Resistenzentwicklung bei *Candida* und *Aspergillus* - ein klinisches Problem?

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Resistenzentwicklung bei *Candida* und *Aspergillus* - ein klinisches Problem?

*Candida auris*
Ausbrüche azolresistenter Stämme

*Candida glabrata*
Vermehrtes Auftreten von Isolaten mit erhöhten Echinocandin MHKs

*Aspergillus fumigatus*
Pandemisches Auftreten azolresistenter Isolate
Candida auris

- Emerging yeast, first described in Japan 2009
- Nosocomial transmission
- Multidrug resistant
- Rapid dissemination worldwide
- Difficult to identify

**Technical Appendix Figure.** Candida auris colonies from an otherwise healthy patient in Austria after 48 hours at 37°C on various chromogenic media: A) Brilliance Candida Agar; B) CHROMagar Candida; C) Candida ID.
Candida auris

global dissemination

source: US gov't, CDC (https://www.cdc.gov/fungal/diseases/candidiasis/tracking-c-auris.html#world)
**Reported cases in DE / AT**

<table>
<thead>
<tr>
<th>Date</th>
<th>City</th>
<th>clinical background</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/2015</td>
<td>Stuttgart</td>
<td>prosthetic joint infection</td>
<td>Unpublished cases data courtesy of Oliver Kurzai (HKI Jena) and Axel Hamprecht (Uni Cologne)</td>
</tr>
<tr>
<td>12/2015</td>
<td>Nuremberg</td>
<td>SIRS</td>
<td></td>
</tr>
<tr>
<td>06/2017</td>
<td>Cologne</td>
<td>intracranial hemorrhage</td>
<td></td>
</tr>
<tr>
<td>07/2017</td>
<td>Munich</td>
<td>neurological disorder</td>
<td></td>
</tr>
<tr>
<td>08/2017</td>
<td>Munich</td>
<td>polytrauma</td>
<td></td>
</tr>
<tr>
<td>11/2017</td>
<td>Aachen</td>
<td>gunshot wound</td>
<td></td>
</tr>
<tr>
<td>12/2017</td>
<td>Regensburg</td>
<td>tetraplegia</td>
<td></td>
</tr>
</tbody>
</table>

MICs for fluconazole high, but low for amphotericin B
Favourable outcome in all cases
Positive travel history in nearly all cases → imported pathogens
There are at least 3 different linages of *C. auris*, with geographically biased distribution.

→ Epidemiology still unclear!
... is now contained in major MALDI-TOF databases

C. auris contained in DB: MBT ✓; VITEK-MS RUO ✓; VITEK-IVD: end 2017
Candida glabrata

Rising incidence in echinocandin resistance?
Echinocandin resistance rates

Echinocandin resistance of 8.0–9.3% was reported in a recent SENTRY program among 1669 bloodstream isolates (BSI) of *C. glabrata*. Similarly, over a 10-year period, echinocandin resistance in *C. glabrata* rose from 2–3% to > 13%.

Italy: (Mencarini, Infection 2018)
Between 2005 and 2015, increased use of echinocandins, but only one *Candida glabrata* isolate was resistant to caspofungin (1.9%) while 30% of *C. glabrata* were resistant to fluconazole.

China: (Hou, AAC 2018)
Among 158 *Candida glabrata* bloodstream isolates 8.9% were FLZ\textsuperscript{R}, 1.9% echinocandin-cross\textsuperscript{R}.

→ Echinocandin resistance rates in *C. glabrata* vary from 2% to 15%, depending on geography and host population.
Echinocandin resistance

Echinocaninds inhibit fungal glucan synthases, encoded by “fks” genes

Where most yeast only have a single gene, *C. glabrata* has two!

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overview of Fks hot spot sequences and amino acid sequence positions resulting in echinocandin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Fks1p</td>
</tr>
<tr>
<td></td>
<td>Hot spot 1</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>641</td>
</tr>
<tr>
<td><em>C. dubliniensis</em></td>
<td>641</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>625</td>
</tr>
</tbody>
</table>

Candida glabrata

CgFKS mutations seen in Germany

2016/17 @ NRZMyk:
- 41 isolates with ANI\textsuperscript{R}
- 12 isolates FLU\textsuperscript{R}, ANI\textsuperscript{R} with limited remaining treatment options
- 80\% with ANI > 0.125 harbour FKS mutations, but only 20\% of those with ANI = 0.125

