NEW CASE OF AZOLE-RESISTANT ASPERGILLUS FUMIGATUS DUE TO TR46/Y121F/T289A MUTATION IN BELGIUM

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Sir,

Azole-resistant Aspergillus fumigatus is an emerging worldwide problem with major epidemiological and clinical implications. The prevalence of azole resistance in A. fumigatus is unknown, but the number of reported cases of therapy failure due to resistant isolates is increasing. A new cyp51A-mediated resistant genotype (TR46/Y121F/T289A) has recently been reported in the Netherlands associated with high-level resistance to voriconazole.1 Similar cases were recently reported for the first time outside the Netherlands, in the neighbouring country of Belgium and more recently in Germany.2,3 Here, we present the second case of invasive aspergillosis (IA) caused by azole-resistant A. fumigatus due to the TR46/Y121F/T289A mutation in a Belgian hospital, confirming the geographical spread of this resistance mechanism.

A patient diagnosed with AML in 2003 received autologous HSCT in 2007. The patient developed chronic graft-versus-host disease (GVHD) requiring bilateral lung transplantation in May 2011. Immunosuppressive therapy after transplantation included azathioprine, tacrolimus and corticosteroids. The patient had three courses of voriconazole therapy for recurrent pulmonary IA (in 2007, 2009 and 2011). In April 2013 the patient was diagnosed with a relapse of acute GVHD, including skin, pulmonary and gastrointestinal tract lesions, and treated with high doses of corticosteroids. At this time, prophylaxis with oral posaconazole (400 mg twice daily) was started in the context of colonization by A. fumigatus. Therapeutic drug monitoring was carried out four times between April and November 2013, with serum concentrations maintained in the range 700–1200 ng/mL (serum concentration >700 ng/mL is recommended in our institution for posaconazole in prophylaxis).

In November 2013, the patient was hospitalized for fever, dyspnoea, cough and headache because of Pseudomonas aeruginosa bacteraemia. On antimicrobial treatment, the patient improved, but a thoracic CT scan showed several ill-defined lesions surrounded by ground glass opacities, suggestive of angio-invasive pulmonary aspergillosis. Galactomannan testing was positive in serum (index 0.88; normal <0.5) and in bronchoalveolar lavage (BAL) fluid (index 2.4; normal <1). A. fumigatus was cultured from BAL fluid and a diagnosis of probable pulmonary IA was concluded. The isolate was tested initially for antifungal susceptibility using Sensititre YeastOne Y010 (Thermo Fisher Scientific, USA) showing high-level resistance to voriconazole (MIC >8 mg/L) according to CLSI epidemiological cut-off values for MIC interpretations.4 Treatment with caspofungin (50 mg daily) then liposomal amphotericin B (3 mg/kg daily) was started, with a stable clinical evolution. After 21 days, the patient was discharged from the hospital on oral posaconazole treatment (400 mg twice daily). One week later, he was admitted again for respiratory failure. Serum galactomannan testing was positive (index 1.2) and thoracic CT scan showed a worsening of initial images, with major left lower lung infarct. Liposomal amphotericin B combined with caspofungin for 11 days was resumed. Eight days later, serum galactomannan values became negative and clinical and radiological evolution slowly improved. The patient died 4 months later in palliative care at home because of hepatic GVHD deterioration.

The isolate was sent to the Belgian National Reference Center for Mycosis. A. fumigatus sensu strictu was confirmed to the species level by β-tubulin sequencing, as described previously.5 The isolate was tested for susceptibility by broth microdilution following the CLSI M38-A2 protocol and genotypic identification of the resistance mechanism was performed by sequencing of the cyp51A gene, as described previously.6,7 MICs obtained were as follows: voriconazole >8 mg/L; itraconazole >16 mg/L; posaconazole 1 mg/L; and amphotericin B 2 mg/L. Finally, resistance was shown to be due to the cyp51A mutation TR46/Y121F/T289A.

