

Case Report

Invasive aspergillosis caused by cryptic *Aspergillus* species: a report of two consecutive episodes in a patient with leukaemia

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We report a case of two consecutive episodes of invasive aspergillosis caused by cryptic *Aspergillus* species in a patient with leukaemia. A first episode of pulmonary infection was caused by *Aspergillus calidoustus* and *Aspergillus novofumigatus*, and the second episode by *A. novofumigatus* and *Aspergillus viridinutans*. Fungal isolates were identified to species level using traditional and sequencing-based molecular methods.

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Introduction

Invasive aspergillosis (IA) is an opportunistic fungal infection that has become a major cause for concern owing to its high morbidity and mortality rates in a variety of immunocompromised patients and the rapid expansion of populations at risk (Maertens *et al.*, 2002). Development and outcome of IA are largely determined by the underlying disease, and IA can be particularly devastating in haematopoietic stem cell recipients with graft-versus-host disease (GVHD). Nevertheless, fungus-related factors, such as species identity and resistance of isolates to antifungal therapy, can also play a critical role in pathogenesis (Denning, 1998; Marr *et al.*, 2002).

The versatile and ubiquitous fungus *Aspergillus fumigatus* has traditionally been considered the main aetiological agent of IA in humans (Denning, 1998; Marr *et al.*, 2002; Muñoz *et al.*, 2006). However, recent findings have

demonstrated that other fungal organisms previously identified as *A. fumigatus* on the basis of standard mycological procedures actually belong to different – yet sometimes closely related – genospecies within the *Aspergillus* subgenus *Fumigati*, section *Fumigati* (Balajee *et al.*, 2005a, b, 2006, 2007; Hong *et al.*, 2005; Katz *et al.*, 2005; Balajee & Marr, 2006; Yaguchi *et al.*, 2007). Correct identification of these cryptic *Aspergillus* species is important, as they can differ in some clinically relevant characteristics, including virulence and antifungal resistance profile (Balajee *et al.*, 2005a, 2006; Alcazar-Fuoli *et al.*, 2008; Alhambra *et al.*, 2008; Montenegro *et al.*, 2009; Sugui *et al.*, 2010; Van Der Linden *et al.*, 2011).

Apart from *A. fumigatus*, several species within *Aspergillus* section *Fumigati* have been implicated in different human infections, namely, *Aspergillus lentulus* (Alhambra *et al.*, 2008; Montenegro *et al.*, 2009), *Aspergillus viridinutans* (Vinh *et al.*, 2009a; Coelho *et al.*, 2011), *Neosartorya fischeri* (Lonial *et al.*, 1997), *Neosartorya pseudofischeri* (Padhye *et al.*, 1994), *Neosartorya spinosa* (Summerbell *et al.*, 1992), *Neosartorya hiratsukae* (Guarro *et al.*, 2002; Koutrotsos *et al.*, 2010) and *Neosartorya udagawae* (Vinh *et al.*, 2009b; Posteraro *et al.*, 2011). Different *Aspergillus* species seem to be simultaneously involved in several such infections

Abbreviations: BAL, bronchoalveolar lavage; COP, cryptogenic organizing pneumonia; CT, computed tomography; GVHD, graft-versus-host disease; IA, invasive aspergillosis; MEC, minimum effective concentration.

The GenBank/EMBL/DDBJ accession numbers for the *benA* sequences of the *Aspergillus* isolates implicated in this case report are provided in Table 2.

(Alhambra *et al.*, 2008; Montenegro *et al.*, 2009). Nevertheless, the pathogenic potential of most *A. fumigatus*-like species in single infections and/or co-infections remains virtually unknown.

In the present article, we report a double episode of IA in a patient with leukaemia, in which a first infection caused by *Aspergillus calidoustus* (*Aspergillus* section *Usti*) and *Aspergillus novofumigatus* (section *Fumigati*) was followed by a second infection caused by *A. novofumigatus* and *A. viridinutans* (section *Fumigati*). Isolates recovered from clinical samples showed resistance to some azole antifungals commonly used for the treatment of IA. Furthermore, to the best of our knowledge, this seems to be the first ever reported isolation of *A. novofumigatus* from a patient with IA.

