Emerging echinocandin resistance in *Candida*

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Advisory board: MSD, Pcovery, Pfizer; Acted as consultant for: Alcimed, Astellas, MSD & Pfizer  
Chairwoman for EUCAST-AFST

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**Agenda**

- Echinocandins  
  - mechanism of action  
  - mechanisms of resistance

- Size of the problem  
  - in general  
  - specifically for *C. glabrata*

- What to do  
  - detection  
  - antifungal stewardship
Echinocandin-use: DK vs. Norway (total DDD)

Note:
NO population is 9% smaller than the DK population


Echinocandins mode of action & resistance

Echinocandins
- Anidulafungin
- Caspofungin
- Micafungin

Glucan synthase
FKS gene(s)

Cell wall β-1,3 glucan

Ergosterol

Lanosterol

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**Echinocandins mode of action & resistance**

**Glucan synthase**

Target gene mutations
Two hot spot regions in:
- **FKS1**
- but in *C. glabrata* also **FKS2**

**Candida Echinocandin resistance: FKS**

<table>
<thead>
<tr>
<th>AFG</th>
<th>EUCAST ECOFF (mg/L)</th>
<th>FKS1p</th>
<th>FKS2p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hot spot 1</td>
<td>Hot spot 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1st AA no. AA sequence</td>
<td>1st AA no. AA sequence</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>0.03</td>
<td>FL TLSLRDP</td>
<td>FL TLSLRDP</td>
</tr>
<tr>
<td><em>C. dublensis</em></td>
<td>0.03</td>
<td>FL TLSLRDP</td>
<td>FL TLSLRDP</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>0.06</td>
<td>FL TLSLRDP</td>
<td>FL TLSLRDP</td>
</tr>
<tr>
<td><em>C. kefyr</em> (0.03)</td>
<td>54* TLSLRDP</td>
<td>769* DLIRYTL</td>
<td></td>
</tr>
<tr>
<td><em>C. krusei</em> (0.06)</td>
<td>655 TLSLRDP</td>
<td>1364 DLIRYTL</td>
<td></td>
</tr>
<tr>
<td><em>C. lusitaniae</em> (0.06)</td>
<td>634* TLSLRDP</td>
<td>** DLIRYTL</td>
<td></td>
</tr>
<tr>
<td><em>C. tropicalis</em> (0.06)</td>
<td>76* TLSLRDP</td>
<td>792* DLIRYTL</td>
<td></td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>4</td>
<td>FL TLSLRDA</td>
<td>1369 DLIRYTL</td>
</tr>
<tr>
<td><em>C. metapsilosis</em> (4)</td>
<td>104* FL TLSLRDA</td>
<td>821* DLIRYTL</td>
<td></td>
</tr>
<tr>
<td><em>C. orthopsilosis</em> (4)</td>
<td>39* FL TLSLRDA</td>
<td>756* DLIRYTL</td>
<td></td>
</tr>
<tr>
<td><em>C. guilliermondii</em> (4)</td>
<td>632 FL TLSLRDP</td>
<td>1347 DLIRYTL</td>
<td></td>
</tr>
<tr>
<td><em>C. lipolectica</em></td>
<td>(4)</td>
<td>FL TLSLRDP</td>
<td>1387 DLIRYTL</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>(1)</td>
<td>FL TLSLRDP</td>
<td>1353 DLIRYTL</td>
</tr>
</tbody>
</table>

- **X** "strong R" mutation; __ letters indicate the codon involves a mutation or deletion; ___ letters indicate the codon involves a mutation or stop codon.
- **X** "weak R" mutation.
- **X** "silent" mutation, acquired or naturally-occurring.
- __ naturally occurring mutation proven or possibly related to the intrinsic lower susceptibility;
- __ naturally occurring mutation of unknown impact; * inaccurate annotation, sequencing of entire gene-sequence required;
- __ Migeafungin ECOFF elevated for *C. krusei* compared to *C. albicans* and *C. glabrata*, but not the anidulafungin ECOFF.

