Journal of Infection (2005) **51**, e225–e228

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CASE REPORT

Voriconazole-resistant disseminated *Paecilomyces variotii* infection in a neutropenic patient with leukaemia on voriconazole prophylaxis

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Accepted 1 February 2005
Available online 23 March 2005

KEYWORDS
Fungal infection;
Paecilomyces;
Resistance;
Voriconazole

Abstract *Paecilomyces variotii*, an emerging hyalohyphomycetes, has been reported to be susceptible in vitro to voriconazole. We describe a case of disseminated *P. variotii* infection in a neutropenic child with relapsed leukaemia who was on voriconazole prophylaxis. The *P. variotii* isolate was resistant to voriconazole in vitro. The patient responded to liposomal amphotericin B.

Introduction

Voriconazole (VRC) is a new extended spectrum triazole with activity against yeasts, dimorphic fungi, aspergilli and emerging opportunistic moulds like *Scedosporium apiospermum* and *Fusarium* species.[1] However, VRC lacks activity against *Zygomycetes*,[2] and its efficacy against rare fungal pathogens is less well studied. VRC has shown in vitro and in vivo activity against the rare opportunistic fungus *Paecilomyces*.[3] We describe the first case of *Paecilomyces* infection breakthrough to VRC, caused by a *Paecilomyces variotii* spp that exhibited in vitro resistance to VRC.

Case report

A 14-year-old boy with a history of autism and developmental delay was diagnosed with acute lymphoblastic leukaemia in June 25 2002. After a short-term remission, the patient relapsed in March 23 2004, with both bone marrow and central nervous system involvement. Salvage chemotherapy was initiated in March 26 2004 with rituximab, hyper-CVAD, and intrathecally administered methotrexate and cytosine arabinoside. The patient remained profoundly neutropenic for 35 days, with leukocyte counts of $<0.1\times10^9$/l.

In April 7 2004, he was placed on anti-fungal prophylaxis with 200 mg twice daily oral voriconazole (VRC) and daily granulocyte colony-stimulating factor. In April 27 2004, 15 days after the second cycle of chemotherapy, he developed neutropenic fever, with a temperature of 38.3 °C, dry cough, and rhinorrhoea. The results of a physical examination were unremarkable, and treatment with

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0163-4453/$30.00
doi:10.1016/j.jinf.2005.02.005

intravenous cefipime, 2 g twice daily, was begun. Over the next 2 days, one blood culture taken from the central venous catheter (port-A-cath) became positive for a fungus that was identified as *P. variotii*. Peripheral blood cultures were negative for the fungus, and thus *P. variotii* was considered a contaminant.

Over the following days, however, the fever persisted, and the patient developed pink macular and nodular skin lesions on his forearms and left cheek. Samples obtained during a punch biopsy of these lesions demonstrated infiltration of the dermis by hyaline septate hyphae, although the results of fungal cultures were negative. In addition, a computed tomography (CT) scan of the chest showed bilateral nodular lung consolidations. Because this disseminated infection occurred during VRC prophylaxis and the *P. variotii* isolate exhibited high minimal inhibitory concentrations (MICs) to VRC in vitro (Table 1), VRC was discontinued, and liposomal amphotericin B (lipo AMB) was administered at a dosage of 5 mg/kg daily. In addition, the central venous catheter was removed.

The patient slowly responded to lipo AMB with resolution of the fever and the skin lesions and with complete regression of pulmonary infiltrates over the following 4 weeks, in conjunction with neutrophil recovery and achievement of a hematologic remission. In July 1 2004, after completing 2 months of lipo AMB treatment, which was remarkably well tolerated, the patient was given liquid itraconazole as secondary prophylaxis. He remains free of infection 5 months after discontinuation of lipo AMB.

Materials and methods

Blood cultures were performed using a Bactec Peds Plus (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) system and subcultured in Sabouraud's dextrose agar and sheep blood agar. The patient's isolate was then sent to the Fungus Testing Laboratory, Department of Pathology, The University of Texas Health Science Centre at San Antonio, TX, for identification and anti-fungal susceptibility testing. Morphologic identification of *P. variotii* was based on its characteristic growth in large yellowish to olive brown powdery colonies and on its particular microscopic features, consisting of irregularly branched conidiophores, large swollen phialides, and fusiform ellipsoid conidia (Fig. 1).

