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Cluster analysis of *Scedosporium boydii* infections in a single hospital

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ABSTRACT

Scedosporiosis is a rare, but often fatal mycotic infection occurring in immunosuppressed as well as in immunocompetent patients. Over a period of 14 months, *Scedosporium boydii* isolates were sent to our reference laboratory from six immunocompetent patients treated at a single hospital in Germany. In analogy to the EORTC/MSG criteria, four patients were classified as proven invasive scedosporiosis cases, and two patients as probable or possible cases. Of note, in five patients scedosporiosis was diagnosed between 1 and 14 months (median 5.0 months) after cardiac surgery. Despite antimycotic treatment two patients died, and three were lost for long-term follow-up. All clinical *S. boydii* isolates were characterized by molecular analysis using multilocus sequence typing (MLST). An identical MLST type was found in five patients who had been treated in the surgery unit, suggesting a link between these infections. The source of *S. boydii* has not been identified. Within an observation period of 2 years before and after this cluster of infections no further cases of scedosporiosis were reported from this hospital.

Introduction

Hyphomycetes of the *Scedosporium apiospermum* species complex and *Scedosporium prolificans*, the latter having been renamed recently as *Lomentospora prolificans*, are opportunistic fungi causing a broad spectrum of mycoses including cerebral infections after near-drowning events, post-traumatic infections and systemic infections in immunocompromised patients (Guarro et al., 2006; Lackner et al., 2014). *Scedosporium* species are frequently isolated from respiratory samples of patients with cystic fibrosis, but this may not necessarily be associated with invasive infection. In contrast, isolation of these molds from deep tissue is a hallmark of invasive infection. Diagnosis of scedosporiosis is challenging as histopathology resembles other agents of hyphomycoses in tissue (Tintelnot, 2013). A rapid and exact diagnosis of scedosporiosis is essential for early guided treatment approaches as these fungi are resistant to many clinically used antifungals.

Here, we report on a series of rare *S. boydii* (formerly *Pseudallescheria boydii*) infections in non-immunocompromised patients over a period of 21 months at a single hospital in Germany, suggesting a cluster of health-care-associated scedosporiosis.

Materials and methods

Patients

Within a period of 13 months (Table 1, Fig. 1), isolates or formalin-fixed paraffin-embedded (FFPE) tissue suspected to belong to the *S. apiospermum* complex from six patients (four male, two female, age between 7 and 80 years) from a single hospital in Germany were sent to the German reference laboratory for scedosporiosis for identification and, if the isolate was available, for susceptibility testing.

The clinical data were extracted from laboratory forms or provided by the clinicians. Adapted to the EORTC/MSG criteria (De Pauw et al., 2008), a case of scedosporiosis was defined as proven if the patient had clinical symptoms compatible with a deep organ infection and the fungus was isolated from a primary sterile site of origin, or if scedosporiosis was diagnosed in FFPE tissue. A case was defined as probable if a patient had a risk factor for a fungal infection, *Scedosporium* was isolated and the patient was treated for a *Scedosporium* infection, and as possible if *Scedosporium* was isolated but no other host or risk factor was known.

Identification and genotyping

Isolates were characterized by conventional phenotypical and molecular methods. These included micromorphology, resistance to cycloheximide, thermotolerance, sequencing of the ITS region

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Table 1
Patients colonized or infected by *S. boydii* from the same hospital.

Patient no.	Reason for hospital admission	Latency between admission and diagnosis of infection	Manifestation	Scedosporiosis	Antifungal treatment	Outcome	Sequence type
1	CAP	Not applicable	Pneumonia	Possible[c]	VCZ	Died	ST14
2	Cardiac surgery	5 Months	Endocarditis	Proven[a]	VCZ + CAS	Died	ST18
3	Cardiac surgery	10 Months	NDA	Probable[b]	VCZ + CAS	No follow up	ST18
4	Cardiac surgery	4 Months	Endophthalmitis	Proven[a]	VCZ (+CAS)	Under treatment	ST18
5	Cardiac surgery	1 Month	Endocarditis, endophthalmitis	Proven[a]	Diagnosis post mortem	Died	NDA[*]
6	Cardiac surgery	13 Months	Endophthalmitis	Proven[a]	VCZ + CAS	No follow up	ST18

CAP, community acquired pneumonia; CAS, caspofungin; NDA, no data available, isolate from wound swab; ST, sequence type; VCZ, voriconazole; ( ), short time treatment.
[a] The patient had clinical symptoms compatible with a deep organ infection and the fungus was isolated from a primary sterile site of origin, or scedosporiosis was diagnosed in FFPE tissue.
[b] The locus of the isolate cannot been clarified, but the re-admission to hospital and the identification of the identical strain in the group of patients after cardiac surgery makes scedosporiosis highly probable but not proven.
[c] *S. boydii* had been isolated from a primarily non-sterile compartment, so colonization cannot be excluded.
[*] No isolate available, diagnosis from formalin-fixed paraffin-embedded tissue by PCR.

