Fungal epidemiology in CF patients & overview of EUCAST breakpoints

Cornelia Lass-Flörl
Division of Hygiene and Medical Microbiology
Innsbruck Medical University

ESCMID 2013, Berlin
Incidence of systemic fungal disease has increased since the 1950s.

The emergence of systemic fungal disease in humans is considered by many to be a 20th-century phenomenon.

Fungi are highly adaptable to new environmental niches including what might be considered “extreme” environments.
This figure illustrates the complex interplay of factors that result in disease at the individual and population levels. The presence of a pathogen is a necessary, but not sufficient, cause of a particular disease.

Snieszko (1974)
Cystic fibrosis (CF)

- An autosomal recessive disease that causes abnormalities of ion transport of epithelial cells and presents as a multisystem disease.
- **Chronic infections in the lungs** are among the most prominent clinical manifestations and are related with the obstruction of respiratory ways by viscous secretions.
- Mucus hypoxia and stasis may contribute to the propensity for bacterial infections, mainly due to *Staphylococcus aureus, Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Burkholderia cepacia*.
The major clinical syndromes due to fungal infections in CF

- Allergic Broncho Pulmonary Aspergillosis (ABPA);
- Indwelling central venous catheter related candidemias;
- Invasive mycoses, especially after lung transplantation.
Prevalence of Aspergillus fumigatus and other fungal species in the sputum of adult patients with cystic fibrosis

Mycoses 2003; 46: 19-23

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Fungal species isolated from 369 sputum samples from 94 cystic fibrosis patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>In patients</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>43</td>
</tr>
<tr>
<td>Other moulds</td>
<td></td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>2</td>
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<tr>
<td>Penicillium sp.</td>
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<tr>
<td>Chrysonilia sp.</td>
<td>1</td>
</tr>
<tr>
<td>Moulds, not further classified</td>
<td>3</td>
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<tr>
<td>Yeasts</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>71</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>3</td>
</tr>
<tr>
<td>Exophila dermatitidis</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Prevalence of Aspergillus fumigatus in sputum of cystic fibrosis patients from different centres.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference, location</td>
<td>Patients, n</td>
</tr>
<tr>
<td>Nelson et al.,² Rochester, USA</td>
<td>37</td>
</tr>
<tr>
<td>Laufer et al.,⁴ Wisconsin, USA</td>
<td>55</td>
</tr>
<tr>
<td>Schoenheyder et al.,⁵ Copenhagen, Denmark</td>
<td>150</td>
</tr>
<tr>
<td>Penketh et al.,³ London, UK</td>
<td>288</td>
</tr>
<tr>
<td>Bauernfeind et al.,¹ Munich, Germany</td>
<td>102</td>
</tr>
<tr>
<td>Mroueh and Spock,¹¹ Durham, USA</td>
<td>236</td>
</tr>
<tr>
<td>Becker et al.,⁶ Seattle, USA</td>
<td>49</td>
</tr>
<tr>
<td>Milla et al.,¹⁵ Delaware, USA</td>
<td>370</td>
</tr>
<tr>
<td>Burns et al.,⁷ USA, different centres</td>
<td>465</td>
</tr>
</tbody>
</table>
Fungal infection developed in 44 % (14 / 32) of patients
• tracheo-bronchial aspergillosis was observed in 9 (in 1 associated with pneumonia)
• isolated pneumonia was observed in 5
• survival was 21 % (3 / 14)
Epidemiology

The actual prevalence and frequency of fungi in CF is not known (data from literature varies substantially).

Why are there such differences?

• genetic heterogeneity in patients
• different lifestyles
• antimycotic prophylaxis (drug, dose?)
• diagnosis: lack of standardization:
  media, incubation time, sample processing, identification (sequencing versus microscopy)

The genus, the species.....

Klich MA. Identification of common *Aspergillus* species (2002). CBS.
Aspergillus species

A. fumigatus
A. niger
A. terreus
A. versicolor
A. flavus

Frequency:
up to 57 % (3.2 % - 57 %)

Culture:
• complete medium (Sab 2 % + antibiotic additives)
• incubation: 3-7 days

Clinical relevance:
• allergic bronchopulmonary Aspergillosis = ABPA
• immune response to A. fumigatus results in severe lung damage
• A. fumigatus leads to hypersensitivity
• invasive infections after lung transplantation

Scedosporium/Pseudallescheria species

P. apiosperma
P. boydii
S. aurantiacum
S. prolificans

Frequency:
up to 14.7%

Culture:
• Selective medium (SceSel+, SelSel3, Benomyl + antibiotic additives)
• incubation: at least 14 days