Case report

A 48-year-old Caucasian woman was diagnosed with high-risk B-lineage acute lymphoid leukaemia in April 2005. She achieved complete remission after a standard 4-week cycle of chemotherapy followed by consolidation and maintenance therapy until January 2008. In November 2008, she experienced a recurrence. Rescue treatment with hyper-CVAD was prescribed, and she reached a second complete remission.

In June 2009, the patient underwent a haploidentical allogeneic transplant of cord blood cells and CD34⁺ cells, depleted from lymphocytes, from a sibling donor. Neutrophil engraftment occurred (>500 cells mm⁻³ on day +12 after transplantation), and complete cord cell chimerism was achieved on day +54.

At day 30 after transplantation, the patient developed interstitial pneumonitis. The thoracic computed tomography (CT) image showed patchy ground glass infiltrates distributed throughout the lungs. Bronchoscopy and bronchoalveolar lavage (BAL) were performed. Histopathology revealed non-specific alveolar and interstitial damage. Screening of BAL and nasopharyngeal samples for respiratory viruses detected parainfluenza. The results of calcofluor white staining, PCR with *Aspergillus*-specific primers and mycological culture of BAL fluid were negative. Nevertheless, the *Aspergillus* antigen was detected in serum using enzyme immunoassay (Platelia *Aspergillus*, Bio-Rad; 0.97–1.24 ng ml⁻¹) and BAL (0.9 ng ml⁻¹). Treatment was started with oral ribavirin (1 g per day) and liposomal amphotericin B (3 mg kg⁻¹ per day) for 20 days, followed by voriconazole and caspofungin (4 mg kg⁻¹ every 12 h and 50 mg kg⁻¹ per day, respectively) for 16 days, although the patient's condition did not improve.

A second bronchoscopy with BAL was performed on day 50 after transplantation, yielding results similar to those of the previous analyses: non-specific alveolar and interstitial damage, persistence of parainfluenza infection and negative results in mycological tests (culture, calcofluor white staining, *Aspergillus* antigenaemia and specific PCR).

Consequently, a diagnosis of cryptogenic organizing pneumonia (COP) was proposed. Corticosteroids were started (methylprednisolone, 125 mg per day, for 5 days), and both the clinical and the radiological response were excellent.

Corticosteroids were progressively withdrawn. Three months after transplantation, the respiratory process reactivated, and corticosteroids were once again prescribed. Cyclosporin continued to be administered for prophylaxis of GVHD, with voriconazole (4 mg kg⁻¹ every 12 h), acyclovir and quinolones being concomitantly used for microbiological prophylaxis.

In the sixth month post-transplantation (December 2009), after a further reduction of the steroid therapy, the patient presented a new episode of COP, with dyspnoea on minimal exertion and cough. Thoracic CT showed multiple nodular infiltrates in both lungs, and bronchiectasis and patchy ground glass infiltrates, mostly in the upper and middle lobes of the right lung. Voriconazole was discontinued owing to elevated liver enzyme levels. Treatment was started with liposomal amphotericin B (3 mg kg⁻¹ per day), but it was discontinued and replaced with caspofungin (50 mg kg⁻¹ per day) due to decreased creatinine clearance.

