CASE REPORT

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Pneumonia with Empyema Caused by *Cladosporium velox* in a Patient with Rheumatoid Arthritis

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No cases of human infections caused by *Cladosporium velox* have been reported. A 69-year-old female patient with rheumatoid arthritis developed pneumonia with empyema. The fungal cultures of pleural fluid were obtained. *Cladosporium* was grown from the pleural fluid. *C. velox* was identified by internal transcribed spacer sequencing. The pathologic results of biopsied lung tissue indicated the presence of fungal hyphae. *C. velox* can cause clinical infection in an immunocompromised setting.

Key Words: *Cladosporium velox*, Empyema, Pneumonia, Rheumatoid arthritis

INTRODUCTION

Cladosporium species are rare fungal pathogens involved in clinical infection. Here, we report on a patient with rheumatoid arthritis who developed pneumonia with empyema caused by *Cladosporium velox*, which was identified by internal transcribed spacer sequencing. This is the first reported human infection with *C. velox*.

CASE PRESENTATION

A 69-year-old female patient with seropositive rheumatoid arthritis, presented with cough and yellowish sputum, was admitted to the Rheumatology Department of Kyung Hee University Hospital in South Korea. The patient had been taking medications including systemic steroids for osteoarthritis for 20 years. She was diagnosed with seropositive rheumatoid arthritis 5 months before being admitted at our institution. For the previous months, the patient was treated with methotrexate, mizoribine (150 mg daily), and celecoxib for rheumatoid arthritis. The initial vital signs were within the normal range. Chest computed tomography (CT) scan showed two cavitary lesions, which were 4 and 2 cm in size, in the left upper lung and a 4-cm cavitary lesion in the left lower lung (Fig. 1A). CT-guided core needle biopsy of a cavitary lung lesion was performed. The pathologic report noted chronic granulomatous inflammation with necrosis. Acid-fast bacilli

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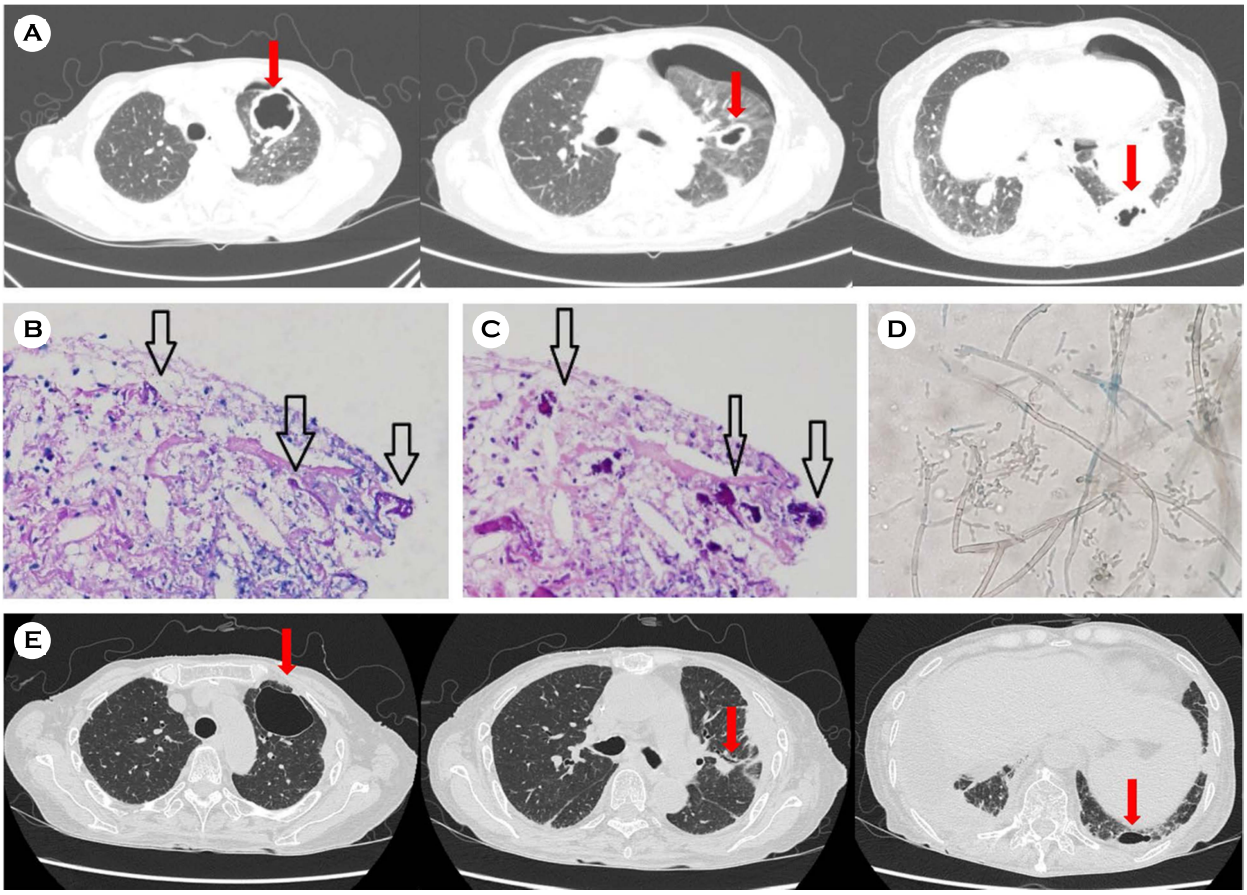