<table>
<thead>
<tr>
<th>mutation</th>
<th>gene</th>
<th># isolates</th>
</tr>
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<tbody>
<tr>
<td>S663P</td>
<td>FKS2</td>
<td>4</td>
</tr>
<tr>
<td>F659Y</td>
<td>FKS2</td>
<td>2</td>
</tr>
<tr>
<td>F659del</td>
<td>FKS2</td>
<td>2</td>
</tr>
<tr>
<td>D666N</td>
<td>FKS2</td>
<td>1</td>
</tr>
<tr>
<td>D666Y</td>
<td>FKS2</td>
<td>1</td>
</tr>
<tr>
<td>L630Q</td>
<td>FKS1</td>
<td>1</td>
</tr>
<tr>
<td>none</td>
<td>-</td>
<td>9</td>
</tr>
</tbody>
</table>

Wagener et al, Der Mikrobiologe 2018
Walther and Kurzai, poster@DMYKG2018 (Innsbruck)
• close association exists between drug exposure and the emergence of resistance.
• *FKS*-mediated resistance is directly linked to prior, prolonged, and/or repeated drug exposure.
• chromosomal instability is rapidly observed following exposure to azoles or echinocandins
• cellular stress increases genetic diversity by altering genome integrity

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Candida glabrata

Fitness cost?

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S. Imbert, CMI 2016;22:891.e5e891.e8
55% of all *C. glabrata* isolates (susceptible and resistant) recovered from patients contain mutations within the MMR gene *MSH2*. Strains with specific *msh2* mutations exhibit a higher frequency of emergence of resistance *in vitro*, and deletion of *MSH2* causes elevated propensity to breakthrough antifungal treatment in a mouse model of gastrointestinal (GI) colonization.

Candida glabrata

C. glabrata population structure

Database: pubmlst.org/cglabrata
MLST tree structure subject to change!
Candida glabrata

C. glabrata population structure

Database: pubmlst.org/cglabrata

MLST tree structure subject to change!
Candida glabrata

C. glabrata population structure

Lott et al 2012

Healey et al 2016

Byun et al 2018

Database: pubmlst.org/cglabrata

MLST tree structure subject to change!
Aspergillus fumgiatus

Global spread of azole resistant linages
Mechanisms ofazole resistance:

**Cyp51A**

- G434C
- G54I,V,E,R,W
- L98H
- F219 C,I
- M220 I,L,K,R,T,V
- E427K
- Y431C
- G138C
- I266N
- G448S
- G432S,A
- E130D
- S400I
- D255E
- M172V,I
- N248T
- F495H
- F46Y
- Y121F
- T289A
- Q141H
- Q88H
- S52T
- N125I
- P216L
- H147Y
- N297T
- A. fish
gcagcattaccg
- A. oerl
gcagcattaccg
- A. fu
 gcagcaccactt
- A. fu TR34
gcagcaccacttcAGAGTTGTC
- A. fu TR46
gcagcaccacttcAGAGTTGTC
- A. fu TR53
gcagcaccacttcAGAGTTGTC

*pandemic* alleles:

- TR34/L98H
- TR46/Y121F/T289A

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

**Aspergillus fumigatus**

Dudakova et al., CMR 2017
**Azole resistance mechanisms in A.f.**

- **A** Normal susceptible cell in absence of azoles
  - Lanosterol → Cyp51A → Ergosterol

- **B** Normal susceptible cell in presence of azoles
  - Lanosterol → Cyp51A → Ergosterol

- **C** Mutations in cyp51A
  - Azole

- **D** Overexpression of cyp51A
  - Azole

- **E** Increased expression of efflux pumps
  - Azole

*Aspergillus fumigatus*

_Dudakova et al., CMR 2017_
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.
Current working hypothesis

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

• presence in hosts
• opportunistic infection
• (selection through azole prophylaxis ???)
• not only humans but also wild animals!
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

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Aspergillus fumigatus

Rößler S, Bader O, et al., AAC 2017
(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)

Aspergillus fumigatus

Rößler S, Bader O, et al., AAC 2017
**antifungal susceptibility testing of the culture isolate (BAL)**

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

AML Case

PSZ and VRZ ineffective in this patient
Strain with PSZ$^R$ and VRZ$^R$ strain with TR$_{46}$/Y121F/M172/T289A cyp51A mutation
Reported isolates in Germany

35 reported clinical isolates

NRZ data 2017
19 isolates, 2/3 with mutations in CYP51A (Walther, Kurzai, Poster@DMYKG2018)

