The First Isolation of *Aspergillus allahabadii* from a Cormorant with Pulmonary Aspergillosis

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ABSTRACT

In this study, we report the first isolation of *Aspergillus allahabadii* from a Japanese cormorant with pulmonary aspergillosis. We performed molecular identification and antifungal susceptibility testing with the E-test. A 7-month-old male cormorant died because of uric acid deposition secondary to dehydration. Whitish nodular lesions were present on the caudal thoracic air sac in the right thoracic cavity. Histopathology revealed multifocal pyogranulomatous necrotic lesions with numerous fungal hyphae in the thoracic air sac. Identification of the etiologic agent was confirmed by comparative analyses of the sequences of the internal transcribed spacer (ITS) region and \(\beta\)-tubulin-encoding genes. According to the E-test, the minimum inhibitory concentrations of the isolate to amphotericin B, fluconazole, itraconazole, and voriconazole were 0.75 µg/ml, >256 µg/ml, 0.38 µg/ml, and 0.38 µg/ml, respectively.

Key words: *Aspergillus allahabadii*, cormorant, pulmonary aspergillosis

Introduction

Aspergillosis is a common opportunistic infection of the respiratory system in domestic, wild, and pet birds. Infection frequently occurs during hatching as a result of inhaling large numbers of conidia from the environment. The most common etiologic agents are *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus nidulans*, and other *Aspergillus* species\(^6\). The antifungal drugs amphotericin B (AMB), fluconazole (FLZ), itraconazole (ITZ), and voriconazole (VRZ) have been used to treat the infection in birds\(^7\). However, drug resistance has been reported in isolates of *A. fumigatus* from birds\(^7\)\(^\sim\)\(^9\), indicating that molecular identification and antifungal susceptibility testing of isolates is necessary in avian medicine\(^9\).

In this study, we report the first isolation of *A. allahabadii* from a Japanese cormorant with pulmonary aspergillosis. We performed molecular identification and antifungal susceptibility testing using the E-test.

Case report

The patient was a cormorant used for traditional cormorant fishing, which has a 400-year history in Japan. In January 2016, Miyoshi City had extraordinarily cold weather, with temperatures around \(-10\)°C. Cormorants were housed indoors. A total of 14 birds died between January and March, probably because of severe dehydration and uric acid deposition in various organs and tissues. Two cormorants were found dead and...
sent to Gifu University for microbiological and pathological examination on March 1, 2016. Necropsy revealed dehydration and multifocal uric acid deposition in various organs and tissues, especially the cloacae.

A 7-month-old male cormorant had whitish nodular lesions on the caudal thoracic air sac in the right thoracic cavity (Fig. 1). Histological examination revealed multifocal pyogranulomatous necrotic lesions with numerous fungal hyphae in the thoracic air sac (Fig. 2). Necrotic areas were encapsulated with granulomatous tissues with infiltrates of macrophages and lymphocytes.

Samples taken from the nodular lesions were inoculated on Sabouraud dextrose agar and potato dextrose agar and incubated at room temperature. Colonies with white, wrinkled, suede-like surfaces developed within 1 week (Fig. 3). Based on gross and microscopic characteristics, isolates were identified as an Aspergillus species. Therefore, the patient was diagnosed with pulmonary aspergillosis.

Isolation of genomic DNA of the isolate was reported previously. The internal transcribed spacer (ITS) region of the isolated Aspergillus was amplified using the universal fungal primers ITS5 (5’ GGA AGT AAA AGTCGTAACAAGC) and ITS4 (5’ TCC TCC GCT TAT TGA TAG C) .

A segment of the β-tubulin-encoding gene was amplified using the universal fungal primers benA-F (5’ AAT TGG TGC CGC TTT CTG G) and benA-R (5’ AGT TGT CGG GAC GGA ATA G) . The PCR amplification and sequence analyses were performed as described previously. The in vitro susceptibilities of the isolate to the antifungal drugs AMB, FLZ, ITZ, and VRZ were assessed by the E-test method. Comparative sequence analyses by nucleotide Basic Local Alignment Search Tool (BLAST) analysis on the National Center for Biotechnology Information website showed that the sequence of the ITS region of the clinical isolate was 99% identical to that of A. allahabadii (Aspergillus allahabadii NRRL 4539 ITS region, from TYPE material GenBank accession no. NR_135399). The β-tubulin-encoding gene of the clinical isolate was also 99% identical to that of A. allahabadii (Aspergillus allahabadii isolate, NRRL 4101 beta-tubulin gene, partial cds. GenBank accession no. EF669533). Therefore, the clinical
isolate was identified as *A. allahabadii*.

According to the E-test drug susceptibility analysis, the minimum inhibitory concentrations (MICs) of this isolate to AMB, FLZ, ITZ, and VRZ were 0.75 µg/ml, >256 µg/ml, 0.38 µg/ml, and 0.38 µg/ml, respectively.

**Discussion**

To our knowledge, this is the first reported case of avian pulmonary aspergillosis due to *A. allahabadii*. This species is included in the section *Terrei* by morphological and molecular studies. The section *Terrei* also includes *A. terreus, A. niveus* (teleomorph: *Fennelia nivea*), *A. carneus, A. niveus var. indicus, A. ambiguus, A. microcysticus*, and other species. *A. terreus* is an important pathogen in humans. *A. niveus* has been reported to cause pulmonary aspergillosis and is genetically similar to *A. allahabadii*. *A. allahabadii* has not been previously reported to cause illness in humans or animals, and its antifungal susceptibility has not been investigated until now. The clinical isolate in our case was resistant to FLZ and susceptible to AMB, ITZ, and VRZ. These drugs have been used to treat avian patients with aspergillosis, including pulmonary aspergillosis. The MICs of antifungal drugs should be determined before choosing therapy for avian patients with aspergillosis.

In this case, the diagnosis of aspergillosis could not have been made while the cormorant was alive. Since the signs of aspergillosis are nonspecific and unreliable for making a diagnosis, aspergillosis is usually diagnosed based on the medical history, clinical presentation, clinical pathology (hematology and biochemistry), serology, mycology, and diagnostic imaging (radiography and endoscopy). Rapid and noninvasive screening tests for avian aspergillosis are needed.

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**Conflict of Interest**

All authors declare no conflict of interest.

**References**