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Arendrup Cur Op Inf Dis 2014

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**C. glabrata in mice “S >> I << R”**

Mice challenged iv with 3 strains: caspofungin MIC 0.25, MIC 1 & MIC 16
Treated ip with caspofungin

- RR#24 Fks1p S629P  
  +1.139 log growth
- R#83 Fks2p S663F  
  -1.850 log reduction
- S#3 wild type  
  -2.728 log reduction

Mice challenged iv with 3 strains: caspofungin MIC 0.25, MIC 1 & MIC 16
Treated ip with caspofungin

**Same Fks1 mutation D → Y ... different impact**

- MIC elevation greater in **C. krusei** D662Y than in **C. albicans** D648Y

<table>
<thead>
<tr>
<th></th>
<th>Anidulafungin EUCAST MIC</th>
<th>Micafungin EUCAST MIC</th>
<th>Caspofungin Etest (MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. krusei</strong></td>
<td>1 step</td>
<td>2 steps</td>
<td>2 steps</td>
</tr>
<tr>
<td><strong>C. albicans</strong></td>
<td>1 step</td>
<td>2 steps</td>
<td>2 steps</td>
</tr>
</tbody>
</table>

**Jensen AAC 2014**  
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### France: 20 cases 5.5 years (2004-10)

- Incidence among *C. albicans*, *C. glabrata* & *C. krusei*
  - 0.4% in the Paris area

- Caspofungin exposure
  - One naïve!
  - Median 26 days (10 days $\Rightarrow$ >8 months)

- Species involved
  - 10 *C. glabrata* – 8 w Fks2p Δ, 1 w Fks1p Δ, 1 w both
  - 8 *C. albicans* – all Fks1p; 7 hot spot 1 Δ, 1 hot spot 2 Δ
  - 2 *C. krusei* – all Fks1p hot spot 1 Δ

- 11/20 were blood isolates
  - 55% Mortality among candidaemia cases
US cancer centre: Caspofungin ≥4 mg/L

- Incidence
  - 1% (7/582 patients)

- Caspofungin exposure preceding 3 months
  - Three naïve ! (but no Fks sequence data)

- Species involved
  - 2 C. glabrata
  - 2 C. albicans
  - 3 C. tropicalis

- 5/7 were blood isolates
  - 1/5 died among candidaemia cases

C. glabrata echinocandin MIC & outcome

- Cancer patients w 93 blood isolates (2005-13)

  - Survival day 28
    - 74.6% (50/67) caspofungin MIC<0.25 mg/L
    - 58.3% (7/12) caspofungin MIC 0.25 mg/L
    - 50% (5/10) caspofungin MIC 0.5 mg/L
    - 25% (1/4) caspofungin MIC >2 mg/L

  - log-rank p = 0.001 for linear trend
Echinocandin resistance in abdominal candidiasis

- Echinocandin resistant isolates with fks mutations
  - 24% (6/25) patients with intraabdominal candidiasis

- Patient characteristics
  - 25 Pts with echinocandin exposure (median 42 d, 4-438 d)
    - 100% (GI) diseases
    - 92% (23/25) GI surgery ≤ 30 days
    - 44% solid-organ transplant recipients

- Presentation
  - abdominal abscesses (13)
  - peritonitis (8)
  - abscesses & peritonitis (2)
  - cholangitis or cholangitis+peritonitis (1 each)
  - 40% (10/25) were echinocandin breakthrough infections
    - caspofungin (9)
    - micafungin (1)

Case

- 59 year-old ♀, 5 days Abdominal pain & Fever

- X-ray: Intra-peritoneal gas

- GI surgery:
  - Perforation, faecal peritonitis, necrosis of sigmoid colon
  - Resection of sigmoid colon & small bowel
  - Salpingo-oophorectomy
  - Ileostomy and colostomy

- ICU
  - Mechanical ventilation
  - Haemodialysis
  - Broad spectrum ABs
Case continued