In February 2010, the patient's condition improved slightly, although immune recovery was still poor (CD3⁺ <10 cells mm⁻³, CD19⁺=360 cells mm⁻³, NK=109 cells mm⁻³, IgG=160 mg dl⁻¹ and IgM <7 mg dl⁻¹). *Aspergillus* antigen in serum reached 2.6 ng ml⁻¹. A third bronchoscopy and BAL were performed. *Aspergillus* antigen in BAL fluid was 8.2 ng ml⁻¹. Calcofluor white staining was positive, and mycological cultures yielded several colonies of *Aspergillus* identified as *A. calidoustus* and *A. novofumigatus* by partial sequencing of the β -tubulin (*benA*) gene and some additional physiological tests (see Methods). Antifungal susceptibility testing was performed using standard procedures (see Methods). The *A. calidoustus* and *A. novofumigatus* isolates showed high MIC values for itraconazole, posaconazole and voriconazole, while both isolates exhibited lower MIC values for amphotericin B and candins (Table 1). Histopathology of a pulmonary biopsy specimen revealed respiratory mucosa with minimal lung parenchyma and no granulomas. In view of these results, the patient was prescribed micafungin (100 mg per day, duration 20 days).

In March 2010, the patient was admitted to hospital with fever and mild respiratory symptoms. A new thoracic and cranial CT revealed pansinusitis with air-fluid levels in both the maxillary and the sphenoid sinus. Thoracic CT images revealed areas of consolidation on the upper left lobe and right apex, as well as multiple bilateral infiltrates. The serum *Aspergillus* antigen level was 7.4 ng ml⁻¹. Calcofluor white staining of sputum isolates was again positive. Mycological cultures of sputum and BAL samples were positive for slow-growing *Aspergillus* species. Partial *benA* gene sequencing identified the isolates as *A.*

Table 1. Antifungal susceptibility (MICs or MECs, in $\mu\text{g ml}^{-1}$) of the three different *Aspergillus* species isolated

AMB, Amphotericin B (MIC); AND, anidulafungin (MEC); CAS, caspofungin (MEC); ITZ, itraconazole (MIC); MYC, micafungin (MEC); POS, posaconazole (MIC); VRC, voriconazole (MIC).

Species (n)*	AMB	AND	CAS	MYC	ITZ	POS	VRC
<i>A. calidoustus</i> (1)	2	0.015	0.125	0.062	8	>16	4
<i>A. novofumigatus</i> (7)	0.5	<0.03	0.25	<0.03	>8	1	8
<i>A. viridinutans</i> (3)	1	<0.03	1	<0.03	1	0.5	4

*Number of isolates tested.

novofumigatus and *A. viridinutans*. *A. calidoustus* was no longer recovered. The antifungal susceptibility profile of the isolates is shown in Table 1. Due to the patient's critical condition, therapy with caspofungin and liposomal amphotericin B (50 mg kg⁻¹ per day and 3 mg kg⁻¹ per day, respectively) was reintroduced before the results of molecular identification and antifungal susceptibility were known. Three days later, the patient's respiratory condition worsened as a result of progression of pulmonary infiltrates, and progressive desaturation was observed. The patient died of cardiac-respiratory arrest. No autopsy was granted.

The aforementioned clinical data suggested a picture of corticosteroid-sensitive post-transplantation COP. The need for prolonged immunosuppressive therapy and the poor immune reconstitution rendered the patient susceptible to subsequent episodes of invasive fungal infection, of which she eventually died.

Methods

Mycological procedures. Mycological culture of sputum and BAL samples was performed on Sabouraud-chloramphenicol medium (Oxoid). The incubation temperature was 37 °C. Fungal isolates were macro- and microscopically examined according to standard criteria (de Hoog *et al.*, 2000). Isolates and their respective origins are listed in Table 2.

Additionally, to confirm the identification of the *A. calidoustus* isolate (HGUGM-14055), we assessed its growth characteristics at 37 °C on Czapek yeast extract agar (CYA; Difco) and its ability to produce indole. These assays were performed as described by Hageskal *et al.* (2011), but using Kovacs' instead of Ehrlich's reagent to test for indole production.

