Update zur Azolresistenz bei *Aspergillus fumigatus*

PEG-Tagung 2018
Bonn

*Oliver Bader*

Institut für Medizinische Mikrobiologie, Universitätsmedizin Göttingen
AML Case

Up to June 2014:
• 71-year old male patient
• treated in a general hospital for AML with adverse-risk cytogenetic features.
• received induction chemotherapy and oral posaconazole for antifungal prophylaxis
• Eventually, a new bone marrow aspirate revealed residual AML
• because of progressive AML, decitabine therapy was initiated

In August 2014:
• referred to Dresden hospital for treatment

Rößler S, Bader O, et al., AAC 2017
AML Case

(C) multiple fungal lesions on the surface of the lung,
(D) fungal abscess in lung parenchyma (PAS reaction),
(E) vascular invasive growth and detection of dichotomously branched and septated fungi in the lumen of a lung vessel
(F) fungal septipyemic focus in the anterior wall of the heart with surrounding granulocytic reaction (PAS reaction)

Rößler S, Bader O, et al., AAC 2017
AML Case

antifungal susceptibility testing of the culture isolate (BAL)

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>E-test MIC</th>
<th>E-test interpretation</th>
<th>EUCAST Microdilution MIC</th>
<th>EUCAST Microdilution breakpoints</th>
<th>EUCAST Microdilution Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>posaconazole</td>
<td>0.5</td>
<td>resistant</td>
<td>0.5</td>
<td>S &lt;= 0.12; R &gt; 0.25</td>
<td>resistant</td>
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<tr>
<td>itraconazole</td>
<td>2</td>
<td>sensitive</td>
<td>1</td>
<td>S &lt;= 1; R &gt; 2</td>
<td>sensitive</td>
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<tr>
<td>voriconazole</td>
<td>&gt;32</td>
<td>resistant</td>
<td>&gt;32</td>
<td>S &lt;= 1; R &gt; 2</td>
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<tr>
<td>amphotericin B</td>
<td>0.25</td>
<td>sensitive</td>
<td>&lt;0.125</td>
<td>S &lt;= 1; R &gt; 2</td>
<td>sensitive</td>
</tr>
</tbody>
</table>

MIC = minimal inhibitory concentration [µg/ml]
EUCAST = European Committee on Antimicrobial Susceptibility Testing

Rößler S, Bader O, et al., AAC 2017
Azole resistance mechanisms in A.f.

A Normal susceptible cell in absence of azoles

B Normal susceptible cell in presence of azoles

C Mutations in cyp51A

D Overexpression of cyp51A

E Increased expression of efflux pumps

Dudakova et al., CMR 2017
Mechanisms of azole resistance: Cyp51A

Emergence of Azole Resistance in Aspergillus fumigatus and Spread of a Single Resistance Mechanism

"pandemic" alleles: TR<sub>34</sub>/L98H TR<sub>46</sub>/Y121F/T289A

Dudakova et al., CMR 2017
AML Case

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PSZ and VRZ ineffective in this patient infection with PSZ\textsuperscript{R} and VRZ\textsuperscript{R} strain with TR\textsubscript{46}/Y121F/M172/T289A  \textit{cyp51A} mutation

Rößler S, Bader O, et al., AAC 2017
Other mechanisms of azole resistance

→ Regulation of sterol biosynthesis pathway
ARAf findings by country

→ if people look for ARAf, they will find it!
Reported isolates in Germany

- Clinical isolates: 35 reported
- Environmental isolates: 55 reported
Current working hypothesis

*A. fumigatus* is one of the best-adapted fungi to dispersal, and its conidia are found world-wide incl. the upper atmosphere, and both polar regions

Kwon-Chung and Segui, PPath, 2013
Current working hypothesis

Unintentional induction of resistance in *A. fumigatus* e.g. TR\textsubscript{34}/L98H or TR\textsubscript{46}/Y121F/T289A (G54?)

- dispersal by wind
- selection over susceptible strains through agricultural azoles?

- presence in hosts
- opportunistic infection
- (selection through azole prophylaxis ???)
- not only humans but also wild animals!
conidia also travel the land route...

# Intercountry Transfer of Triazole-Resistant Aspergillus fumigatus on Plant Bulbs

**Katie Dunne,**1 Ferry Hagen,**2,3** Niamh Pomeroy,**1** Jacques F. Meis,**2,3** and Thomas R. Rogers**3**

1Department of Clinical Microbiology, Trinity College Dublin, Ireland; 2Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, and 3Centre of Expertise in Mycology, Radboud University Medical Center/Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

