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Case Report

# A Case Report on *Aspergillus lentulus* Pneumonia

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## ABSTRACT

**Background:** *Aspergillus lentulus* was described as a new species in 2005 but it was isolated from Turkey for the first time.

**Case report:** *A. lentulus* was isolated as the cause of pneumonia from a patient who had renal transplantation 4 months ago. The patient received immunosuppressive treatment after transplantation. *A. lentulus* was isolated from his sputum as an agent in pneumonia developed 4 months after the transplantation. Leukocytes, blastospores, and hyphae were seen in both Gram- and Giemsa-stained smears of the sputum. The isolate was identified by using the Maren A. Klich algorithm and molecular methods and confirmed by the reference laboratory of the CBS Fungal Biodiversity Centre (The Netherlands). In the susceptibility tests of the isolate, minimal inhibitory concentrations for amphotericin B, voriconazole, posaconazole, and caspofungin were found to be 0.5 µg/mL, 0.25 µg/mL, 0.125 µg/mL, and 0.25 µg/mL, respectively. The patient recovered with voriconazole treatment (2x200 mg/day).

**Conclusion:** The use of the molecular tests is important for identification of *A. lentulus* strains because they are very easily confused with *A.fumigatus* strains according to phenotypic characteristics.

**Key Words:** Invasive pulmonary aspergillosis, immunocompromised host, *Aspergillus lentulus*

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## Introduction

*Aspergillus* fungi are widely distributed all over the world in soil and decayed materials (1). At the same time, these *Aspergillus* species are the most common agents of invasive infections caused by mould in the world (2). They are in second place after *Candida* spp. when considering all invasive fungal infections (3).

Invasive aspergillosis is usually seen in an immunocompromised host and is an illness related to haematological malignancy, bone marrow/solid organ transplantation, or corticosteroid therapy (4, 5). Today, as the mentioned risk factors increase, the incidence of this disease increases at the same rate (5). The most common cause of invasive aspergillosis is *Aspergillus fumigatus* (6, 7). Other causative agents are *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus flavus*, and other species belonging to section Fumigati (5). Recently, *A. lentulus* has also been reported as a cause of invasive aspergillosis (6, 8, 9).

*A. lentulus* was described by Balajee et al. (9) as a new species in 2005. Firstly, a variant of the weak sporulated *A. fumigatus* was isolated from patients with haematopoietic stem cell transplantation as the agent of invasive infection. These strains were found to be different from other species according to characteristics of the phylogenetic origins as well as their different antifungal sensitivities, and they were named as *A. lentulus* (9). In this paper, the characteristics of the first isolate of *A. lentulus* in Turkey are presented.

## Case Report

The case was a 36-year-old male with chronic renal failure who had been having continuous ambulatory peritoneal dialysis for 8 years. Cadaveric renal tissue was transplanted to the patient approximately 4 months ago. Anti-thymocyte globulin and corticosteroid were initially administered to the patient for immunosuppressive treatment after transplantation. Later, the patient's therapy was continued with tacrolimus, mycophenolate mofetil, and low-dose corticosteroid. The case was admitted by the Trakya University Hospital in treatment of a cough complaint. He had auscultation sign and radiological infiltration in the right lung. In laboratory investigation, his leucocyte count was 6000/µL in haemogram, ESR was 74 mm/hour, and CRP was 8.44 mg/dL. Leukocytes, blastospores, and hyphae were seen in both Gram- and Giemsa-stained smears of his sputum. *A. lentulus* was isolated from his sputum as a causative agent of pneumonia that developed 4 months after the renal transplantation. It was again isolated in the second sputum and bronchoalveolar lavage samples. Invasive pulmonary aspergillosis was diagnosed according to clinical, radiological, and microbiological findings in this immunosuppressed host.

The strain was identified by using the Maren A. Klich algorithm and molecular methods. It was incubated for 7 days after inoculation of Czapek Agar (25˚C), 20% sucrose Czapek Yeast Agar (25˚C), malt extract agar (25˚C), and Czapek Yeast Agar (25-37˚C). The diameter of *Aspergillus* colonies were 32 mm, 35 mm, 36 mm, and 41-47 mm, respectively (Figure 1).

