicating probable genus-level identification. For confirmation, sequence analysis was performed on a MicroSeq 500 system (Applied Biosystems, Foster City, CA, USA) using a MicroSeq D2 LSU rDNA fungal identification kit. The sequences were analyzed using an ABI PRISM 3730xl DNA analyzer. The sequence from one isolate showed 100% identity with that of *C. parapsilosis* and the sequence from another isolate showed 98.23% identity with that of *Trichosporon faecale* in MicroSeq ID Fungal Gene Library v2013, which did not include a *T. asahii* reference sequence. The latter sequence was confirmed to share 100% identity with a *T. asahii* sequence by using NCBI BLAST. When antifungal susceptibility testing was performed using the VITEK-2 yeast susceptibility test (AST-YS01; bioMérieux, Hazelwood, MO, USA), *C. parapsilosis* was confirmed to be susceptible to all 4 anti-fungal drugs tested (amphotericin B, MIC ≤0.5 mg/L; fluconazole, ≤1 mg/L; voriconazole, ≤0.12 mg/L; flucytosine, ≤1 mg/L). The same fungi were isolated from a blood sample taken at HD 54. Combination therapy of amphotericin B and voriconazole was initiated, and *T. asahii* was not isolated in subsequent blood culture tests performed with samples taken at HD 60 and later. The sample taken at HD 60 was the last specimen from which *C. parapsilosis* was isolated. Although clinical symptoms originating from pulmonary tuberculosis persisted, sustained high fever and hypotension, which probably arose from fungemia, subsided and the extent of pulmonary consolidation was confirmed to be decreased by follow-up imaging. No other bacteria or fungi were isolated from the patient's blood. Following clinical improvement, the patient was discharged at HD 98, and ambulatory care was used to manage underlying tuberculosis, without recurrence of fungemia.

DISCUSSION

Fungi are present as normal flora in the natural environment and human body, and generally do not cause clinical disease [1, 2]. Yet, these microorganisms can be the source of opportunistic infections when the barrier integrity in the body is disrupted. The incidence of nosocomial fungal infections in patients with known risk factors [13-15] is consistently increasing [1, 2].

In the present case, the patient visited a hospital because of respiratory symptoms and was initially diagnosed with active pulmonary tuberculosis in accordance with clinical symptoms, radiologic findings, and results of sputum smear staining. No evidence of blood stream infection was observed during the 2 weeks of hospitalization, and the patient was discharged. Despite regular medication administered according to instructions, the symptoms became aggravated and the patient was re-admitted to hospital. *Mycobacterium* culture was converted to negative soon after administration of anti-tuberculosis drugs and antibiotics, but the clinical symptoms did not improve significantly. After long-term use of antibiotics and parenteral nutrition support, blood culture tests became positive and a sepsis-like event occurred and 2 fungal pathogens, *C. parapsilosis* and *T. asahii*, were isolated from the patient's blood. Symptoms of high fever and hypotension were relieved after antifungal treatment (amphotericin B plus voriconazole), and the fungi became undetectable in blood culture a few days later.

In this case, the patient initially did not have any known risk factors for opportunistic fungal infections. During admission, antibiotics such as moxifloxacin and amikacin were added to treat active tuberculosis, which was refractory to first-line regimen. Metronidazole and vancomycin were injected to treat diarrhea. Parenteral nutrition support was started to address ongoing weight loss likely caused by diarrhea and loss of appetite. As described in previous studies, these conditions, including prior antibiotic therapy, use of intravascular catheter, and parenteral nutrition, can be risk factors for opportunistic fungal infections [13, 14].

Candida and *Trichosporon* species largely contribute to the current increase in fungal infections, and some species, such as *C. parapsilosis* and *T. asahii*, can cause systemic infections such as fungemia [5, 7, 11]. Many cases of blood stream infections caused by each pathogen have already been reported in Korea [7, 18, 19], but to the best of our knowledge this is the first Korean case of *C. parapsilosis* and *T. asahii* co-infection identified through multiple diagnostic methods, including culture-base methods, MALDI-TOF analysis, and molecular testing. Systemic fungal infections can be deleterious when the diagnosis is incorrect or delayed, and it is challenging to diagnose fungal infections and identify the pathogen. Future studies should establish rapid and sophisticated diagnostic strategies using various diagnostic techniques for patients with high risk of nosocomial fungal infections.

요 약

호중구 감소, 면역억제, 광범위 항생제의 사용, 혈관내 도관 거치

와 비경구 영양법이 병원 내 진균감염의 위험 요인으로 작용하는 것은 과거의 연구를 통하여 보고된 바 있다. 본 환자는 상기의 위험 요인을 갖지 않은 상태로 활동성 결핵을 주소로 입원하여, 치료 과정 중 광범위 항생제와 중심정맥관 삽입, 비경구 영양법 적용 등의 위험 요인에 노출된 후, 혈액검체로부터 *Candida parapsilosis*와 *Trichosporon asahii*의 동시 감염에 의한 진균혈증의 발생이 MALDI-TOF MS와 직접 염기서열분석을 통하여 확인되었다. 항진균제 투여 후 배양검사에서 음성 소견을 보일 때까지 상기의 균종은 혈액검체에서 각각 3회와 2회에 걸쳐 동정되었고, 이는 한국에서 상기 균종의 동시 감염에 의하여 발생한 진균혈증이 진단된 첫 번째 사례이다.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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