Fig. 1. Radiologic and pathologic findings of the current case (A) Contrasted-enhanced chest CT initial showing multiple cavitary lung lesions (B) Hematoxylin and eosin stain, ×200: interstitial fibrosis and chronic inflammation with a focal necrosis containing fungal hyphae (arrow) (C) Periodic-acid Schiff stain, ×200: Fungal hyphae (arrow) (D) Lactophenol cotton blue stain of the cultured colony, ×400: conidophores with oval conidia chains and septate hyphae (E) The cavitary mass at the left lung field improved gradually after the 5th month of treatment.

stain and polymerase chain reaction for *Mycobacterium tuberculosis* with the obtained tissue were both negative. No bacterial and fungal pathogens were isolated from the sputum and bronchoalveolar lavage fluid. The patient was prescribed only mizoribine for rheumatoid arthritis and did not develop fever during hospitalization. A chest tube was inserted for drainage of pleural effusion on the left lung field after 45 days of admission. In laboratory examinations, white blood cell count was 5.36 × 10^9/L (62.6% neutrophil), and the level of C-reactive protein (normal value < 48 nmol/L) was 34.7 nmol/L. Pleural fluid analysis revealed a white blood cell count of 161, 300/μL (neutrophil 96%), and the bacterial culture of pleural fluid was negative. CT-guided biopsy of the left cavitary mass was repeatedly performed. The pathologic results indicated the presence of interstitial fibrosis and chronic inflammation with a focal necrosis containing fungal hyphae at the pulmonary tissue (Fig. 1B and 1C). *Cladosporium* was identified from three sets of fungal culture of the pleural fluid. Dry, dark, and olivaceous green colonies were grown on Sabouraud dextrose agar at 25℃ after 7 days of incubation. Septate hyphae with a separated, branched, and acropetal chain of spherical conidia was suggestive for *Cladosporium* (Fig. 1D). A fungal culture of the tissue was negative. She was treated with amphotericin B deoxycholate (0.6 mg/kg per day), vancomycin (1 g per day), and imipenem (2 g per day) for about 5 months. The cavitary mass at the left lung fields improved gradually (Fig. 1E). However, the patient died due to progression of hospital-acquired pneumonia caused by an unknown pathogen 8 months since the first admission.

Identification of fungal isolates was assessed via internal

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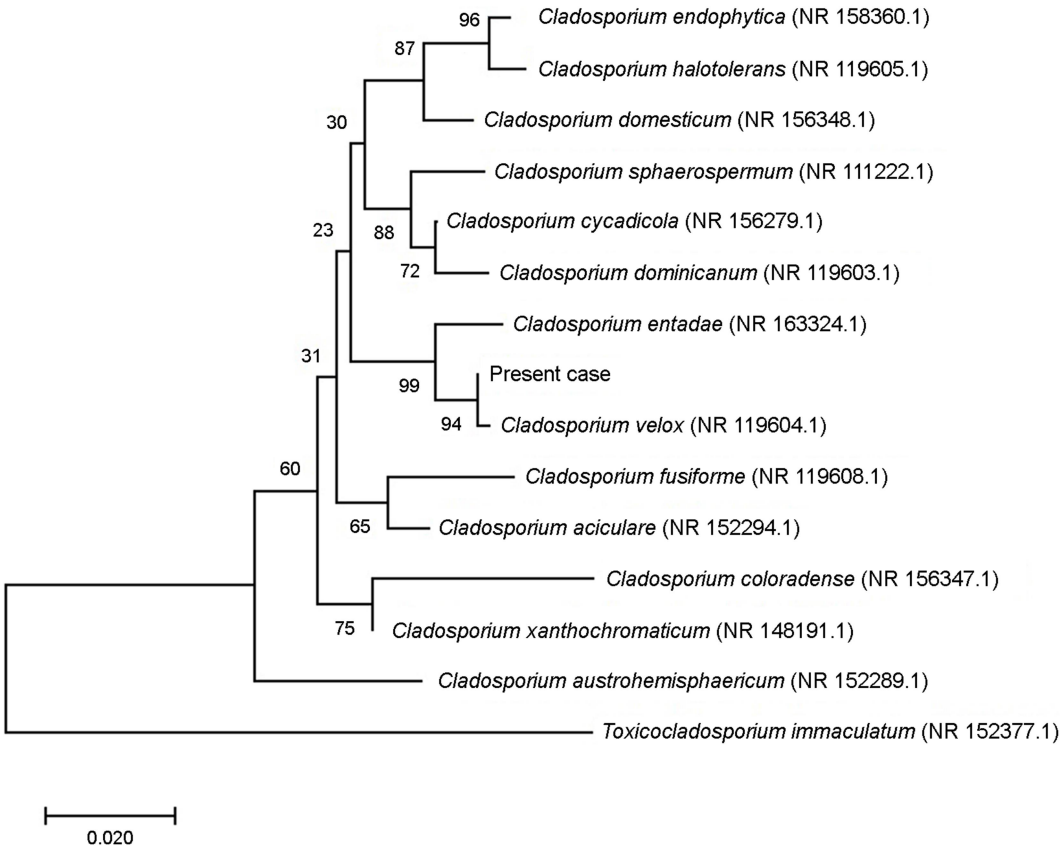