The number of clinical cases of TR46/Y121F/T289A A. fumigatus described until now in the literature is limited and diverse in terms of underlying diseases andazole exposure. This new resistance mechanism was isolated for the first time in the Netherlands. IA was diagnosed in eight patients; most of them were immunocompromised because of haematological or solid organ transplantation. All patients who received primary therapy with voriconazole died, whereas patients primary treated with amphotericin B were still alive 12 weeks after the initial diagnosis.1 The first case described in Belgium was an HSCT patient complicated with GVHD requiring high-dose corticosteroid therapy. The patient was receiving fluconazole prophylaxis, but had never been exposed to mould-active azoles. Initial treatment wasazole with a later switch to liposomal amphotericin B. However, the patient died 19 days after his first presentation with dyspnoea.2 More recently, in Germany, TR46/Y121F/T289A A. fumigatus was isolated from a cystic fibrosis patient receiving itraconazole therapy for allergic bronchopulmonary aspergillosis.3

We describe the second clinical case of A. fumigatus harbouring the TR46/Y121F/T289A mutation in Belgium: a patient immunocompromised because of HSCT and bilateral lung transplantation with favourable clinical evolution of IA after treatment with liposomal amphotericin B combined with caspofungin. Although the number of TR46/Y121F/T289A A. fumigatus clinical cases is limited, patients treated with liposomal amphotericin B appeared to respond better than those treated with
voriconazole. Primary treatment with amphotericin B or quickly switching from azoles to amphotericin B seems to be mandatory for good clinical outcome. There is no evidence of a clear correlation between the MICs of amphotericin B and outcome of treatment, but high MICs should be taken into consideration for antifungal alternatives. The combination of amphotericin B with caspofungin could have been beneficial in this case.

The patient described here was exposed to azoles previously, in contrast to the majority of azole-naive patients described in the Netherlands. Resistance in antifungal-exposed patients may be expected as any antimicrobial treatment is associated with selective pressure and therefore with a risk of resistance emergence. A. fumigatus resistant to medical azoles has also been recovered from the environment. Molecular typing confirmed that environmental and clinical isolates harbouring cyp51A mutations were clustered, suggesting environmental transmission to azole-naive patients. This suggests that selection through a fungicide-based route may have taken place. TR46/Y121F/T289A-bearing strains have been found throughout the environment in the Netherlands and Belgium, but also recently in India. TR46/Y121F/T289A environmental strains in India were similar to the Dutch clinical strains. All these cases may indicate the rapid and worrisome geographical spread of these new resistance mechanisms, possibly following the same path as TR34/L98H, which now causes therapy-refractory infections worldwide. 1–3,11

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References


Combination testing of colistin with telavancin and daptomycin

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Sir,

Recently, there has been increased interest in testing unorthodox combinations of antimicrobials brought about by an alarming increase in antimicrobial resistance and a paucity of existing and new antimicrobials for the treatment of infections caused by multidrug-resistant organisms. One such example is the organisms colonizing the respiratory tracts and causing infective exacerbations in individuals with cystic fibrosis. We investigated a small sample of 17 multidrug-resistant Pseudomonas aeruginosa recovered from the respiratory secretions from such individuals by testing them against telavancin and daptomycin in combination with colistin. The Etest method used to assess the combinations and the calculation of the fractional inhibitory concentration index (FICI) have been described previously.

By CLSI breakpoint criteria, 14 (82.3%), 2 (11.8%) and 1 (5.9%) of the strains were susceptible, intermediate and resistant to colistin, respectively. As expected, telavancin and daptomycin had no activity against the strains. An MIC50 of 1 mg/L and MIC90 of 4 mg/L were observed for colistin when tested alone and in combination with telavancin. In combination with daptomycin, the MIC50 and MIC90 of colistin increased to 3.0 and 8.0 mg/L, respectively. The results of the combination testing are presented in Table 1. Hornsey et al.1 also reported synergy with colistin+telavancin in combination when used against various Gram-negative organisms, but voiced concerns about the potential for this combination to cause nephrotoxicity. Using Galleria mellonella larvae infected with the multidrug-resistant AB210