What is the role of in-vitro susceptibility testing in current routine practice?

Cornelia Lass-Flörl
Innsbruck Medical University
Faculty disclosure

- Invited speaker: Pfizer, Gilead, MSD, Schering-Plough
- Consultant: Pfizer, Gilead, Schering-Plough
- Research Grants: Pfizer, Gilead, Schering-Plough
Roadmap

In vitro susceptibility methods
EUCAST breakpoints
MICs and clinical value
Increased interest in antifungal susceptibility testing

1. Changing epidemiology
2. New drugs – more choices
3. More immunocompromised patients
4. Antifungal susceptibility testing becoming more commonplace
A brief history of antifungal susceptibility testing standardization

- **1982**: Established subcommittee
- **1986**: Develop reproducible method
- **1992**: M27-P method introduced
- **1997**: M27-A method introduced

- 20% hospitals performing testing for yeast; intra/inter-laboratory agreement poor
- Synthetic medium (RPMI)
  - Broth-based method
  - 0.5-2.5 x10^3
  - Breakpoints

- Conidia forming filamentous fungi
- Disk-diffusion method-yeast
- Disk-diffusion method-moulds

- Higher glucose; 24 hour endpoint; spectrophotometer

Minimum inhibitory concentration (MICs)

1. MICs are not an absolute measurement: MICs vary based on medium, temperature, ...
2. Suggests that target fungal species is susceptible to antifungal drug
3. MIC values might not necessarily correlate with in vivo efficacy noted
4. Host factors play a crucial role in clinical practice
MICs

1. MICs are not an absolute measurement: MICs vary based on medium, temperature, ...
2. Suggests that target fungal species is susceptible/resistant to antifungal drug
3. MIC values might not necessarily correlate with in vivo efficacy noted
4. Host factors play a crucial role in clinical practice
Disc diffusion (M44-P)

- no MIC obtained
- screening method
- qualitative results
  susceptible or resistant or intermediate

E-test strips
- MIC value obtained
- sometimes problematic endpoints
  - quantitative results
    a value in µg/ml and an interpretation in S/R/I
....there are also other test systems

- An array of commercial formats
  - Vitek-2
  - YeastOne
  - Fungitext
  - Others...

Some are more equal than others!

- Molecular methods
CLSI M27-A, 74.0%
Etest, 83.8%
Sensititre YeastOne, 64.1%
Disk, 80.6%
Fungitext, 76.6%
Integral System Yeasts, 28.3%
Candifast, 27.4%

All methods except Candifast and Integral System Yeasts showed good agreement with CLSI M27-A results for both C. albicans and non-C. albicans isolates.

(Morace et al., JCM, 2002)
**Candida spp.**

**EUCAST Antifungal Clinical Breakpoint Table v. 5.0, valid from 2013-01-09**

**MIC method (EUCAST standardised broth microdilution method)**
- **Medium:** RPMI1640-2% glucose, MOPS buffer
- **Inoculum:** Final 0.5x10^5 – 2.5x10^5 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

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>C. albicans</th>
<th>C. glabrata</th>
<th>C. krusei</th>
<th>C. parapsilosis</th>
<th>C. tropicalis</th>
<th>C. guilliermondii</th>
<th>Non-species related breakpoints</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flucytosine</td>
<td>S ≤ R &gt;</td>
<td>S ≤ R &gt;</td>
<td>S ≤ R &gt;</td>
<td>S ≤ R &gt;</td>
<td>S ≤ R &gt;</td>
<td>S ≤ R &gt;</td>
<td>S ≤ R &gt;</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.12^4</td>
<td>0.12^4</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
<td></td>
</tr>
<tr>
<td>Caspofungin</td>
<td>Note^3</td>
<td>Note^3</td>
<td>Note^3</td>
<td>Note^3</td>
<td>Note^3</td>
<td>Note^3</td>
<td>IE2</td>
<td></td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>0.03</td>
<td>0.03</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>-</td>
<td>IE2</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td></td>
</tr>
<tr>
<td>Micafungin</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td></td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.06</td>
<td>0.06</td>
<td>IE^2</td>
<td>IE^2</td>
<td>IE^2</td>
<td>0.06</td>
<td>0.06</td>
<td></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.

