Azole-resistant Aspergillus fumigatus with the environmental TR46/Y121F/T289A mutation in India

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Sir,

Triazole antifungals are the mainstay of therapy for patients with aspergillosis. Notably, the mortality associated with aspergillosis is high and the rate of treatment failure is much higher if patients are infected with multiple-triazole-resistant (MTR) Aspergillus fumigatus.1-3 MTR A. fumigatus strains with the mutation TR46/Y121F/L98H occur both in azole-treated as well as in azole-naive patients and have increasingly been reported from Dutch patients and their environment and from other European and Asian countries.1-7 Molecular studies from Europe and India suggested that use of azole fungicides in the environment selects MTR A. fumigatus TR46/Y121F/L98H strains.4,5 This issue is further complicated by the emergence of a new resistance mechanism, TR46/Y121F/T289A in the A. fumigatus genome responsible for voriconazole resistance in A. fumigatus, which was detected in 2009 in a Dutch patient and has been reported from various European countries.4-7

For phylogenetic analysis, 32 resistant strains of A. fumigatus from the Netherlands (TR46, 14 clinical; TR34, 2 clinical and 3 environmental), India (TR46, 6 environmental; TR34, 2 clinical and 3 environmental), France (TR34, 1 clinical) and Germany (TR34, 1 clinical) were included along with 30 wild-type strains from India (8 clinical and 14 environmental) and the Netherlands (n = 8). The Indian environmental TR46/Y121F/T289A strains were similar to Dutch clinical TR46/Y121F/T289A strains and clustered separately from TR34/L98H A. fumigatus strains (Figure 1).

In the in vitro activity of azole antifungals and fungicides against 6 TR46/Y121F/T289A and 14 wild-type environmental A. fumigatus strains obtained from the same geographical locality were determined using the CLSI M38-A2 broth microdilution method. The azole fungicides tested were bromeconazole, cyproconazole, difenoconazole, epoxiconazole, penconazole, tebuconazole, triadimefon, metconazole (gifted by Dr P. E. Verweij, The Netherlands), hexaconazole (Rallis India, Mumbai, India) and trifloxizole (Cheminova India, Mumbai, India). All six TR46/Y121F/T289A isolates showed high MICs of voriconazole (>16 mg/L) and isavuconazole (8 mg/L), reduced susceptibility to itraconazole (range, 1–2 mg/L) and posaconazole (range, 0.25–0.5 mg/L) and cross-resistance to all the fungicides tested (range, 4 to >32 mg/L). However, all the wild-type isolates revealed low MICs of the azole antifungals (range, 0.06–0.5 mg/L) and fungicides (range, 0.125–8 mg/L) tested. The emergence of a new azole resistance mechanism in environmental A. fumigatus strains in India raises concern, as this mechanism, TR46/Y121F/T289A, has been associated with invasive infections and therapeutic failure with voriconazole. So far, this

1A. fumigatus strains originated from Varanasi, five from a potato (Solanum tuberosum) field and one from a fenugreek (Trigonella foenum-graecum) field, which were located 2 km apart from each other. Four of the eight itraconazole-resistant A. fumigatus strains originated from rose (Rosa species) bed soil and red chilli (Capsicum annuum) fields, Delhi. Of the remaining four itraconazole-resistant A. fumigatus strains, one each originated from fields of aubergine (Solanum melongena), mustard (Brassica juncea), potato and fenugreek in Varanasi. Notably, 5.7% of the soil samples harboured voriconazole-resistant A. fumigatus and 7.6% harboured itraconazole-resistant A. fumigatus. The overall isolation rate of both itraconazole- and voriconazole-resistant A. fumigatus was found to be higher in Varanasi (26.3%; 10/38) than in Delhi (4.5%; 4/88). Identification of resistant A. fumigatus strains was confirmed by sequencing of the internal transcribed spacer region, β-tubulin and the calmodulin gene.7

Further, A. fumigatus strains were screened for triazole resistance mutations using a mixed-format real-time PCR.7 All six voriconazole-resistant A. fumigatus strains exhibited the TR46/Y121F/T289A mutation and the eight itraconazole-resistant strains showed the TR34/L98H mutation. Microsatellite genotyping of TR46/Y121F/T289A A. fumigatus strains was performed with a panel of nine short tandem repeats.7 For phylogenetic analysis, 32 resistant strains of A. fumigatus from the Netherlands (TR46, 14 clinical; TR34, 2 clinical and 3 environmental), India (TR46, 6 environmental; TR34, 2 clinical and 3 environmental), France (TR34, 1 clinical) and Germany (TR34, 1 clinical) were included along with 30 wild-type strains from India (8 clinical and 14 environmental) and the Netherlands (n = 8).

