MICROBIOLOGY AND INVASIVE FUNGAL INFECTION

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What tools we can offer to optimize antifungal treatment?

From lab Bench to Bedside:

✓ Improve the IFI diagnostic

✓ Optimize information about *in vitro* antifungal susceptibility
What tools we can offer to optimize IFI diagnosis?

- **↑ the sensibility of techniques:**
  - Adding Direct Microscopy to culture
  - Sending new samples if culture (-) after 5 days

- **Quicker results:**
  - Direct microscopy
  - Molecular techniques (Septifast®)
  - New techniques (PNA FISH®)
Direct microscopy

- **Utility:**
  - Quick presumptive diagnosis (min)
  - Early antifungal treatment

- **Efficacy:**
  - Depends on observer’s experience

- **Inconvenience:**
  - The identification of causal agent is not possible
Sensibility of diagnosis: 15 - 20% > Culture alone

Denning, CID 1998
Samples:
- Sterile fluids, biopsies
- Respiratory (BAL, TA, BAS)
- Abscess, wound, ...

Techniques:
- KOH (wet mount)
- Phase contrast (wet mount)
- Specific stains:
  - Calcofluor white (wm)
  - Methenamine-silver
  - PAS
Direct microscopy

© J. Pontón

Calcofluor white
What tools we can offer to optimize IFI diagnosis?

- If fungal cultures (-) after 5 days …
  → collect and culture new samples

Old legends never die...
# Time of fungal pathogen growth in clinical samples

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Number strains</th>
<th>Detection average (d)</th>
<th>Detection maximum (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>191</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>21</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Candida spp. no albicans</td>
<td>45</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>10</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Rhodotorula spp.</td>
<td></td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td></td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Phycomycetes spp.</td>
<td>4</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Trichosporon asahii</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Acremonium spp.</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Morris, J Clin Microbiol 1996**

If (-) after 5 days, → new cultures

Mean 4.5 days
Blood culture
Speed of growth

- 415 candidemias La Fe Univ. Hospital (2004-2010):
  - Average: 36.8 h (2.2 h – 7.5 d)
  - Median: 31.5 h

- **C. albicans**: 33.7 h (8.7 h - 5.6 d)
- **C. parapsilosis**: 30.7 h (2.2 h - 5 d)
- **C. glabrata**: 35.5 h (7.5 h - 5.1 d)
- **C. tropicalis**: 18.1 h
Blood culture
Speed of growth


**Time**

- 24 h: 28%
- 36 h: 62%
- 48 h: 85%
- 72 h: 93%

If (-) after 2 days, → new blood cultures
What tools we can offer to optimize IFI diagnosis?

- Sensibility of techniques:
  - Adding Direct Microscopy to culture
  - Sending new samples if culture (-) after 5 days

- Quicker results:
  - Direct microscopy
  - Molecular techniques (Septifast®)
  - New techniques (PNA FISH®)
PNA FISH

Peptide Nucleic Acid Fluorescent In-situ Hybridization

Positive Blood Culture

Gram Stain

Results (2.5 Hrs.)

S. aureus
PNA FISH™

C. albicans
PNA FISH™

E. faecalis
PNA FISH™

S. aureus
non-S. aureus GPCC

C. albicans
non-C. albicans Yeast

E. faecalis - Green
other enterococci GPCPC

non-enterococci - Red

AdvanDX; Woburn, MA
Peptide Nucleic Acid Fluorescent In-situ Hybridization

- C. glabrata / C. krusei
- C. tropicalis
- C. albicans / C. parapsilosis

Yeast Traffic Light PNA FISH™

Forrest GN, Curr Fun Infect Rep 2008; 2:221-6
Molecular diagnosis

LightCycler® SeptiFast Test

• The first PCR technique for multiple detection of sepsis pathogens

• Real time PCR (qualitative)