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**Table 1. Characteristics of some cases with colonisation or infection caused by Aspergillus lentulus in the literature**

| Underlying disease | Infection/ Colonisation | Immunosuppressive therapy history | Isolation sample | Reference |
|---|---|---|---|---|
| Chronic obstructive pulmonary disease (COPD) | Exacerbation of COPD | Yes | Bronchial aspirate | Alhambra et al. (3) |
| 4 haematopoietic stem cell transplant recipients | Invasive aspergillosis | No data | Not data | Balajee et al. (9) |
| Arterial hypertension and end-stage chronic kidney disease | Pneumonia | Yes | Bronchoalveolar lavage fluid (BAL) | Montenegro et al. (8) |
| Cystic fibrosis | Colonisation of the airways | No | Sputum | Symoens et al. (13) |
| Heart transplantation for ischaemic cardiomyopathy | Pneumonia | Yes | 1 BAL, 1 sputum, and 2 tracheal bronchial secretions | Zbinden et al. (14) |
| Renal transplant recipient | Pneumonia | Yes | Sputum | The present study |

The appearance of the colony was downy to powdery in texture and greenish to white in colour. The reverse colour was cream. Branched conidiophore structures were observed in direct microscopic analysis of the strain prepared with Saboraud Dextrose Agar. Biseriate phialides arising from the round vesicles were also observed (Figure 2). The identification of the *A. lentulus* strain was confirmed with molecular methods by the reference laboratory of the CBS Fungal Biodiversity Centre (The Netherlands). Its susceptibility was tested by the broth dilution method (10). In the susceptibility tests of the strain, minimal inhibitory concentrations (MICs) for amphotericin B, voriconazole, posaconazole, and caspofungin were found to be 0.5 µg/mL, 0.25 µg/mL, 0.125 µg/mL, and 0.25 µg/mL, respectively. The patient recovered with voriconazole treatment (2x200 mg/day).

## Discussion

Fungal spores of *Aspergillus* or the other saprophytes in the air may reach the respiratory system and can easily cause infections in an immunocompromised host (1). According to the order of frequency, *A. fumigatus*, *A. flavus*, and *A. niger* were recently isolated as agents of invasive aspergillosis in our hospital (11). Isolations of *A. lentulus* defined in 2005 as a kind of new *Aspergillus* species were reported in America, Japan, South Korea, Australia, and Spain (9, 12). The characteristics of some cases with colonisation or infection caused by *A. lentulus* in the literature are shown in Table 1 (3, 8, 9, 13, 14). However, an isolation of or infection caused by *A. lentulus* was not reported in Turkey until today.

Recently, some fungi in section Fumigati have often been misdiagnosed as *A. fumigatus*. Today, the use of molecular methods is recommended to prevent false identification of the species in the *A. fumigatus* complex (2, 6). The strain isolated in the present study resembled *A. fumigatus* according to many features but its branched conidiophore on microscopic examination attracted attention; therefore, it was thought to be of a different kind. The strain was identified as *A. lentulus* by the reference laboratory to which it was sent for confirmation by molecular methods.

Although the strains of *A. fumigatus* are intrinsically sensitive to itraconazole, voriconazole, posaconazole, and amphotericin B, *A. lentulus* strains usually have higher MIC values for these drugs and caspofungin (6, 7, 15). Average MIC values of eight *A. fumigatus* strains, agents of invasive aspergillosis in our hospital, were 2 µg/mL, 0.25 µg/mL, 0.064 µg/mL, and 0.064 µg/mL for amphotericin B, voriconazole, posaconazole, and caspofungin, respectively (16). The *A. lentulus* strain in the present study had a fourfold lower MIC for amphotericin B, similar MIC for voriconazole, twofold higher MIC for posaconazole, and fourfold higher MIC for caspofungin in comparison with the *A. fumigatus* strains. As pointed out by Staab et al. (17), *A. lentulus* may show in vitro low and variable susceptibility for amphotericin B, itraconazole, voriconazole, and echinocandins. It is interesting that the susceptibility characteristics of the present strain are slightly different to that antifungal susceptibility reported in the literature. It is also interesting that two out of 14 *A. lentulus* strains are susceptible to itraconazole in the literature (5). Although these susceptibility tests were repeated eight times, the results were not uniform. Thus, modifications in evaluation of the susceptibility tests to solve different susceptibility results were recommended by the authors (5). In the present study, the susceptibility tests were repeated twice and similar results were obtained. It was evaluated positive in terms of its compliance susceptibility tests due to a good clinical response to voriconazole therapy of the patient.