In vitro susceptibility of the *P. variotii* isolate was evaluated using the broth microdilution susceptibility testing reference method for filamentous fungi proposed by the National Committee for Clinical Laboratory Standards (NCCLS M38-A)[4] and with Epsilometer strips (Etest; AB Biodisk, Solna, Sweden) using standard methods.[5] The *Candida parapsilosis* ATCC 22019 strain was used as quality control isolate. The MICs of AMB, fluconazole (FLC), itraconazole (ITC), VRC, and posaconazole were defined as the lowest drug concentration resulting in complete inhibition of visual growth. The minimal effective concentration (MEC) of caspofungin (CAS) was defined as the lowest drug concentration that resulted in the formation of aberrantly growing hyphal tips.[6] Minimal fungicidal concentrations (MFCs) for AMB, ITC, and VRC were determined using the previously described method.[7] The MICs for the *E*-test method were determined using AMB, FLC, ITZ, and VRC strips. *Candida parapsilosis* ATCC 22019 was again used as a control. The MICs were read at 24 and 48 h as the drug concentration at the point where the growth ellipse intersected the strip.[5] All MIC and MFC determinations were carried out in triplicate at different times, and the mean 24- and 48-h MIC/MFC values were reported.

Table 1 In vitro susceptibilities of the *Paecilomyces variotii* isolate to AMB, ITC, VRC, CAS and POS (NCCLS M38-A and *E*-test methods)

Anti-fungal agent	NCCLS M38-A			*E*-test	
	MIC (24 h)	MIC (48 h)	MFC (48 h)	MIC (24 h)	MIC (48 h)
AMB	0.25	0.25	0.5	0.047	0.047
VRC	8	16	16	32	32
ITR	0.125	0.125	1.0	0.25	0.25
FLC	16	16	NA	64	64
CAS[a]	1	8	NA	NA	NA
POS	0.06	0.125	NA	NA	NA

Abbreviations: AMB, amphotericin B; VRC, voriconazole; ITR, itraconazole; FLC, fluconazole; CAS, caspofungin; POS, posaconazole; NA, not available.

[a] MECs are presented instead of MICs.

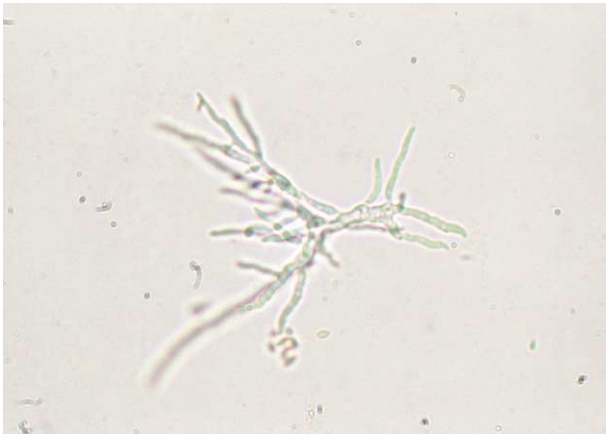

Figure 1 Characteristic irregularly branched conidiophores, large swollen phialides, and fusiform ellipsoid conidia of *Paecilomyces variotii* (×400 magnification).

Discussion

We report the first case of *P. variotii*, occurring as disseminated (blood, skin, lungs) breakthrough infection to VRC. This patient had several classic risk factors for an opportunistic mould infection, such as a history of active leukaemia, long-term profound neutropenia, significant corticosteroid use, and possibly the presence of a central venous catheter (CVC). Although we did not measured VRC serum levels at the time of the *Paecilomyces* infection, we believe that the prolonged exposure to VRC as prophylaxis, along with the high MICs of the corresponding mould for VRC, suggests the occurrence of a breakthrough opportunistic mould infection that was resistant to VRC.