Molecular identification of fungal isolates. DNA extraction from *Aspergillus* isolates was performed as described previously (Álvarez-Pérez *et al.*, 2010a, b). PCR mixtures for *benA* gene amplification contained 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 20 pmol each forward primer (5'-AATTGGTGCCGCTTTCTGG-3') and reverse primer (5'-AGTTGTCGGGACGGAATAG-3') (Isogen Life Science) (Balajee *et al.*, 2005a, b), 200 μM each dNTP, 1.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and 2 μl

DNA extract. The final volume was adjusted to 50 μl with MilliQ sterilized water (Millipore). Amplification was carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) and consisted of a denaturation step of 2 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 68 °C, and a final extension at 68 °C for 5 min. PCR amplicons were purified with the QIAquick PCR Purification kit (Qiagen Iberia), according to the manufacturer's instructions. Both strands were sequenced (using the same primer sets as in PCR amplifications) with the ABI Prism Big Dye Terminator v3.0 Ready Reaction Cycle Sequencing kit (Applied Biosystems) and analysed on an ABI Prism 3730 sequencer (Applied Biosystems). The sequences obtained were compared with reference sequences from GenBank using the Basic Local Alignment Search Tool (BLAST) software (<http://www.ncbi.nlm.nih.gov/blast>).

Antifungal susceptibility testing. Antifungal susceptibility testing was performed using two broth microdilution methods: EUCAST (2008) and that of the Clinical and Laboratory Standards Institute (CLSI, 2008). The antifungal agents tested were amphotericin B (Sigma-Aldrich), itraconazole (Janssen Pharmaceutical), voriconazole (Pfizer), posaconazole (Merck), caspofungin (Merck), micafungin (Astellas) and anidulafungin (Pfizer). Susceptibility tests were performed at least three times on different days with each strain. *Aspergillus flavus* ATCC 204304 and *A. fumigatus* ATCC 204305 were used as quality control strains.

Discussion

In the past, it was generally accepted, sometimes without significant mycological or clinical evidence, that most cases of IA were caused by *Aspergillus* isolates susceptible to azoles. However, acquired resistance to azoles has been described for *A. fumigatus* (Snelders *et al.*, 2008; Verweij *et al.*, 2009; Howard & Arendrup, 2011), and reports of aspergillosis caused by non-*fumigatus* *Aspergillus* species from different sections showing reduced susceptibility to azoles have multiplied in recent years (Pavie *et al.*, 2005; Panackal *et al.*, 2006; Varga *et al.*, 2008; Vinh *et al.*, 2009a, b; Coelho *et al.*, 2011; Posteraro *et al.*, 2011). Therefore, established dogma on the aetiology and management of IA should be revised. Data from clinical reports could prove invaluable for such a task.

In this article, we present what we believe to be the first description of two consecutive episodes of IA caused by different cryptic species of *Aspergillus*. The first of these episodes was caused by two species belonging to different sections, namely *A. calidoustus* (section *Usti*) and *A. novofumigatus* (section *Fumigati*), while the second episode was caused by two *Aspergillus* section *Fumigati* members (*A. novofumigatus* and *A. viridinutans*). The relevance of this case is also highlighted by the fact that all three *Aspergillus* species implicated showed decreased susceptibility to the azoles commonly used for the treatment of IA.

A. calidoustus is as an emerging mould pathogen (Hageskal *et al.*, 2011). This species is usually confounded with *A. ustus*, and isolates typically show resistance to multiple antifungal drugs (Varga *et al.*, 2008; Alastruey-Izquierdo *et al.*, 2010). *A. viridinutans* was originally isolated in 1954 from rabbit dung (McLennan *et al.*, 1954), but it was later reported in retrospective analyses of culture collections

Table 2. *Aspergillus* isolates recovered and GenBank accession numbers of partial *benA* sequences

Episode	Date	Fungal species	Isolate	Origin	GenBank accession no.
First	11 February 2010	<i>A. calidoustus</i>	HGUGM-14055	Sputum	JX888460
		<i>A. novofumigatus</i>	HGUGM-14028	Sputum	HQ127280
Second	5 March 2010	<i>A. novofumigatus</i>	HGUGM-14158	Sputum	HQ127264
	8 March 2010	<i>A. novofumigatus</i>	HGUGM-14159	Sputum	HQ127265
		<i>A. novofumigatus</i>	HGUGM-14164	Sputum	HQ127263
		<i>A. novofumigatus</i>	HGUGM-14165	Sputum	HQ127273
		<i>A. novofumigatus</i>	HGUGM-14219	BAL	HQ127274
		<i>A. viridinutans</i>	HGUGM-14162	Sputum	HQ127259
		<i>A. viridinutans</i>	HGUGM-14163	Sputum	HQ127257
		<i>A. viridinutans</i>	HGUGM-14220	BAL	HQ127256
	13 March 2010	<i>A. novofumigatus</i>	HGUGM-14222	Sputum	HQ127271