## Table: Intercountry Transfer of Triazole-Resistant A. fumigatus

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Date of Sample</th>
<th>Type of Sample</th>
<th>Origin</th>
<th>No. of Triazole-Resistant/Total A. fumigatus Colonies/Plant Bulb Pack</th>
<th>MIC, mg/L&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Resistance Mechanism</th>
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<tr>
<td>P1</td>
<td>Jan 2016</td>
<td>Double mixed tulip bulbs (30°)</td>
<td>Lisse, the Netherlands</td>
<td>1/5</td>
<td>0.5 &gt;8 0.5</td>
<td>TR&lt;sub&gt;46&lt;/sub&gt;/Y121F/T289A</td>
</tr>
<tr>
<td>P2, P3</td>
<td>Jan 2016</td>
<td>Bastogne tulip bulbs (6°)</td>
<td>Lisse, the Netherlands</td>
<td>2/3</td>
<td>1 4 1</td>
<td>TR&lt;sub&gt;46&lt;/sub&gt;/Y121F/T289A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8 &gt;8 0.5</td>
<td>TR&lt;sub&gt;34&lt;/sub&gt;/L98H</td>
</tr>
<tr>
<td>P4</td>
<td>Jan 2016</td>
<td>Triumph tulip bulbs (6°)</td>
<td>Breezand, the Netherlands</td>
<td>1/4</td>
<td>8 4 0.5</td>
<td>TR&lt;sub&gt;34&lt;/sub&gt;/L98H</td>
</tr>
<tr>
<td>P5, P6</td>
<td>Jan 2016</td>
<td>Narcissus bulbs (8°)</td>
<td>Breezand, the Netherlands</td>
<td>2/5</td>
<td>0.5 &gt;8 1</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8 4 0.5</td>
<td>TR&lt;sub&gt;34&lt;/sub&gt;/L98H</td>
</tr>
<tr>
<td>P7</td>
<td>Jan 2016</td>
<td>Tall Triumph mixed tulip bulbs (10°)</td>
<td>The Netherlands (region not specified)</td>
<td>1/2</td>
<td>1 &gt;8 1</td>
<td>TR&lt;sub&gt;46&lt;/sub&gt;/Y121F/T289A</td>
</tr>
<tr>
<td>D6</td>
<td>Feb 2016</td>
<td>Soil (2 g)</td>
<td>Hospital campus, Dublin, Ireland</td>
<td>...</td>
<td>8 4 0.5</td>
<td>TR&lt;sub&gt;34&lt;/sub&gt;/L98H</td>
</tr>
</tbody>
</table>
International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*

Paul E. Verweij, Michelle Ananda-Rajah, David Andes, Maiken C. Arendrup, Roger J. Brüggemann, Anuradha Chowdhary, Oliver A. Cornely, David W. Denning, Andreas H. Groll, Koichi Izumikawa, Bart Jan Kullberg, Katrien Lagrou, Johan Maertens, Jacques F. Meis, Pippa Newton, Iain Page, Seyedmojtaba Seyedmousavi, Donald C. Sheppard, Claudia Viscoli, Adilia Warris, J. Peter Donnelly


- microbiological diagnostics critical to guiding therapy
- if azole resistant isolates are obtained,
  - the underlying mechanism should be identified for epidemiological reasons,
  - but neither therapy initiation/modification nor MIC determination delayed

- 2-10% environmental prevalence → alternative therapies must be considered
- 10% environmental prevalence → definitely re-evaluate azole therapy

- at least 5 independent colonies should tested for AST
Concomitant occurrence of itraconazole-resistant and -susceptible strains of *Aspergillus fumigatus* in routine cultures

Ahmad et al, 2015 JAC 70:412-415

**CORRESPONDENCE**

Voriconazole-Susceptible and Voriconazole-Resistant *Aspergillus fumigatus* Coinfection

To the Editor:

Azeole resistance is an increasing problem in *Aspergillus fumigatus* infection (1). Two mutations, TRH/00H and TRH/Y121/F209A, are frequently recovered from isolates of patients with azole-resistant invasive aspergillosis and are believed to originate from the environment (2, 3). In regions with these environmental mutations, azole-resistant *Aspergillus* diseases may develop in patients not previously treated with azoles, and mortality rates are very high (4-6). We review the clinical course of three patients with proven invasive aspergillosis resulting from voriconazole-resistant and voriconazole-resistant *A. fumigatus* strains. We hypothesized that in regions with TRH/00H and TRH/Y121/F209A environmental mutations, individual pulmonary lesions may arise from *A. fumigatus* strains with different azole resistance profiles.