Clinical relevance:
• allergic bronchopulmonary Scedosporiosis = ABPS
• immune response to Scedosporium spp. results in severe lung damage
• invasive infections after lung transplantation
• Contraindication for lung transplantations? 3 cases were lethal!
Trichosporon species

Trichosporon mycotoxinivorans (polymorph)
T. asahii, T. mucoides, T. inkin, T. ovoides, T. cutaneum and T. asteroides

Frequency:
Increasing, lack of epidemiological studies
(only 2 publications; first case in 2009)

Culture:
• complete medium (Sab 2 % + antibiotic additives)
• incubation: at least 14 days

Clinical relevance:
• leads to pneumonia in CF patients
• colonisation associated with worsening of lung function
• empiric treatment with voriconazole, based on the fact that the isolate is susceptible

**Geosmithia species**

**Geosmithia argillacea** (Penicillium-like fungi)

**Frequency:**
Increasing, lack of epidemiological studies (only 2 publications; first case in 2009)

**Culture:**
- complete medium (Sab 2 % + antibiotic additives)
- incubation: at least 7days

**Clinical relevance:**
- frequently found in patients with F508del mutation
- clinical relevance unknown
- dissemination after lung transplantation?
- Caspofungin in vitro active (≤ 0.5 µg/mL)
- confusion with Penicillium?

Exophiala species (Wangiella)

Exophiala dermatitidis (dimorph)

Frequency:
Up to 19%

Culture:
• Erythritol-Chloramphenicol Agar (ECA)
• incubation: at least 21 days

Clinical relevance:
• clinical relevance unknown
• associated with pancreatic insufficiency ($P = 0.0198$) or advanced lung diseases
• lack of prospective studies
• lowest in vitro MIC for Voriconazole and Posaconazole*


Analysis of 8 sputum samples of 4 patients

<table>
<thead>
<tr>
<th>Mycological culture</th>
<th>Molecular analysis</th>
<th>Pyrosequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nested PCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rt-PCR</td>
<td></td>
</tr>
<tr>
<td><strong>DE</strong></td>
<td>Fungi</td>
<td>Aspergillus spp. (also A. lentulus, A. penicillioides)</td>
</tr>
<tr>
<td>0</td>
<td><em>Candida albicans</em> Geotrichum sp</td>
<td>Eurotium halophilicum</td>
</tr>
<tr>
<td>0</td>
<td><em>C. albicans</em></td>
<td>Candida spp. (also Candida lusitaniae)</td>
</tr>
<tr>
<td>0</td>
<td><em>C. albicans</em></td>
<td>Geotrichum spp.</td>
</tr>
<tr>
<td>0</td>
<td><em>Aspergillus fumigatus</em> <em>C. albicans</em></td>
<td>Pneumocystis jirovecii</td>
</tr>
<tr>
<td>0</td>
<td><em>A. fumigatus</em> <em>Aspergillus flavus</em></td>
<td>Malassezia spp. (M. globosa, M. sympodialis)</td>
</tr>
<tr>
<td><strong>PH,H</strong></td>
<td><em>A. fumigatus</em> <em>C. albicans</em></td>
<td>Penicillium spp. (Penicillium camemberti)</td>
</tr>
<tr>
<td>0</td>
<td><em>C. albicans</em></td>
<td>Didymella exitialis</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td><em>C. albicans</em> <em>A. fumigatus</em></td>
<td>Cryptococci</td>
</tr>
</tbody>
</table>

4 different species

24 different species

- case reports
- allergic reactions and/or asthma
- skin infections
- up to now several fungi have not been detected from clinical samples

Fungi & clinical relevance

• A short-term worsening of lung function is proven.
• The clinical relevance of *Exophiala* spp., *Trichosporon* spp. and *Geosmithia* spp. is unknown.
• More rapid worsening of lung function in patients suffering from ABPA than in patients without ABPA; especially due to the inflammatory reaction to the fungus, but also because of the structural injury of the lung tissue.
• *Scedosporium/Pseudoallescheria* species can also cause a ABPS with ABPA-similar course.
• *Scedosporium* spp., *Trichosporon* spp. and *Aspergillus* spp. can cause invasive and disseminated infections in immunosuppressed patients after lung transplantation. There is an ongoing debate about whether colonisation with these fungi should be considered as contraindication for lung transplantation.
• In 11 % of lung transplanted CF-patients *Aspergillus* causes an invasive infection within 3 months after lung transplantation.