- Day 4: Antifungal Prophylaxis Fluconazole 400 mg/day
- Day 8: yeast in blood culture, flu → Caspofungin
- GI surgery due to perforation
  - Day 11: Small bowel resection
  - Day 27: Small bowel resection

Case continued

- Imaging
  - Day 31: leakage
  - Day 35: mesenteric oedema, liver abscesses, abd abscesses

- GI surgery
  - Surgical debridement, drainage of liver abscesses and rinsing. Day 41, 42 and 43

- Death due to multi-organ failure day 45
### Case cont. Mycology & Treatment

<table>
<thead>
<tr>
<th>Sample day</th>
<th>Sample type</th>
<th>Result</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Abdominal Pus:</td>
<td>Faecal flora</td>
<td>-</td>
</tr>
<tr>
<td>Day 4</td>
<td>Blood, trachea:</td>
<td>C. albicans S</td>
<td>Flu</td>
</tr>
<tr>
<td>Day 7</td>
<td>Blood:</td>
<td>C. albicans S</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 17</td>
<td>Trachea</td>
<td>C. albicans S</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 21</td>
<td>Urine</td>
<td>C. albicans S</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 22</td>
<td>Wound, trachea:</td>
<td>C. albicans ND + yeast</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 24</td>
<td>Trachea:</td>
<td>C. albicans S</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 29</td>
<td>Urine &amp; pus</td>
<td>C. albicans S &amp; ND</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 35</td>
<td>Catheter tip:</td>
<td>C. albicans Caspo R</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 38</td>
<td>Urine/Tracheal suction:</td>
<td>C. albicans Caspo R / Mould</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 39</td>
<td>Peritoneal cavity</td>
<td>C. albicans ND</td>
<td>Caspo</td>
</tr>
<tr>
<td>Day 40</td>
<td>Tracheal suction:</td>
<td>C. albicans / Mould</td>
<td>Caspo+Flu</td>
</tr>
<tr>
<td>Day 43</td>
<td>K-Urine/Tracheal suction:</td>
<td>C. albicans / Mould</td>
<td>Caspo+Flu</td>
</tr>
<tr>
<td>Day 45</td>
<td>Death</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Acquired Resistance

<table>
<thead>
<tr>
<th></th>
<th>C. albicans Caspofungin S</th>
<th>C. albicans Caspofungin R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS:</td>
<td>&gt;32 µg/ml</td>
<td>&lt;0.06 µg/ml</td>
</tr>
<tr>
<td>Anid:</td>
<td>0.5 µg/ml</td>
<td>≤0.002 µg/ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C. albicans Caspofungin S</th>
<th>C. albicans Caspofungin R</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUCAST:</td>
<td>2 µg/ml</td>
<td>&gt;32 µg/ml</td>
</tr>
<tr>
<td>Anid:</td>
<td>0.125 µg/ml</td>
<td>0.06 µg/ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C. albicans Caspofungin S</th>
<th>C. albicans Caspofungin R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI:</td>
<td>1 µg/ml</td>
<td>&gt;32 µg/ml</td>
</tr>
<tr>
<td>Anid:</td>
<td>0.5 µg/ml</td>
<td>0.06 µg/ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C. albicans Caspofungin S</th>
<th>C. albicans Caspofungin R</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUCAST:</td>
<td>0.25 µg/ml</td>
<td>&gt;32 µg/ml</td>
</tr>
<tr>
<td>Anid:</td>
<td>0.03 µg/ml</td>
<td>0.06 µg/ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C. albicans Caspofungin S</th>
<th>C. albicans Caspofungin R</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI:</td>
<td>≤0.06 µg/ml</td>
<td>&gt;32 µg/ml</td>
</tr>
<tr>
<td>Anid:</td>
<td>0.015 µg/ml</td>
<td>0.06 µg/ml</td>
</tr>
</tbody>
</table>
In vivo susceptibility - IV mouse model