The different sensitivity characteristics of *A. lentulus* strains compared with *A. fumigatus* strains should be considered in the choice of empirical treatment. The first condition of suitable empirical antifungal therapy is also correct identification of the agent. The use of molecular tests is important for identification of *A. lentulus* strains because they are very easily confused with *A. fumigatus* strains according to phenotypic characteristics.

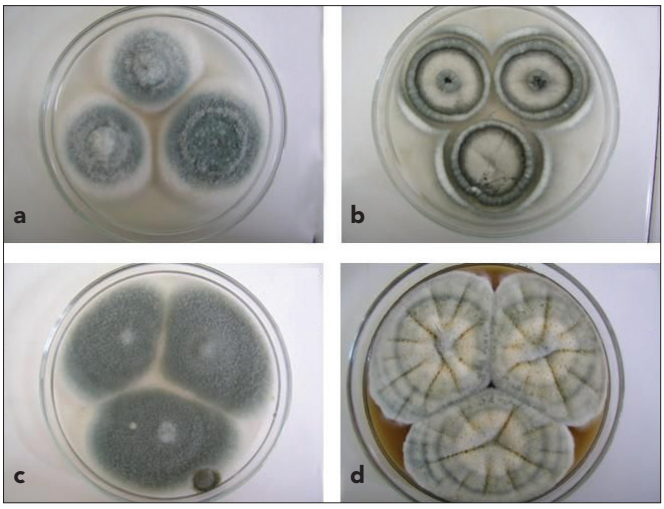


**Figure 1. a-d. Colony appearance in Czapek Agar (37˚C) (a), Czapek Yeast Agar (37˚C) (b), 20% sucrose Czapek Yeast Agar (25˚C) (c), malt extract agar (25˚C) (d)**

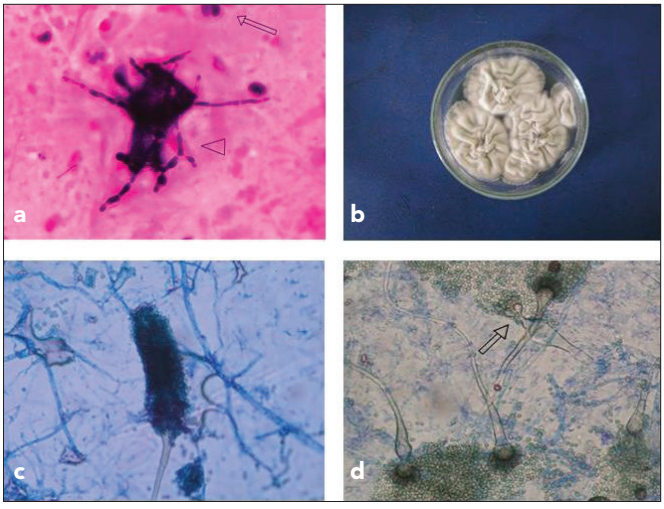


**Figure 2. a-d. Leucocytes (arrow) and hyphae (arrowhead) in direct microscopic analysis of sputum (Gram staining) (a), colony appearance in Saboraud Dextrose Agar (SDA; 42˚C, 3 days) (b), conidiospore structure of the strain in slide culture (SDA, 25˚C) (c), and microscopic appearance of the branched conidiophores (arrow) in SDA (25˚C) (d)**

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