(Katz *et al.*, 2005; Yaguchi *et al.*, 2007; Alcazar-Fuoli *et al.*, 2008) and implicated in clinical cases of chronic IA (Vinh *et al.*, 2009a; Coelho *et al.*, 2011). The more recently described *A. novofumigatus* (Hong *et al.*, 2005) has not been implicated in clinical infections (Balajee & Klaassen, 2009). In fact, to our knowledge, the present work is the first to report the isolation of this *A. fumigatus*-like species from clinical samples.

In conclusion, *A. novofumigatus*, *A. viridinutans* and other *A. fumigatus*-like species can cause refractory infections in immunocompromised patients. Multicentre studies assessing the incidence of these 'unusual' species as causal agents of IA, as well as their antifungal resistance profiles and other clinically relevant traits, should be a priority for investigation.

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References

- Alastruey-Izquierdo, A., Cuesta, I., Houbraken, J., Cuenca-Estrella, M., Monzón, A. & Rodríguez-Tudela, J. L. (2010). In vitro activity of nine antifungal agents against clinical isolates of *Aspergillus calidoustus*. *Med Mycol* **48**, 97–102.
- Alcazar-Fuoli, L., Mellado, E., Alastruey-Izquierdo, A., Cuenca-Estrella, M. & Rodríguez-Tudela, J. L. (2008). *Aspergillus* section *Fumigati*: antifungal susceptibility patterns and sequence-based identification. *Antimicrob Agents Chemother* **52**, 1244–1251.
- Alhambra, A., Catalán, M., Moragues, M. D., Brena, S., Pontón, J., Montejo, J. C. & del Palacio, A. (2008). Isolation of *Aspergillus lentulus* in Spain from a critically ill patient with chronic obstructive pulmonary disease. *Rev Iberoam Micol* **25**, 246–249.

Álvarez-Perez, S., Blanco, J. L., Alba, P. & García, M. E. (2010a). Mating type and invasiveness are significantly associated in *Aspergillus fumigatus*. *Med Mycol* **48**, 273–277.

Álvarez-Perez, S., Mateos, A., Domínguez, L., Martínez-Nevado, E., Blanco, J. L. & García, M. E. (2010b). Polyclonal *Aspergillus fumigatus* infection in captive penguins. *Vet Microbiol* **144**, 444–449.

Balajee, S. A. & Klaassen, C. H. W. (2009). Molecular methods for species identification and strain typing of *Aspergillus fumigatus*. In *Aspergillus fumigatus and Aspergillosis*, pp. 15–28. Edited by J.-P. Latgé & W. J. Steinbach. Washington, DC: American Society for Microbiology.

Balajee, S. A. & Marr, K. A. (2006). Phenotypic and genotypic identification of human pathogenic aspergilli. *Future Microbiol* **1**, 435–445.

Balajee, S. A., Gribskov, J., Brandt, M., Ito, J., Fothergill, A. & Marr, K. A. (2005a). Mistaken identity: *Neosartorya pseudofischeri* and its anamorph masquerading as *Aspergillus fumigatus*. *J Clin Microbiol* **43**, 5996–5999.

Balajee, S. A., Gribskov, J. L., Hanley, E., Nickle, D. & Marr, K. A. (2005b). *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. *Eukaryot Cell* **4**, 625–632.

Balajee, S. A., Nickle, D., Varga, J. & Marr, K. A. (2006). Molecular studies reveal frequent misidentification of *Aspergillus fumigatus* by morphotyping. *Eukaryot Cell* **5**, 1705–1712.