Aspergillus fumigatus

55 reported environmental isolates
North-South imbalance?

environmental isolates

clinical isolates
Aspergillus fumigatus

ARAf findings by country

→ if people look for ARAf, they will find it!
No fitness cost associated with ARAf

Fitness Studies of Azole-Resistant Strains of *Aspergillus fumigatus*

Isabel Valsecchi, Emilia Mellado, Rémi Beau, Shriya Raj, Jean-Paul Latgé

Unité des Aspergillus, Institut Pasteur, Paris, France; Mycology Reference Laboratory, Instituto de Salud Carlos III, Madrid, Spain

Antimicrobial Agents and Chemotherapy

December 2015 Volume 59 Number 12

qPCR-based growth estimation after competition

on agar in mouse model
“International expert opinion”
(in the absence of clinical guidelines)

International expert opinion on the management of infection caused by azole-resistant *Aspergillus fumigatus*

Paul E. Verweij^a,*, Michelle Ananda-Rajah^b, David Andes^c, Maiken C. Arendrup^d,
Roger J. Brüggemann^e, Anuradha Chowdhary^f, Oliver A. Cornely^g, David W. Denning^h,
Andreas H. Groll^i, Koichi Izumikawa^j, Bart Jan Kullberg^k, Katrien Lagrou^l,
Johan Maertens^m, Jacques F. Meis^a,n, Pippa Newton^b, Iain Page^h,
Seyedmojtaba Seyedmousavi^a, Donald C. Sheppard^b, Claudio Viscoli^p, Adilia Warris^q,
J. Peter Donnelly^r


- 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:

Aspergilllus fumigatus infection is an increasing problem in Aspergillus fumigatus infection [1]. Two mutations, TRG1208 and TRG11211/2909, are frequently recovered from isolates of patients withazole-resistant invasive aspergillosis and are believed to originate from the environment [2, 3]. In regions with these environmental mutations, aza-resistant Aspergillus fumigatus 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 invasive aspergillosis resulting from voriconazole-susceptible and voriconazole-resistant A. fumigatus strains. We hypothesized that in regions with TRG1208 and TRG11211/2909 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

unit because of pulmonary deterioration requiring mechanical ventilation. The BAL showed heavy growth of A. fumigatus. Resistance screening of four colonies indicated voriconazole-susceptible infection, and voriconazole was added to the regimen. Ten days after voriconazole was started, routine follow-up bronchial aspirate yielded a voriconazole-resistant A. fumigatus colony (Table 1). Because the patient was improving clinically with adequate voriconazole plasma levels, voriconazole was continued. However, a bronchial aspirate taken on day 13 showed heavy growth of voriconazole-resistant A. fumigatus. Despite continued clinical improvement, azithromycin was added. Almost 2 weeks after intubation, ventilator support could be withdrawn, and immunosuppressive drugs were reintroduced. A follow-up computed tomography scan of the chest showed cavity formation in the right upper lobe lesion, and multiple nodular lesions. The patient suddenly died after having received voriconazole and azithromycin combination therapy for 34 days. At autopsy, multiple pulmonary fungal lesions were found, as well as one fungal lesion in the kidney transplant. A. fumigatus colonies cultured from the cavitation...
large-scale screening

- molecular mechanisms
- a scheme to screen large scale collections
- tools to locally analyse sequencing data
Take-home messages

- *Candida auris* is a globally emerging, fluconazole resistant species associated to (nosocomial?) outbreaks

- Echinocandin resistance is emerging in *Candida glabrata*
- Potentially tied to hypermutability phenotype (*MSH2*), already present in parts of the *C. glabrata* population

- Spread of azole resistance in *A. fumigatus* 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
Acknowledgement

*Candida auris* and *Candida glabrata* epidemiologic data:
O Kurzai (Würzburg/Jena), A. Hamprecht (Köln)

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

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

**Contributions of strains:**
J. Steinmann (Nürnberg), A. Hamprecht (Köln), S. Rößler (Dresden)
Multiplex -PCR approach
(AsperGenious 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

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 University, United Kingdom; Infection, Immunity and Biochemistry, School of Medicine, Cardiff University, Cardiff University, 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
company statement: multi-platform RT-PCR
Detects A. fumigatus, A. terreus, A. spec. from BAL samples
From A. fumigatus: L98H, TR34, T289A, Y121F

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).
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