Sequencing of FSK1 S645P mutation

Echinocandin R in C. glabrata
Echinocandin-R *C. glabrata* Duke Hosp

- 2001-10: 274 pt; 293 episodes; 313 *C. glabrata*

<table>
<thead>
<tr>
<th>Resistance to</th>
<th>2001-2</th>
<th>2009-10</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinocandin</td>
<td>4.9%</td>
<td>12.3%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>18%</td>
<td>30.1%</td>
<td>24.9%</td>
</tr>
<tr>
<td>Echinocandin res in fluconazole res isolates</td>
<td></td>
<td></td>
<td>14.1%</td>
</tr>
</tbody>
</table>

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  - in general
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- What to do
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### Echinocandin Breakpoints for Candida spp

#### Breakpoints (BPs): S: ≤X; R: >Y

<table>
<thead>
<tr>
<th></th>
<th>CLSI M27-S3</th>
<th>CLSI Revised 2011 (M27-S4)</th>
<th>EUCAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF</td>
<td>≤2</td>
<td>≤0.25; &gt;0.5 (alb, krus, trop)</td>
<td>≤0.032; &gt;0.032 (alb)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤0.125; &gt;0.25 (glab)</td>
<td>≤0.06; &gt;0.06 (glab, krus, trop)</td>
</tr>
<tr>
<td>CSF</td>
<td>≤2</td>
<td>≤2; &gt;4 (para, guillier)</td>
<td>≤0.002; &gt;4 (para)</td>
</tr>
<tr>
<td>MFG</td>
<td>≤2</td>
<td>≤0.25; &gt;0.5 (alb, krus, trop)</td>
<td>≤0.016; &gt;0.016 (alb)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤0.06; &gt;0.125 (glab)</td>
<td>≤0.002; &gt;2 (glab, krus, trop)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤2; &gt;4 (para, guillier)</td>
<td>(guillier IE)</td>
</tr>
</tbody>
</table>

#### Etest: Caspofungin and CLSI BP

<table>
<thead>
<tr>
<th>Species</th>
<th>CLSI (mg/L)</th>
<th>C. albicans (278)</th>
<th>C. glabrata (136)</th>
<th>C. krusei (26)</th>
<th>C. tropicalis (24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.07</td>
<td>0.016</td>
<td>0.03</td>
<td>0.06</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>&gt;32</td>
</tr>
<tr>
<td>CLSI (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4283)</td>
<td>(996)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.07</td>
<td>0.016</td>
<td>0.03</td>
<td>0.06</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>&gt;32</td>
</tr>
<tr>
<td>50%</td>
<td>30%</td>
<td></td>
<td>20%</td>
<td></td>
<td>5%</td>
</tr>
</tbody>
</table>

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Echinocandin-R *C. glabrata*

Caspofungin BP proposal for Etest and *C. glabrata*

$S \leq 0.25 \text{ mg/L}$

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Echinocandin use and resistance

- Fluconazole & Echinocandin use (a Paris tertiary centre)

<table>
<thead>
<tr>
<th>Year</th>
<th>Patients on echinocandins</th>
<th>DDD/patient</th>
<th>Breakthrough rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>213</td>
<td>16.7</td>
<td>3.3%</td>
</tr>
<tr>
<td>2012</td>
<td>216</td>
<td>13.3</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

Mean exposure for patients with “R” isolates:
- 33 days
- Week 1: 0
- Week 2: 2
- Week 4: 1
- Week 5: 1
- Week 8: 1
- Week 9: 1

Treatment choice after species ID (ESCMID guidelines)

- C. parapsilosis
  - Change to Fluconazole

- C. glabrata, C. krusei or another Fluconazole “I/R” strains
  - Continue echinocandin treatment

- C. albicans, C. tropicalis and other fluconazole “S” strains
  - Consider Step down to fluconazole
    - If clinically stable and responding
    - After 10 days (ECCMID – based on the Reboli study)
    - Earlier?
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