Kolwijck et al, 2016 Am J Respir Crit Care Med 193
large-scale screening

- molecular mechanisms
- a scheme to screen large scale collections
- tools to locally analyse sequencing data
Multiplex -PCR approach

(AsperGenious Assay, PathoNostics, NL)

corporate statement: multi-platform RT-PCR

Detects A. fumigatus, A. terreus, A. spec. from BAL samples

From A. fumigatus: L98H, TR34, T289A, Y121F

Analytical and Clinical Evaluation of the PathoNostics AsperGenius Assay for Detection of Invasive Aspergillosis and Resistance to Azole Antifungal Drugs during Testing of Serum Samples

P. Lewis White, Raquel B. Posso, Rosemary A. Barnes

Public Health Wales Microbiology, Cardiff, Cardiff, United Kingdom; Infection, immunity and Biochemistry, School of Medicine, Cardiff University, Cardiff, United Kingdom

The commercially developed PathoNostics AsperGenius species assay is a multiplex real-time PCR capable of detecting aspergillosis and genetic markers associated with azole resistance. The assay is validated for testing bronchoalveolar lavage fluids, replacing the requirement for culture and benefiting patient management. Application of this assay to less invasive, easily obtainable samples (e.g., serum) might be advantageous. The aim of this study was to determine the analytical and clinical performance of the AsperGenius species and resistance assays for testing serum samples. For the analytical evaluations, serum samples were spiked with various concentrations of Aspergillus genomic DNA for extraction, following international recommendations. For the clinical study, 124 DNA extracts from 14 proven/probable invasive aspergillosis (IA) cases, 2 possible IA cases, and 33 controls were tested. The resistance assay was performed on Aspergillus fumigatus PCR-positive samples when a sufficient fungal burden was evident. The limits of detection of the species and resistance assays for A. fumigatus DNA were 10 and ≥75 genomes/sample, respectively. Nonreproducible detection at lower burdens was achievable for all markers. With a positivity threshold of 39 cycles, the sensitivity and specificity of the species assay were 78.6% and 100%, respectively. For 7 IA cases, at least one genetic region potentially associated with azole resistance was successfully amplified, although no resistance markers were detected in this small cohort. The AsperGenius assay provides good clinical performance with the added ability to detect azole resistance directly from noninvasive samples. While the available burden will limit application, it remains a significant advancement in the diagnosis and management of aspergillosis.

White, Posso, Barnes (2015) JCM 53:2155-21
Multiplex -PCR approach
(AsperGenius Assay, PathoNostics, NL)

**company statement:** multi-platform RT-PCR
Detects A. *fumigatus*, A. *terreus*, A. *spec.* from BAL samples
From A. *fumigatus*: L98H, TR34, T289A, Y121F

**Journal of Antimicrobial Chemotherapy Advance Access published August 15, 2016**

PCR-based detection of Aspergillus *fumigatus* Cyp51A mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay in 201 patients with haematological disease suspected for invasive aspergillosis


**Results:** Two hundred and one patients each contributed one BAL sample, of which 88 were positive controls and 113 were negative controls. The optimal cycle threshold cut-off value for the Aspergillus species PCR was ~28. With this cut-off, the PCR was positive in 74/88 positive controls. The sensitivity, specificity, positive predictive value and negative predictive value were 84%, 80%, 76% and 87%, respectively. 32/74 BAL samples were culture negative. Azole treatment failure was observed in 6/8 patients with a RAM compared with 12/45 patients without RAMs (P=0.01). Six week mortality was 2.7 times higher in patients with RAMs (50.0% versus 18.6%; P=0.07).
nested-PCR approach / AML case

(Spieß & Buchheidt; Mannheim)

Amplification of specific cyp51A fragments from autopsy material (lung, heart) of the patient shown at this talk’s beginning → confirms TR_{46}, Y121F, and T289A (region with M172 not covered)

Rößler S, Bader O, et al., AAC 2017
summary

• Emergence of azole resistance is not an academic, but a clinical problem.

• It is not restricted to The Netherlands, but of global concern.

• Environmental Screening makes sense to estimate the prevalence in local clinics/wards.

• Methods for analysis are available, and they are not complicated.
Acknowledgement

**MykoLabNet-D-Partners:** (in alphabetical order)

**Collectors of environmental samples:** (in order of samples contributed)

**Bereitstellung von Stämmen aus anderen Studien:**
J. Steinmann (Nürnberg), A. Hamprecht (Köln), S. Rößler (Dresden)
## Current EUCAST breakpoints

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>MIC breakpoint (mg/L)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. <em>flavus</em></td>
<td>A. <em>fumigatus</em></td>
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<tr>
<td>Amphotericin B</td>
<td>IE²</td>
<td>IE²</td>
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<tr>
<td>Anidulafungin</td>
<td>IE</td>
<td>IE</td>
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<tr>
<td>Caspofungin</td>
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<td>IE</td>
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<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Itraconazole⁴</td>
<td>1</td>
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<tr>
<td>Micafungin</td>
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<tr>
<td>Posaconazole⁴</td>
<td>IE²</td>
<td>IE²</td>
</tr>
<tr>
<td>Voriconazole⁴</td>
<td>IE²</td>
<td>IE²</td>
</tr>
</tbody>
</table>

¹ Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for organisms that do not have specific breakpoints.
### MIC value distribution across the literature

#### EUCAST

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
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<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
<td>R</td>
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<tr>
<td>TR34/L98H</td>
<td>9</td>
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<td>TR34/L98H+S297T+F495I</td>
<td>8</td>
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