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A total of 105 soil samples from the agricultural fields of the river Yamuna bank, Delhi (n = 63) and Varanasi, Uttar Pradesh (n = 42), located 800 km apart, were investigated during 2012–13. Samples were processed as described previously.7 A total of 37 (35%) samples (Delhi, n = 27; Varanasi, n = 10) yielded 126 A. fumigatus strains, including 88 from Delhi and 38 from Varanasi, on Sabouraud dextrose agar (SDA) plates. All A. fumigatus strains were screened for resistance on SDA plates supplemented with 4 mg/L itraconazole and 1 mg/L voriconazole. Six A. fumigatus strains grew on voriconazole plates and eight grew on itraconazole plates. Voriconazole-resistant A. fumigatus strains originated from Varanasi, five from a potato (Solanum tuberosum) field and one from a fenugreek (Trigonella foenum-graecum) field, which were located 2 km apart from each other. Four of the eight itraconazole-resistant A. fumigatus strains originated from rose (Rosa species) bed soil and red chilli (Capsicum annuum) fields, Delhi. Of the remaining four itraconazole-resistant A. fumigatus strains, one each originated from fields of aubergine (Solanum melongena), mustard (Brassica juncea), potato and fenugreek in Varanasi. Notably, 5.7% of the soil samples harboured voriconazole-resistant A. fumigatus and 7.6% harboured itraconazole-resistant A. fumigatus. The overall isolation rate of both itraconazole- and voriconazole-resistant A. fumigatus was found to be higher in Varanasi (26.3%; 10/38) than in Delhi (4.5%; 4/88). Identification of resistant A. fumigatus strains was confirmed by sequencing of the internal transcribed spacer region, β-tubulin and the calmodulin gene.7

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mutation has been reported in clinical and environmental A. fumigatus strains from the Netherlands and Belgium.9,11 Interestingly, in the Netherlands, strains with TR46/Y121F/T289A mutations were also recovered from patients’ homes and found to be genotypically similar to clinical strains.9

Previously, 90% of azole-resistant clinical A. fumigatus strains from the Netherlands were due to TR34/L98H. This pan-azole resistance mechanism is now widespread in Europe and Asia with a prevalence of 0.8%–9.5%1–4 and is attributed to the use and persistence of triazole fungicides in the environment. Triazole fungicides can reduce the environmental population of azole-susceptible strains and select for azole resistance. Also, clinical MTR A. fumigatus TR34/L98H strains showed cross-resistance to agricultural triazole fungicides.8 Similarly, TR46/Y121F/T289A A. fumigatus strains were cross-resistant to the azole fungicides tested. This new resistance mechanism possibly also has a fungicide-driven route of development.

In the present study, both TR34/L98H and TR46/Y121F/T289A coexisted in the soil of potato and fenugreek fields of Varanasi, where fungicides were used. Previously, genotypic analysis of Indian TR34/L98H revealed that Indian strains were probably an extremely adaptive recombinant progeny derived from a cross between azole-resistant strains that migrated from outside of India and a native azole-susceptible strain from within India, followed by mutation.7 Recent reports suggest evidence of rapid dispersal of this mutation within Asia.6 Likewise, in the present study, all the Indian TR46/Y121F/T289A strains were similar to Dutch clinical strains at eight of the nine loci. Considering that the environmental mutation TR46/Y121F/T289A in A. fumigatus was detected in the Netherlands only 4 years ago, its occurrence in the Indian environment in such a short time span is worrisome. It is anticipated that strains with the TR46/Y121F/T289A mutation will spread rapidly to other geographical regions by producing a large number of airborne conidia in the environment. It is noteworthy that two major types of azole-resistant mutations, TR34/L98H and TR46/Y121F/T289A, first detected in the Netherlands in 1998 and 2009, respectively, are now observed in India. Furthermore, it is highly likely that other mutations resulting in multiple azole resistance could emerge from environmental sources and spread among human populations.

Figure 1. Minimum spanning tree showing wide genotypic diversity between the TR46/Y121F/T289A, TR34/L98H and wild-type A. fumigatus strains studied.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian TR46</td>
<td>6</td>
</tr>
<tr>
<td>Dutch TR46</td>
<td>14</td>
</tr>
<tr>
<td>Indian TR34</td>
<td>5</td>
</tr>
<tr>
<td>Dutch TR34</td>
<td>5</td>
</tr>
<tr>
<td>German TR34</td>
<td>1</td>
</tr>
<tr>
<td>French TR34</td>
<td>1</td>
</tr>
<tr>
<td>Dutch wild-type</td>
<td>8</td>
</tr>
<tr>
<td>Indian wild-type</td>
<td>22</td>
</tr>
</tbody>
</table>

This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
Identification of a marker for two lineages within the GC1 clone of Acinetobacter baumannii

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Sir,

Isolates from a collection of 177 antibiotic-resistant Acinetobacter baumannii recovered at Westmead Hospital, Sydney over the period 1995–2002 were further characterized. The relationships among these isolates had previously been examined by PFGE of Apal-digested DNA, determination of MICs of several antibiotics and PCR screening to determine whether the blaOXA-23 gene or class 1 integrons were present. Eight PFGE pulsotypes were detected. The majority of isolates were A. baumannii but five isolates (PFGE pulsotypes III and IV) were not, and these were not examined here. One or two isolates from each year for each remaining pulsotype were tested using triplex PCR targeting the blaOXA-23, intI1 and homA genes, to determine whether they belong to global clone 1 (GC1) or global clone 2 (GC2). Pulsotype I isolates were GC1 and isolates from pulsotypes VI, VII and VIII, which first appeared in 1999, were GC2. The 54 GC2 isolates were the only isolates resistant to imipenem and carried the intI1, intI1, class 1 integron.

Keywords: A. baumannii, global clone 1, antibiotic resistance islands, AabR0, ISAba1

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