2. The ECOFFs for these species are in general higher than for *C. albicans*.

3. Due to significant inter-laboratory variation in MIC ranges for caspofungin, EUCAST breakpoints have not yet been established.

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.

IE: insufficient evidence
IE2: insufficient evidence; testing is not recommended; report as “R”
IP: in preparation

ESCMID Online Lecture Library © by author
MICs and clinical relevance
Acquired resistance to echinocandins in *Candida albicans*: case report and review

Marie-Thérèse Baixench¹, Naji Aoun², Marie Desnos-Ollivier³, Dea García-Hermoso³, Stéphane Bretagne³, Sandrine Ramires², Christophe Piletty² and Eric Dannaoui¹,³*
## AST recommendation

<table>
<thead>
<tr>
<th>Isolated from</th>
<th>FOR patient management</th>
<th>FOR Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood and other deep</td>
<td>All isolates and particularly:</td>
<td>• All isolates should be tested using a reference method or a validated commercial method</td>
</tr>
<tr>
<td>sites</td>
<td>1. Strains from patients exposed to antifungal agents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Clinical failures</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Rare and emerging species</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Species that are known to be resistant or less susceptible to antifungal drug(s) in clinical use</td>
<td></td>
</tr>
<tr>
<td>Superficial sites</td>
<td>• Failed to respond or relapsing infection</td>
<td>• Periodical epidemiological studies should be done</td>
</tr>
<tr>
<td></td>
<td>• Surveillance cultures from patients exposed to antifungal agents</td>
<td></td>
</tr>
</tbody>
</table>

### References:
1. CLSI M27-A3, M27-S3, M44-A2
2. EUCAST Discussion Document E.Dis 7.1
4. EUCAST-AFST. Clin Microbiol Infect 2008;14:193-95
5. EUCAST-AFST. Clin Microbiol Infect 2008;14:985-987

AST: Antifungal Susceptibility Testing
Moulds - the Aspergilli
Echinocandins and *Aspergillus* Different from routine tests

1. Activity does not fit classic definition of fungicidal – no reduction in the number of colony count.

2. In *Aspergillus* the 1,3 β-glucane synthase complex is localized in the apical tips of the growing hyphae.
   - Inhibition results in profound changes in growth, morphology.
   - Changes decrease ability to invade blood vessels but do not decrease colony count.
   - Colony counts ≠ number of viable cells → traditional endpoints are not useful.
   - MEC: concentration where microscopically swollen, distorted hyphae.
CASPOFUNGIN / Aspergillus flavus
Aspergillus fumigatus
### EUCAST Antifungal Clinical Breakpoint Table v. 5.0, valid from 2013-01-09

#### MIC method (EUCAST standardised broth microdilution method)
- **Medium:** RPMI1640-2% glucose, MOPS as buffer
- **Inoculum:** Final 1x10⁵ – 2.5x10⁵ 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 18-24h) or C. krusei ATCC 6258 (read after 18-24h)

<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>
</tr>
<tr>
<td>Amphotericin B</td>
<td>IE²</td>
<td>IE²</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>IE</td>
<td>IE</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>IE</td>
<td>IE</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Itraconazole⁴</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Micafungin</td>
<td>IE</td>
<td>IE</td>
</tr>
<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.
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.
Amphotericin B MICs: clinical relevance

• Non-WT amphotericin B MICs > 1 µg/ml have been suggestive of clinical failure because they only approximate achievable serum concentrations at high dosages of ≥1 mg/kg of body weight/day.

• Incidence of amphotericin B MICs > 1 µg/ml for A. terreus has been consistently high by both methods (> 45.9 % [EUCAST] ≥ 68 % [CLSI]) and response to treatment has been poor in animal models.