• Detects 90% of causal agents of bacteriemia/fungemia

• Commercialized in Europe (CE Mark) January 2006

• Not in USA
Detect and identify DNA of 25 bacterial and fungal pathogens directly from whole blood (EDTA) in less than 6 hours:

<table>
<thead>
<tr>
<th>Gram (-)</th>
<th>Gram (+)</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td><em>Staphylococcus aureus</em></td>
<td><em>Candida albicans</em></td>
</tr>
<tr>
<td><em>Klebsiella (pneumoniae / oxytoca)</em></td>
<td><em>CoNS (Coagulase negative Staphylococci)</em></td>
<td><em>Candida tropicalis</em></td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td><em>Streptococcus pneumoniae</em></td>
<td><em>Candida parapsilosis</em></td>
</tr>
<tr>
<td><em>Enterobacter (cloacae / aerogenes)</em></td>
<td><em>Streptococcus spp.</em></td>
<td><em>Candida krusei</em></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td><em>Enterococcus faecium</em></td>
<td><em>Candida glabrata</em></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td><em>Enterococcus faecalis</em></td>
<td><em>Aspergillus fumigatus</em></td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*S. epidermidis, S. haemolyticus  **S. pyogenes, S. agalactiae, S. mitis*
Clinical impact of a commercially available multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis

Christine Dierkes*1, Boris Ehrenstein1, Sylvia Siebig1, Hans-Jörg Linde2, Udo Reischl2 and Bernd Salzberger1

- 77 patients with suspected sepsis
- 101 blood samples:
  - Blood culture (BC): Bactec 9240
  - Septifast (SF)

- Concordant negative results: 62%
- Concordant positive results: 13%
- BC positive only: 9%
- SF positive only: 13%

Table 3: Isolated Pathogens: Pathogens identified in BC or SF; 7 samples yielded polymicrobial results.

<table>
<thead>
<tr>
<th>Species</th>
<th>SeptiFast®</th>
<th>Blood culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter cloacae/aerogenes</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcus species</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida krusei</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>34</td>
<td>28</td>
</tr>
</tbody>
</table>

Fungal pathogens:
- Detected by SF: 6
- Detected by BC: 4
- Detected by SF only: 3

Dierkes C. BMC Infect Dis 2009, 9:126
What tools we can offer to optimize antifungal treatment?

From lab Bench to Bedside:
✓ Improve the IFI diagnostic
✓ Optimize information about *in vitro* antifungal susceptibility
To improve in vitro susceptibility techniques:

- Faster techniques:
  - Sensititre YeastOne
  - Etest

Wider knowledge about IFI epidemiology:

- Own hospital vs. other hospitals
- Own geographical region vs. other regions
- Own country vs. other countries
Correlation using logistic regression between percentage reduction of *C. albicans* isolation rates and fluconazole use [DDDs per year].

C. albicans and Candida non albicans isolate rates during study period.

*Bassetti M et al. BMC Infectious Diseases 2006;6:80*
FUNGEMYCA Study (1,383 episodes)

13 months of candidemias in Spain (2009-2010)
Number of participant institutions from Spanish regions

Total: 43
FUNGEMYCA Study (1,383 episodes)

13 months of candidemias in Spain (2009-2010)

Isolates distribution by hospital unit

%
FUNGEMYCA Study (1,383 episodes)

13 months of candidemias in Spain (2009-2010)

% species isolated

- **C. albicans**: 43%
- **C. parapsilosis**: 30%
- **C. glabrata**: 11%
- **C. tropicalis**: 8%
- **Otras**: 8%
FUNGEMYCA Study (1,383 episodes)

13 months of candidemias in Spain (2009-2010)

% species isolated

30
54
4 6 5

Hospital Univ La Fe (79 episodes)

C albicans
C parapsilosis
C glabrata
C tropicalis
Otras

FUNGEMYCA Study (1,383 episodes)

13 months of candidemias in Spain (2009-2010)