Balajee, S. A., Houbraken, J., Verweij, P. E., Hong, S. B., Yaguchi, T., Varga, J. & Samson, R. A. (2007). *Aspergillus* species identification in the clinical setting. *Stud Mycol* **59**, 39–46.

CLSI (2008). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi*, 2nd edn. Approved Standard, document M38-A2. Wayne, PA: Clinical and Laboratory Standards Institute.

Coelho, D., Silva, S., Vale-Silva, L., Gomes, H., Pinto, E., Sarmiento, A. & Pinheiro, M. D. (2011). *Aspergillus viridinutans*: an agent of adult chronic invasive aspergillosis. *Med Mycol* **49**, 755–759.

de Hoog, G. S., Guarro, J., Gené, J. & Figueras, M. J. (2000). *Atlas of Clinical Fungi*, 2nd edn. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures.

Denning, D. W. (1998). Invasive aspergillosis. *Clin Infect Dis* **26**, 781–803.

EUCAST (2008). EUCAST Technical Note on the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds. *Clin Microbiol Infect* **14**, 982–984.

Guarro, J., Kallas, E. G., Godoy, P., Karenina, A., Gené, J., Stchigel, A. & Colombo, A. L. (2002). Cerebral aspergillosis caused by *Neosartorya hirsutiae*, Brazil. *Emerg Infect Dis* **8**, 989–991.