In vitro amphotericin B resistance associated with molecular mechanisms and/or treatment outcome in patients or animal models

<table>
<thead>
<tr>
<th>Species and agents</th>
<th>MIC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mechanism/Mutation&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amphotericin B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. flavus</td>
<td>&gt; 1</td>
<td>Cell wall change</td>
<td>NA</td>
</tr>
<tr>
<td>A. terreus</td>
<td>&gt; 4</td>
<td>Catalase production</td>
<td>NA</td>
</tr>
<tr>
<td>A. terreus</td>
<td>≥ 2</td>
<td>ND</td>
<td>Death</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>≥ 2</td>
<td>ND</td>
<td>Death</td>
</tr>
<tr>
<td>P. boydii</td>
<td>≥ 2</td>
<td>ND</td>
<td>No response</td>
</tr>
<tr>
<td>A. strictum</td>
<td>8</td>
<td>ND</td>
<td>No response</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIC= minimal inhibitory concentration. MEC= minimal effective concentration (applies only to caspofungin).

<sup>b</sup> Mutation of the *FKS1* gene (applies only to caspofungin).

<sup>c</sup> Laboratory mutant; WT= caspofungin MEC ≤ 0.5 µg/ml according to the newly defined epidemiologic cutoff value (ECV) for *Aspergillus fumigatus* and caspofungin.

<sup>d</sup> CLSI/Etest values; over-expression of *FKS1* gene without known mutation (applies only to caspofungin).

NA= isolate not available or not applicable; ND= not determined; ?= unknown or not reported.
Triazole MICs: clinical relevance

- Data from isolated cases have clearly shown a relationship between non-WT triazole MICs (> 1 µg/ml) for *A. fumigatus* and *A. flavus* and a poor or lack of response to triazole therapy as well as an incidence of breakthrough infections during azole therapy.
- In some cases single or multiple point mutations are responsible for an increased level of *cyp51A* expression.

Genetic mutations associated with cross-resistance between itraconazole, posaconazole and voriconazole in *Aspergillus fumigatus* isolates from clinical or animal studies

<table>
<thead>
<tr>
<th>Treatment agent</th>
<th>MIC increase (µg/ml) of itra/posa/vori (Method)</th>
<th>Single and (multiple) gene mutations</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itra (8)</td>
<td>WT→ &gt;16/0.5/8 (E)</td>
<td>(P216L,F2191,G54E)</td>
<td>Relapse</td>
</tr>
<tr>
<td>Itra (6)</td>
<td>WT→ &gt;8/0.5/8/1-8 (E)</td>
<td>(M220I,G54E),G54R,G448S, H147,P216L</td>
<td>Failure/no response</td>
</tr>
<tr>
<td>Vori (1)</td>
<td>WT→ &gt;8/ND/8 (E)</td>
<td>ND</td>
<td>No response</td>
</tr>
<tr>
<td>Pos (3)</td>
<td>WT→ &gt;16/2 (C)</td>
<td>M2201,G54W, TR/L98H G448S</td>
<td>Lower response</td>
</tr>
<tr>
<td>Vor (2)</td>
<td>WT→ &gt;8/2 (C)</td>
<td>G448S</td>
<td>Failure</td>
</tr>
<tr>
<td>Itra (4)</td>
<td>WT→ &gt;16/0.25 (C)</td>
<td>(M2201,G54R)</td>
<td>Relapse</td>
</tr>
<tr>
<td>Vor (3)</td>
<td>WT→ &gt;16/8-16/0.5 (C)</td>
<td>L98H</td>
<td>Relapse</td>
</tr>
<tr>
<td>Itra (5)</td>
<td>&gt;16/0.5 (C)</td>
<td>L98H</td>
<td>Breakthrough</td>
</tr>
</tbody>
</table>

*WT MICs= itraconazole <1 µg/ml; posaconazole <0.5 µg/ml and voriconazole <1 µg/ml; non-WT= itraconazole >1 µg/ml; posaconazole >0.5 µg/ml and voriconazole >1 µg/ml, according to the newly defined epidemiologic cutoff values (ECVs) for *Aspergillus fumigatus*.*

*Isolates from single or several patients.*

*C=CLSI and E=EUCAST (Bolded values are WT MICs for the corresponding species/agent combination.)*

*Animal model*

Espinol-Ingroff et al. JCM 2010
Echinocandin MECs: clinical relevance

- A breakthrough *A. fumigatus* infection during caspofungin treatment was reported in the absence of characteristic *FKS1* resistant mutations, but the MEC result was not provided.
- Overexpression in the absence of *FKS* gene mutations in *A. fumigatus* isolate from another patient failing caspofungin therapy was reported; the CLSI MEC was below the proposed ECV (0.5 μ/mg).