Paecilomyces spp. are ubiquitous hyaline filamentous fungi typically found in nature as saprophytes.[8] They are frequently encountered us contaminants of sterile solutions and culture specimens as they are highly resistant to conventional sterilization methods.[9] *Paecilomyces* spp. have been rarely reported as human pathogens. Only 2 of 31 species, *P. variotii* and *Paecilomyces lilacinus*, cause the vast majority of infections.[3,8] Because *Paecilomyces* spp. are closely related morphologically to *Penicillium* spp., also a common saprophyte, careful macroscopic and microscopic assessment is required to distinguish between them. The presence of characteristic phialides that are swollen at the base and elliptical conidia in chains favors the diagnosis of *Paecilomyces* spp.[3]

Most *Paecilomyces* infections are associated with the presence of biomedical devices, such as central venous or peritoneal catheters, surgically implanted lenses, prosthetic cardiac valves, cerebrospinal fluid shunts, and breast implants.[3,8,9] Immunosuppression is a critical risk factor for severe *Paecilomyces* infections. Most described cases have involved soft tissues, sinuses, lungs, and bones.[3,9] The reported relatively high rate of positive blood cultures frequently encountered in heavily immunocompromised patients[3,9] has been attributed to the ability of *Paecilomyces* to adventitiously sporulate during tissue invasion, similar to the case of *Fusarium*, *Acremonium*, and *Scedosporium* infections.[8]

Classification of *Paecilomyces* at the species level is based on the morphologic characteristics of colonies: white cottony colonies gradually become lilac in the case of *P. lilacinus*, and powdery yellow-brown ones in the case of *P. variotii*.[3] Differentiation between *Paecilomyces* spp. is clinically important because these species have entirely different susceptibility to anti-fungal agents. *P. lilacinus* is typically resistant to AMB, fluconazole, and itraconazole in vitro, with associated high rates of treatment failure.[3,8-10] The echinocandins have mediocre activity against *P. lilacinus* in vitro (e.g. MECs of caspofungin range from 3.12 to >100 μg/ml), while there is no clinical experience with this class of anti-fungal agents.[11] However, VRC and the investigational triazoles posaconazole and ravuconazole have demonstrated promising in vitro activity, with MICs for VRC ranging from 0.25 to 4 μg/ml,[3,8,12] and an increasing number of refractory localized and disseminated *P. lilacinus* infections have been successfully treated with VRC.[13-15]

In contrast, *P. variotii* is universally susceptible both in vitro and in vivo to AMB, which is considered the agent of choice.[3,8,10,] In addition, with the sole exception of fluconazole, *P. variotii* is susceptible in vitro to most of the azoles.[3,8,9] The echinocandins also have promising in vitro activity against *P. variotii*, with caspofungin MEC <0.09 μg/ml in one study.[11] In the case of the newer triazoles, VRC had activity against *P. variotii* in some[3,8] but not all in vitro studies: in a recent in vitro study, 2 out of 4 *P. variotii* clinical isolates tested exhibited high MICs.[12] However, it should be emphasized that in most in vitro studies, only a small number of isolates is tested. In addition, these isolates might not always be representative of those recovered from immunocompromised patients heavily pre-exposed to anti-fungals, as the latter isolates have a higher likelihood of primary and secondary resistance.

To our knowledge, this is the first report that documents breakthrough to VRC *P. variotii* infection by a VRC-resistant isolate. Because VRC is generally regarded as one of the most broad-spectrum agents for a variety of opportunistic

mould infections, including those caused by *Paecilomyces* spp., one needs to be aware that VRC may exhibit dissimilar activity against different *Paecilomyces* species.

In vitro susceptibility methods play a central role in the management of bacterial infections, providing essential information on anti-microbial activity, especially against multidrug-resistant pathogens.[16] However, the susceptibility end points of antifungal agents against filamentous fungi have not been established yet, and uncertainty remains concerning correlation of in vitro susceptibilities and clinical outcome.[17] We believe that for rare, refractory, or breakthrough opportunistic fungal infections, when clinical experience to guide antifungal therapy is lacking, these in vitro susceptibility methods might be particularly helpful.[17]

Acknowledgements

We thank J. Lemoine for assistance in the preparation of Fig. 1.

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