- Hageskal, G., Kristensen, R., Fristad, R. F. & Skaar, I. (2011). Emerging pathogen *Aspergillus calidoustus* colonizes water distribution systems. *Med Mycol* **49**, 588–593.
- Hong, S.-B., Go, S.-J., Shin, H.-D., Frisvad, J. C. & Samson, R. A. (2005). Polyphasic taxonomy of *Aspergillus fumigatus* and related species. *Mycologia* **97**, 1316–1329.
- Howard, S. J. & Arendrup, M. C. (2011). Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. *Med Mycol* **49** (Suppl 1), S90–S95.
- Katz, M. E., Dougall, A. M., Weeks, K. & Cheetham, B. F. (2005). Multiple genetically distinct groups revealed among clinical isolates identified as atypical *Aspergillus fumigatus*. *J Clin Microbiol* **43**, 551–555.
- Koutrotsos, K., Arabatzis, M., Bougatsos, G., Xanthaki, A., Toutouza, M. & Velegraki, A. (2010). *Neosartorya hiratsukae* peritonitis through continuous ambulatory peritoneal dialysis. *J Med Microbiol* **59**, 862–865.
- Lonial, S., Williams, L., Carrum, G., Ostrowski, M. & McCarthy, P., Jr (1997). *Neosartorya fischeri*: an invasive fungal pathogen in an allogeneic bone marrow transplant patient. *Bone Marrow Transplant* **19**, 753–755.
- Maertens, J., Theunissen, K. & Boogaerts, M. (2002). Invasive aspergillosis: focus on new approaches and new therapeutic agents. *Curr Med Chem: Anti-Infect Agents* **1**, 65–81.
- Marr, K. A., Patterson, T. & Denning, D. (2002). Aspergillosis. Pathogenesis, clinical manifestations, and therapy. *Infect Dis Clin North Am* **16**, 875–894, vi.
- McLennan, E. I., Tucker, S. C. & Thrower, L. B. (1954). New soil fungi from Australian heathland: *Aspergillus*, *Penicillium*, *Spegazzinia*. *Aust J Bot* **2**, 355–364.
- Montenegro, G., Sánchez Puch, S., Jewtuchowicz, V. M., Pinoni, M. V., Relloso, S., Temporitti, E., Iovannitti, C. A. & Mujica, M. T. (2009). Phenotypic and genotypic characterization of *Aspergillus lentulus* and *Aspergillus fumigatus* isolates in a patient with probable invasive aspergillosis. *J Med Microbiol* **58**, 391–395.
- Muñoz, P., Guinea, J. & Bouza, E. (2006). Update on invasive aspergillosis: clinical and diagnostic aspects. *Clin Microbiol Infect* **12** (s7), 24–39.
- Padhye, A. A., Godfrey, J. H., Chandler, F. W. & Peterson, S. W. (1994). Osteomyelitis caused by *Neosartorya pseudofischeri*. *J Clin Microbiol* **32**, 2832–2836.
- Panackal, A. A., Imhof, A., Hanley, E. W. & Marr, K. A. (2006). *Aspergillus ustus* infections among transplant recipients. *Emerg Infect Dis* **12**, 403–408.
- Pavie, J., Lacroix, C., Hermoso, D. G., Robin, M., Ferry, C., Bergeron, A., Feuilhade, M., Dromer, F., Gluckman, E. & other authors (2005). Breakthrough disseminated *Aspergillus ustus* infection in allogeneic hematopoietic stem cell transplant recipients receiving voriconazole or caspofungin prophylaxis. *J Clin Microbiol* **43**, 4902–4904.
- Posteraro, B., Mattei, R., Trivella, F., Maffei, A., Torre, A., De Carolis, E., Posteraro, P., Fadda, G. & Sanguinetti, M. (2011). Uncommon *Neosartorya udagawae* fungus as a causative agent of severe corneal infection. *J Clin Microbiol* **49**, 2357–2360.
- Snelders, E., van der Lee, H. A., Kuijpers, J., Rijs, A. J., Varga, J., Samson, R. A., Mellado, E., Donders, A. R., Melchers, W. J. & Verweij, P. E. (2008). Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* **5**, e219.
- Sugui, J. A., Vinh, D. C., Nardone, G., Shea, Y. R., Chang, Y. C., Zelazny, A. M., Marr, K. A., Holland, S. M. & Kwon-Chung, K. J. (2010). *Neosartorya udagawae* (*Aspergillus udagawae*), an emerging agent of aspergillosis: how different is it from *Aspergillus fumigatus*? *J Clin Microbiol* **48**, 220–228.
- Summerbell, R. C., de Repentigny, L., Chartrand, C. & St Germain, G. (1992). Graft-related endocarditis caused by *Neosartorya fischeri* var. *spinosa*. *J Clin Microbiol* **30**, 1580–1582.
- Van Der Linden, J. W. M., Warris, A. & Verweij, P. E. (2011). *Aspergillus* species intrinsically resistant to antifungal agents. *Med Mycol* **49** (Suppl 1), S82–S89.
- Varga, J., Houbaken, J., Van Der Lee, H. A., Verweij, P. E. & Samson, R. A. (2008). *Aspergillus calidoustus* sp. nov., causative agent of human infections previously assigned to *Aspergillus ustus*. *Eukaryot Cell* **7**, 630–638.
- Verweij, P. E., Snelders, E., Kema, G. H., Mellado, E. & Melchers, W. J. (2009). Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? *Lancet Infect Dis* **9**, 789–795.
- Vinh, D. C., Shea, Y. R., Jones, P. A., Freeman, A. F., Zelazny, A. & Holland, S. M. (2009a). Chronic invasive aspergillosis caused by *Aspergillus viridinutans*. *Emerg Infect Dis* **15**, 1292–1294.
- Vinh, D. C., Shea, Y. R., Sugui, J. A., Parrilla-Castellar, E. R., Freeman, A. F., Campbell, J. W., Pittaluga, S., Jones, P. A., Zelazny, A. & other authors (2009b). Invasive aspergillosis due to *Neosartorya udagawae*. *Clin Infect Dis* **49**, 102–111.
- Yaguchi, T., Horie, Y., Tanaka, R., Matsuzawa, T., Ito, J. & Nishimura, K. (2007). Molecular phylogenetics of multiple genes on *Aspergillus* section *Fumigati* isolated from clinical specimens in Japan. *Nippon Ishinkin Gakkai Zasshi* **48**, 37–46.