% species isolated

30
54
4 6 5

Hospital Univ La Fe (79 episodes)

C albicans
C parapsilosis
C glabrata
C tropicalis
Otras
% species isolated in ICU

427 episodes (36,8%)
Increasing incidence of *Candida parapsilosis* candidemia with caspofungin usage

Graeme N. Forrest a,*, Elizabeth Weekes b, Jennifer K. Johnson c

<table>
<thead>
<tr>
<th>Species</th>
<th>FY02</th>
<th>FY03</th>
<th>FY04</th>
<th>FY05</th>
<th>FY06</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>0.28</td>
<td>0.40</td>
<td>0.31</td>
<td>0.39</td>
<td>0.48</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>0.23</td>
<td>0.17</td>
<td>0.21</td>
<td>0.19</td>
<td>0.20</td>
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<tr>
<td>C. parapsilosis</td>
<td>0.05</td>
<td>0.06</td>
<td>0.12</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>0.09</td>
<td>0.09</td>
<td>0.06</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>C. krusei</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>Overall</td>
<td>0.69</td>
<td>0.73</td>
<td>0.71</td>
<td>0.80</td>
<td>0.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>FY02</th>
<th>FY03</th>
<th>FY04</th>
<th>FY05</th>
<th>FY06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspofungin</td>
<td>3.91</td>
<td>4</td>
<td>6.96</td>
<td>11</td>
<td>6.61</td>
</tr>
<tr>
<td>Micafungin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>84.2</td>
<td>86.2</td>
<td>104.4</td>
<td>84.4</td>
<td>85.7</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0</td>
<td>47.6</td>
<td>80.1</td>
<td>61.3</td>
<td>72.5</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>23.8</td>
<td>13.9</td>
<td>3.2</td>
<td>3.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>31.6</td>
<td>27</td>
<td>18</td>
<td>16.6</td>
<td>10.9</td>
</tr>
</tbody>
</table>

* Lipid formulation
Candidemias ICUs
H Univ. La Fe

392 episodes

%
Candidemias adults ICUs
H Univ La Fe, 2008-2009

75 episodes

% species isolated

Surgery ICU

Medical ICU

C albicans
C parapsilosis
C glabrata
C tropicalis
Otras

41
6
6
27
2
5
2
64
47
Candidemias in Spain

Comparison between different multicenter studies
(1997-1999 vs. 2009)

% species isolated

2009 (1,383 episodes)

1997-1999 (290 episodes)

Candidemias in Europe

Comparison between Spain and Finland

% species isolated

1997-1999 (290 episodes)

1995-1999 (479 episodes)
Poikonen E, Emerg Infect Dis 2003; 9:985-90
Study of antifungal susceptibility of clinical isolates
<table>
<thead>
<tr>
<th></th>
<th>MIC</th>
<th>CAS</th>
<th>ANI</th>
<th>FLZ</th>
<th>ITR</th>
<th>VOR</th>
<th>POS</th>
<th>5FC</th>
<th>AMB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. albicans</strong></td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td>1.8</td>
<td>2.4</td>
<td>2.2</td>
<td>2.2</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td><strong>C. parapsilosis</strong></td>
<td>1.4</td>
<td>0</td>
<td>1.7</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>C. tropicalis</strong></td>
<td>1.1</td>
<td>0</td>
<td>1.1</td>
<td>7.4</td>
<td>16</td>
<td>8.5</td>
<td>11</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>C. glabrata</strong></td>
<td>0</td>
<td>0</td>
<td>0.8</td>
<td>7.8</td>
<td>24</td>
<td>0.6</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>C. krusei</strong></td>
<td>4.1</td>
<td>8.3</td>
<td>4.1</td>
<td>***</td>
<td>4.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>2.3</td>
<td>2.6</td>
<td>2</td>
<td>4</td>
<td>5.8</td>
<td>2.1</td>
<td>3.9</td>
<td>0.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

(1,152 isolates)
Kiitos...