

A Case Report on *Aspergillus lentulus* Pneumonia

Şaban Gürcan¹, Melek Tikveşli¹, Sedat Üstündağ², Beyza Ener³

¹Department of Medical Microbiology, Trakya University Faculty of Medicine, Edirne, Turkey

²Department of Nephrology, Trakya University Faculty of Medicine, Edirne, Turkey

³Department of Medical Microbiology, Uludağ University Faculty of Medicine, Bursa, Turkey

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*

Received: 17.01.2013

Accepted: 02.08.2013

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).



This study was presented at the 35th Turkish Microbiology Congress, 3-7 November 2012, Aydın, Turkey.

Address for Correspondence: Dr. Şaban Gürcan, Department of Medical Microbiology, Trakya University Faculty of Medicine, Edirne, Turkey.
Phone: +90 284 226 02 32 e-mail: sabangurcan@trakya.edu.tr

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 Sabouraud 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.

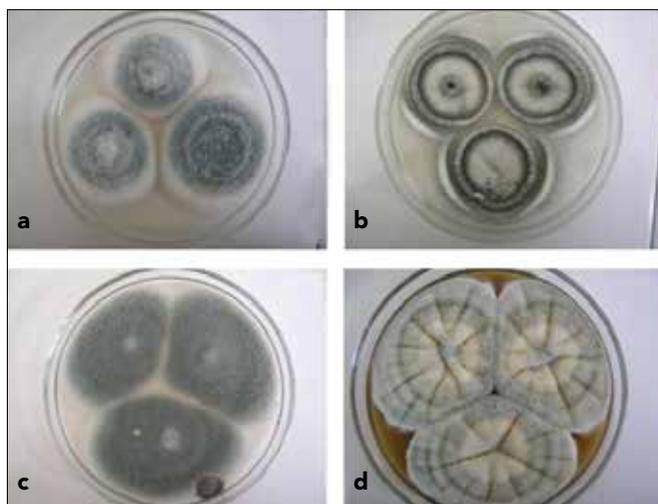


Figure 1. a-d. Colony appearance in Czapek Agar (37°C) (a), Czapek Yeast Agar (37°C) (b), 20% sucrose Czapek Yeast Agar (25°C) (c), malt extract agar (25°C) (d)

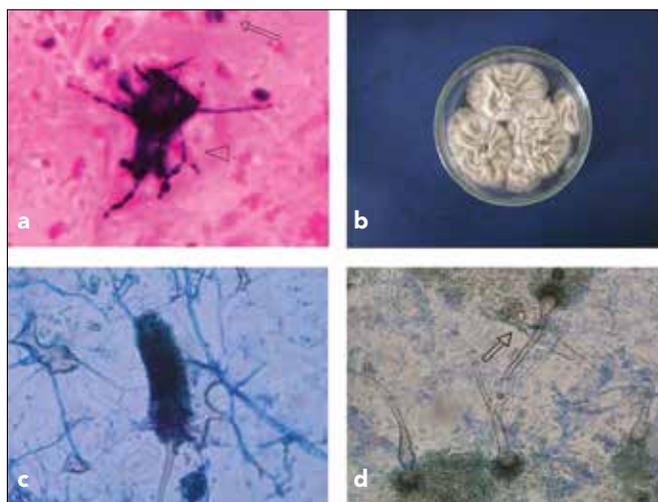


Figure 2. a-d. Leucocytes (arrow) and hyphae (arrowhead) in direct microscopic analysis of sputum (Gram staining) (a), colony appearance in Saboraud Dextrose Agar (SDA; 42°C, 3 days) (b), conidiospore structure of the strain in slide culture (SDA, 25°C) (c), and microscopic appearance of the branched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

Informed Consent: Written informed consent was obtained from patients.

Peer-review: Externally peer-reviewed.

Author contributions: Concept – Ş.G., M.T., S.Ü.; Design – Ş.G., M.T., S.Ü., B.E.; Supervision – Ş.G., M.T., S.Ü.; Design – Ş.G., M.T., S.Ü., B.E.; Resource – Ş.G., M.T., S.Ü.; Materials – Ş.G., M.T., S.Ü.; Data Collection&/or Processing – Ş.G., M.T.; Analysis&/or Interpretation – Ş.G., M.T., B.E.; Literature Search – Ş.G., M.T.; Writing – Ş.G.; Critical Reviews – Ş.G., B.E.