Fig. 2. Neighbor-joining phylogenetic tree based on ITS of *Cladosporium* was constructed using MEGA-X software[2] using Kimura's two-parameter model. GenBank accession numbers are given in parentheses. *Toxicocladosporium immaculatum* was used as an outgroup. The genetic distance was determined according to the bar, which indicates 0.02 changes per nucleotide position.

transcribed spacer (ITS) sequencing and polymerase chain reaction (PCR) of the β-tubulin gene[1]. In the ITS sequencing of the cultured colony, the best matched taxa obtained using NCBI BLAST against the database for ITS from reference material was *C. velox* (sequence ID: NR119604.1) with a similarity of 100% (478/478). The second highest match was *Cladosporium entadae* (Sequence ID: NR_163324.1) with a similarity of 98.4% (479/487). The neighbor-joining tree by MEGA-X[2], based on ITS, showed *C. velox* as the nearest taxa (Fig. 2). Antifungal susceptibilities were determined using the broth dilution method. The minimal inhibitory concentrations (MICs) of each antifungal agent were as follows: 2.0 mg/L for voriconazole, 0.5 mg/L for itraconazole, 64 mg/L for fluconazole, 0.5 mg/L for posaconazole, and 1.0 mg/L for amphotericin B deoxycholate. The real-time PCR of formalin-fixed paraffin-embedded (FFPE) lung tissue containing fungal hyphae was performed using the LC480 (Roche Diagnostics, Mannheim, Germany) with primers recognizing *benA* and ITS, subsequently followed by melting curve analysis. However, fungal isolate was not amplified by tissue real-time PCR. This study was approved by the institutional review board (KHUH 2020-04-028), and it was conducted in compliance with the principles of the Declaration of Helsinki.

DISCUSSION

Cladosporium are ubiquitous and frequently isolated from environmental sources worldwide[3]. The genus *Cladosporium* has undergone extensive revisions by polyphasic approaches[4]. The number of species under *Cladosporium* has increased up to 218 due to continuous isolation from clinical samples and environment[5]. *Cladosporium* species are often isolated from the respiratory tract and superficial/deep tissue infection. Transmission of *Cladosporium* are usually via the airborne route because of their small conidia and tendency to detach[6].

Multilocus gene analysis of the ITS, D1/D2, *EF-1α*, and actin gene is useful for accurate identification of *Cladosporium*[4]. The analysis of *EF-1α* and *actin* genes identify a high degree of divergence among species, while ITS shows a poor species resolution[7,8]. However, *C. velox* in this case was successfully differentiated from other *Cladosporium* by ITS gene analysis. PCR amplification of β-tubulin gene from the tissue samples was attempted, but the result was not successful. This may be due to problems with DNA extraction and the amount/length of DNA for PCR from FFPE tissue.

Cladosporium may cause clinically important infections in immunocompromised hosts, albeit rarely. Deep tissue infection, lymphadenitis, mycotic aneurysm, keratitis, pneumonia, meningitis, brain abscess, and pulmonary fungal ball caused by *Cladosporium* have been reported previously[9-12]. Notably, no evidence of human infections caused by *C. velox* had been reported. To our knowledge, this is the first study to report pneumonia and empyema due to *C. velox* infection. *C. velox* was the most likely pathogen, as the patient improved with antifungal therapy.

The antifungal susceptibilities of *Cladosporium* showed variability according to the species. Overall, amphotericin B, terbinafine, micafungin, anidulafungin, itraconazole, and posaconazole exhibit good antifungal activity against *Cladosporium*, while flucytosine, caspofungin, and voriconazole showed variable *in vitro* activity[13]. The case patient received amphotericin B deoxycholate (MIC = 1.0 mg/L) for pneumonia /empyema due to *C. velox*, resulting in a gradual improvement in cavitary pneumonia with empyema. In our case, the patient with rheumatoid arthritis has been prescribed only mizoribine during hospitalization. Mizoribine is a strong immunosuppressive drug, interrupting DNA synthesis[14]. The rheumatoid arthritis itself combined with the strong immunosuppressive effect of mizoribine may have influenced the response to antifungal therapy for pneumonia/empyema caused by *C. velox* and affected the development of hospital-acquired bacterial pneumonia.

CONCLUSION

We present here a case of pneumonia and empyema caused by *C. velox* in a female patient with rheumatoid arthritis. It is significant to report this case of human infection due to *C. velox*. *Cladosporium* may cause clinically important infections in immunocompromised hosts.

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The authors declare that there are no acknowledgment.

CONFLICT OF INTEREST

In relation to this article, we declare that there is no conflict of interest.

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