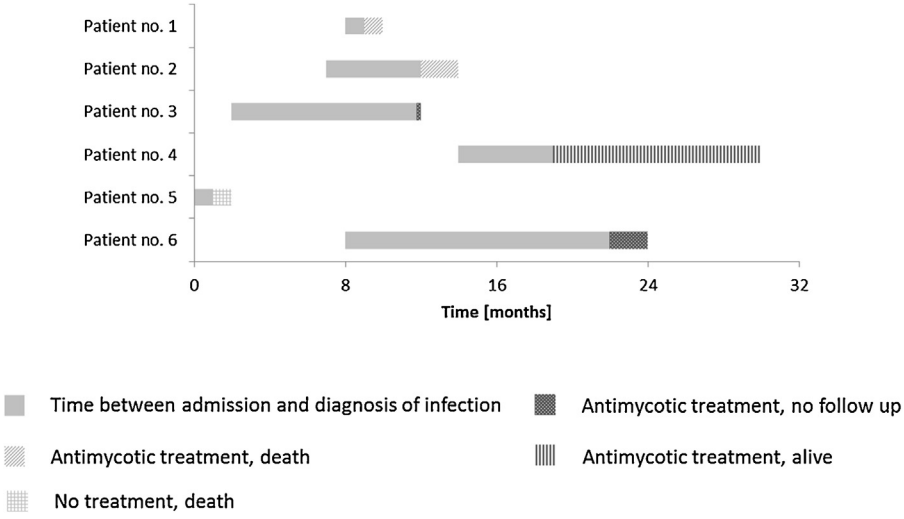

Fig. 1. Timeline of proposed acquisition of infection and diagnosis.

of the ribosomal DNA gene cluster and/or hybridization to specific gene probes targeting the ITS2 region of the rDNA. The gene probes had been developed in cooperation with Chipron, Berlin, Germany. If no isolate was available but invasive hyalohyphomycosis was diagnosed histologically, fungal DNA was extracted and amplified from FFPE tissue. The amplicon of the ITS2 region of rDNA was identified by hybridization and sequencing as described (Bernhardt et al., 2015).

Multilocus sequence typing (MLST) for isolates was performed, including parts of the *actin* (*ACT*), *β-tubulin*, (*BT2*, exon 2–4), *calmodulin* (*CAL*, exon 3–4), *second largest subunit of RNA polymerase II* (*RPB2*) and *manganese superoxide dismutase 2* (*SOD2*) genes as described by Bernhardt et al. (2013). Primers for *SOD2* amplification had been modified (SOD2-F-Pb-comp3 5′-CACCACCAGACCTACGTCAATG and SOD2-R-Pb-comp2 5′-CAAGAGAGGAGCRAGGTTRC) because the published primer did not reliable amplify this locus of all isolates of the *S. apiospermum* complex.

The alleles were aligned with sequences of *Scedosporium* species deposited at the MLST-database for *Scedosporium* (http://mlst.mycologylab.org) (Bernhardt et al., 2013). Phylogenetic analysis included sequences of the patients' isolates from the same hospital (patient nos. 1–4 and 6, Table 1), an isolate from a patient with cystic fibrosis (CF) from a different hospital in the same city (patient no. 7) and epidemiologically unrelated strains of the same species as well as reference strains of *S. boydii* (CBS 101.22^T, CBS 418.73, CBS 301.79, CBS 117432 and CBS 120157) and *S. apiospermum* (CBS 117407^T, CBS 100392, CBS 100395 and CBS 117410). Except for the five reference strains, all *S. boydii* isolates were from Germany and included 42 unique isolates of human patients and one veterinary isolate.

Simpson's index of diversity which is a numerical index and is indicative of the difference between unrelated strains was calculated according to Hunter and Gaston (1988) to evaluate the discriminatory power of the typing method. It has been suggested that values exceeding 0.95 reflect typing methods suitable for outbreak investigation (Debourgogne et al., 2010; van Belkum et al., 2007).

In vitro *susceptibility testing*

In vitro susceptibility testing of the isolates was performed according to CLSI guidelines M38-A2 (CLSI, 2008).

Results

Patients

Scedosporiosis was proven in four patients while it was probable in one patient and possible in another (Table 1). The age of the