Sequeiros IM et al. Chron Respir Dis 2012; 9: 9-16
Solé et al. Rev Iberoam Micol 2011
Miraldi F et al. Transpl Infect Dis 2012
Sources

**Trichosporon spp.:**
Environment (soil), skin (humans and animals); first isolation in termites.

**Geosmithia argillacea:**
Food contaminants (heat-resistant fungus), environment, soil.

**Exophiala spp.:**
Dishwasher, sauna, steam bath, hand-warm habitats.

**Scedosporium/Pseudoallescheria spp.:**
Potted plants, compost, soil and water (esp. contaminated soil and water).

**Aspergillus spp.:**
In-door environment, air, soil, food, etc.

Harun et al. Med Mycol 2010; 48: S70-76
Azole resistance in Aspergillus
Antifungal Resistance

Although in vitro susceptibility testing is often used to select antimicrobial agents that are most likely to be active clinically, perhaps the most important role of such testing is in detecting resistance (eg, determining those agents that will not work).
Epidemiological cutoff values (ECOFFs)

- ECOFFs discriminate the wild-types strains from acquired resistance mechanisms if drugs display a bimodal MIC distribution.

- Define upper limit of wild type MIC distribution with no acquired resistance mechanisms.

- Helps identify organisms requiring further characterization.
Overlap between WT and non-WT

A. *Fumigatus* and itraconazole

EUCAST-AFST (Arendrup MC, Hope WW, Cuenca-Estrella M, Lass-Flörl C)
CLSI approach to the development of clinical breakpoints (CBPs)

- ECVs are not always the same as CBPs.
- Whereas the CBPs are used to indicate those isolates that are likely to respond to treatment with a given antimicrobial agent administered at the approved dosing regimen for that agent, the ECV may serve as the most sensitive measure for the emergence of strains with reduced susceptibility (acquired resistance mechanisms) to that agent.

**Candida spp.**

EUCAST Antifungal Clinical Breakpoint Table v. 4.1, valid from 2012-03-05

**MIC method (EUCAST standardised broth microdilution method)**
- **Medium:** RPMI1640-2% glucose, MOPS buffer
- **Inoculum:** Final $0.5 \times 10^5$ – $2.5 \times 10^5$ cfu/mL
- **Incubation:** 18-24h
- **Reading:** Spectrophotometric, full inhibition for amphotericin B but 50% growth inhibition for other compounds
- **Quality control:** *C. parapsilosis* ATCC 22019 or *C. krusei* ATCC 6258

### MIC Breakpoint (mg/L)

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>MIC breakpoint (mg/L)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. albicans</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. glabrata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. kru sei</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. parapsilosis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. tropicalis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. guillermontii</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-species related breakpoints¹</td>
<td></td>
</tr>
</tbody>
</table>
| Amphotericin B   | S ≤ R > S ≤ R > S ≤ R > S ≤ R > S ≤ R > S ≤ R > S ≤ R > S ≤ R > | 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.
| Anidulafungin    | 0.03 0.03 0.06 0.06 0.06 0.06 - - 0.06 0.06 | 2. The ECOFFs for these species are in general higher than for *C. albicans*.
| Caspofungin      | Note³ Note³ Note³ Note³ Note³ Note³ - - Note³ Note³ | 3. Due to significant inter-laboratory variation in MIC ranges for caspofungin, EUCAST breakpoints have not yet been established.
| Fluconazole      | 2 4 IE² IE² - - 2 4 2 4 IE² IE² 2 4 | 4. Strains with MIC values above the S/I breakpoint are rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant.
| Itraconazole     | IP IP IP IP IP IP IP IP IP IP |       |
| Micafungin       | IP IP IP IP IP IP - - IP IP IP IP IP |       |
| Posaconazole     | 0.06 0.06 IE² IE² IE² IE² 0.06 0.06 0.06 0.06 IE² IE² IE² IE² |       |
| Voriconazole     | 0.12⁴ 0.12⁴ IE IE IE IE 0.12⁴ 0.12⁴ 0.12⁴ 0.12⁴ IE² IE² IE² IE² |       |

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.
### Aspergillus spp.

"-" indicates that susceptibility testing is not recommended as the species is a poor target for therapy with the drug. Isolates may be reported as R without prior testing. "IE" indicates that there is insufficient evidence that the species in question is a good target for therapy with the drug.