Acknowledgements: The authors would like to thank Robert A. Samson and Neriman Yilmaz (CBS Fungal Biodiversity Centre, The Netherlands) for contributing to the molecular identification process of the strain.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

- Mortensen KL, Mellado E, Lass-Flörl C, Rodriguez-Tudela JL, Johansen HK, Arendrup MC. Environmental study of azole-resistant *Aspergillus fumigatus* and other aspergilli in Austria, Denmark, and Spain. *Antimicrob Agents Chemother* 2010;54:4545-9. [CrossRef]
- Serrano R, Gusmão L, Amorim A, Araujo R. Rapid identification of *Aspergillus fumigatus* within the section *Fumigati*. *BMC Microbiol* 2011;11:82. [CrossRef]
- Alhambra A, Catalán M, Moragues MD, Brena S, Pontón J, Montejo JC, et al. Isolation of *Aspergillus lentulus* in Spain from a critically ill patient with chronic obstructive pulmonary disease. *Rev Iberoam Micol* 2008;25:246-9. [CrossRef]
- Mortensen KL, Johansen HK, Fuursted K, Knudsen JD, Gahrn-Hansen B, Jensen RH, et al. A prospective survey of *Aspergillus* spp. in respiratory tract samples: prevalence, clinical impact and antifungal susceptibility. *Eur J Clin Microbiol Infect Dis* 2011;30:1355-63. [CrossRef]
- Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, Cuenca-Estrella M, Rodriguez-Tudela JL. *Aspergillus* section *Fumigati*: antifungal susceptibility patterns and sequence-based identification. *Antimicrob Agents Chemother* 2008;52:1244-51. [CrossRef]
- Mellado E, Alcazar-Fuoli L, Cuenca-Estrella M, Rodriguez-Tudela JL. Role of *Aspergillus lentulus* 14- α sterol demethylase (Cyp51A) in azole drug susceptibility. *Antimicrob Agents Chemother* 2011;55:5459-68. [CrossRef]
- Alcazar-Fuoli L, Cuesta I, Rodriguez-Tudela JL, Cuenca-Estrella M, Sanglard D, Mellado E. Three-dimensional models of 14- α -sterol demethylase (Cyp51A) from *Aspergillus lentulus* and *Aspergillus fumigatus*: an insight into differences in voriconazole interaction. *Int J Antimicrob Agents* 2011;38:426-34. [CrossRef]
- Montenegro G, Sánchez Puch S, Jewtuchowicz VM, Pinoni MV, Relloso S, Temporitti E, et al. Phenotypic and genotypic characterization of *Aspergillus lentulus* and *Aspergillus fumigatus* isolates in a patient with probable invasive aspergillosis. *J Med Microbiol* 2009;58:391-5. [CrossRef]
- Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. *Eukaryot Cell* 2005;4:625-32. [CrossRef]
- Clinical Laboratory Standard Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard. M38-A. PA: Wayne; 2002.
- Gürcan S, Demir M, Altıay G, Tikveşli M, Kiliç H, Otkun M. *Aspergillus* spp. isolations from respiratory tract samples in Trakya University Hospital. *Tuberk Toraks* 2007;55:160-6.
- Balajee SA, Kano R, Baddley JW, Moser SA, Marr KA, Alexander BD, et al. Molecular identification of *Aspergillus* species collected for the Transplant-Associated Infection Surveillance Network. *J Clin Microbiol* 2009;47:3138-41. [CrossRef]
- Symoens F, Haase G, Pihet M, Carrere J, Beguin H, Degand N, et al. Unusual *Aspergillus* species in patients with cystic fibrosis. *Med Mycol* 2010;48:S10-6. [CrossRef]
- Zbinden A, Imhof A, Wilhelm MJ, Ruschitzka F, Wild P, Bloemberg GV, et al. Fatal outcome after heart transplantation caused by *Aspergillus lentulus*. *Transpl Infect Dis* 2012;14:E60-3. [CrossRef]
- Staab JF, Kahn JN, Marr KA. Differential *Aspergillus lentulus* echinocandin susceptibilities are Fksp independent. *Antimicrob Agents Chemother* 2010;54:4992-8. [CrossRef]
- Gürcan Ş, Tikveşli M, Eryıldız C, Evcı C, Ener B. Antifungal susceptibilities of *Aspergillus* spp. strains isolated from invasive aspergillosis cases. *Mikrobiyol Bul* 2010;44:273-8.
- Staab JF, Balajee SA, Marr KA. *Aspergillus* section *Fumigati* typing by PCR-restriction fragment polymorphism. *J Clin Microbiol* 2009;47:2079-83. [CrossRef]