Echinocandin MECs: clinical relevance

Less information is available for the other echinocandins, but low anidulafungin and micafungin MECs (≤ 0.06 µ/ml) and/or wide disk diameters (> 20 mm) for the infecting Aspergillus isolates in experimental aspergillosis correlated with good response to therapy.

In vitro caspofungin resistance associated with molecular mechanisms and/or treatment outcome in patients or animal models.

<table>
<thead>
<tr>
<th>Species and antifungal agent</th>
<th>MEC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mechanism/Mutation&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caspofungin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus (mutant)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>WT → 16</td>
<td>S678P</td>
<td>NA</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>NA</td>
<td>None</td>
<td>Breakthrough</td>
</tr>
<tr>
<td>A. fumigatus&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.25/32</td>
<td>over-expression</td>
<td>Failure</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>4-8</td>
<td>ND</td>
<td>Breakthrough</td>
</tr>
<tr>
<td>A. flavus</td>
<td>?</td>
<td>ND</td>
<td>Death</td>
</tr>
<tr>
<td>F. solani</td>
<td>&gt; 8</td>
<td>Y639 residue</td>
<td>NA</td>
</tr>
<tr>
<td>S. prolificans</td>
<td>&gt; 8</td>
<td>Y639 residue</td>
<td>NA</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIC= minimal inhibitory concentration. MEC= minimal effective concentration (applies only to caspofungin).

<sup>b</sup> Mutation of the *FKS1* gene (applies only to caspofungin).

<sup>c</sup> Laboratory mutant; WT= caspofungin MEC ≤ 0.5 μg/ml according to the newly defined epidemiologic cutoff value (ECV) for *Aspergillus fumigatus* and caspofungin.

<sup>d</sup> CLSI/Etest values; over-expression of *FKS1*gene without known mutation (applies only to caspofungin).

NA= isolate not available or not applicable; ND= not determined; ?= unknown or not reported.
Others
### Triazole MICs: clinical relevance

<table>
<thead>
<tr>
<th>Voriconazole*</th>
<th>Response rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. Prolificans (9)</td>
</tr>
<tr>
<td>MICs ≥ 4 µg/ml</td>
<td>37 %</td>
</tr>
<tr>
<td>MICs &lt; 4 µg/ml</td>
<td>48 %</td>
</tr>
</tbody>
</table>

*During phase III voriconazole clinical trials

### Scedosporiosis and response rate %

<table>
<thead>
<tr>
<th>Voriconazole</th>
<th>Scedosporiosis and response rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICs &lt; 1 µg/ml</td>
<td>54 % (P. boydii)</td>
</tr>
<tr>
<td>MICs &gt; 4 µg/ml</td>
<td>40 % (S. prolificans)</td>
</tr>
</tbody>
</table>

Correlations of posaconazole MICs and treatment outcome in experimental mucoraceous infections have been variable, but higher rates of survival were reported in mice infected with *R. oryzae*.
Conclusions EUCAST and M38-A

- can identify amphotericin B resistance among isolates of the same species.
- can discriminate non-WT from WT strains of most fungi when testing amphotericin B, triazoles and caspofungin.
- poor response to therapy or failure has been linked to non-WT or high MICs of amphotericin B and especially itraconazole and voriconazole in isolated cases.
- less information is available for the echinocandins, but non-WT caspofungin MECs have also been associated with failure or breakthrough during therapy with this agent.
- since ECVs are species specific, identification of the infecting isolate to the species level is also helpful.
- more than one fungus may be involved.
Conclusions
In vitro susceptibility testing

**Yeast**s
- Sterile body site
- plus non-C. albicans
- Azole (?)
- Non-responder
- Rare species

**Molds**
- Non A. fumigatus
- all:
  - Non responder
  - Long treatment & azole
  - Rare species
Thank you very much for your attention!