#### MIC method
- EUCAST standardised broth microdilution method

- **Medium:** RPMI1640-2% glucose, MOPS as buffer
- **Inoculum:** Final 1x10(5) – 2.5x10(5) cfu/mL
- **Incubation:** 48h
- **Reading:** Visual

#### Quality control
- A. fumigatus ATCC 204305, A. flavus ATCC 204304, A. fumigatus F 6919, A. flavus CM 1813, C. parapsilosis ATCC 22019 (read after 18-24 h) or C. krusei ATCC 6258 (read after 18-24 h)

#### Antifungal agent

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>MIC breakpoint (mg/L)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. flavus</td>
<td>A. fumigatus</td>
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<tr>
<td>Amphotericin B</td>
<td>IE²</td>
<td>IE²</td>
</tr>
<tr>
<td>Anidulafungin</td>
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<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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<td>Itraconazole⁴</td>
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<td>2</td>
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<tr>
<td>Micafungin</td>
<td>IE</td>
<td>IE</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>

#### Notes

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.

2. The ECOFFs for these species are in general one step higher than for A. fumigatus.

3. There are too few MIC data to establish ECOFFs and hence to suggest any breakpoints.

4. Monitoring of azole trough concentrations in patients treated for fungal infection is recommended.

5. The MIC values for isolates of A. niger and A. versicolor are in general higher than those for A. fumigatus. Whether this translates into a poorer clinical response is unknown.

6. Provided adequate drug exposure has been confirmed using therapeutic drug monitoring (TDM). There remains some uncertainty regarding cut-off values for posaconazole concentrations that separate patients with a high probability of clinical success from those with a low probability of clinical success. In some circumstances (e.g. patients with persistent and profound neutropenia, large lesions, or those with other features associated with a poor clinical outcome) a relatively high trough concentration should be sought. Preclinical and clinical data suggest this value should be >1 mg/L at steady state. For other patient groups a lower trough concentration may be acceptable. For prophylaxis a target concentration of >0.7 mg/L has been suggested.
## EUCAST Aspergillus BPs 2012

BPs indicated as $S \leq x / R > y$

<table>
<thead>
<tr>
<th>AF compound</th>
<th>flavus</th>
<th>fumigatus</th>
<th>nidulans</th>
<th>niger</th>
<th>terreus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin</td>
<td>IE*</td>
<td>1/2</td>
<td>Note</td>
<td>1/2</td>
<td>Poor target</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>IE*</td>
<td>1/2</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>IE*</td>
<td>0.125/0.25**</td>
<td>IE*</td>
<td>IE*</td>
<td>Note</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>IE*</td>
<td>1/2</td>
<td>Note</td>
<td>IE*</td>
<td>IE*</td>
</tr>
</tbody>
</table>

* MICs are higher than for A. fumigatus

** provided sufficient levels can be achieved

Note: the MICs are similar to A. fumigatus but insufficient clinical data for BP setting
What is the role of MIC? Help to guide antifungal treatment!

- Drug interactions?
- Prior treatment?
- Localisation of infection?
- Liver & renal functions?
- Gastrointestinal tract?
- Colonisation of catheter? Biofilm?
- Immunosuppression & Reconstitution?
- Dosis, time, grade immunosuppression?
- Resistance?
- PK/PD?
- Pathogens, virulence?
- Diagnostic?
- Coinfection?
CF & azole resistance in *Aspergillus*

8% prevalence of itraconazole resistance in CF patients, mostly driven by TR/L98H (6%). Data confirm that TR/L98H occurs in France and can be highly prevalent in CF patients. 2012

(4.5%) harbored azole-nonsusceptible or -resistant *A. fumigatus* isolates, and five of those six patients had isolates with Cyp51A alterations (M220K, tandem repeat [TR]/L98H, TR/L98H-S297T-F495I, M220I-V101F, and Y431C). All six patients were previously exposed to azoles. Genotyping suggested selection of resistance in the patient as well as resistance being achieved in the environment. 2011

Development of resistance in *A. fumigatus* to antifungals appears to be rare amongst CF patients. 1 of 159 isolates showed higher posaconazole MIC. 2010

Morio F, JAC, 2012
The emergence of azole resistance in the *A. terreus* isolates in patients with CF is of particular concern:

1. patients may later develop *Aspergillus* bronchitis or severe complications, such as ABPA.
2. in patients undergoing lung transplantation, an azole-resistant invasive infection may develop.
3. this species is intrinsically a poor target for amphotericin B, leaving very few treatment options for infections involving isolates with acquired azole resistance.

Arendrup M et al. JID 2012
Conclusions

- Mucus hypoxia and stasis may contribute to the propensity for fungal colonisation
- Outcome unclear; s short-term worsening of lung function is proven
- The actual prevalence and frequency of fungi in CF is not known, epidemiological differences
- A. fumigatus is leading, others follow
- Azole resistance may be an issue
Thank you very much for your attention!

Klich MA. Identification of common *Aspergillus* species (2002). CBS.