Fungal Update
- antifungal susceptibility testing

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Factors affecting outcome of IFI

HOST
- Immunological status
- Underlying disease
- Site of disease

THERAPY
- Prompt aggressive therapy
- Drug pharmacokinetics
- Surgical intervention

FUNGUS
- Virulence
- Propensity to disseminate
- Infecting species
- Drug resistance
Factors influencing the outcome of antifungal therapy

Van t' Wout J. 1996

- HOST
- FUNGUS
- ANTIFUNGAL DRUG

**HOST**
- resistance

**FUNGUS**
- Cell type (yeast / mould)
- Genomic stability
- Size of population
- Biofilms

**ANTIFUNGAL DRUG**
- Fungistatic / fungicidal
- Dosing
- Pharmacokinetics
- Drug interactions

**efficacy**
‘The MIC predicts how best to treat an infected test tube’

H.B. Levine
Susceptibility testing - issues

Format - disc test / agar incorporation / broth dilution
Inoculum standardization
Medium / drug solvents
Temperature
Duration
Static or shaken
Reading - endpoint interpretation
Breakpoint interpretation - *in vivo* - *in vitro* correlation
  - normal ranges
  - attainable blood concentrations
Oropharyngeal candidosis in AIDS

- Homogeneity of underlying disease
- Emerging resistance encountered
- Visible condition
- Treated with oral antifungals
- Differing regimens
- Response easily evaluable
CLSI (NCCLS) broth dilution (M27-A2)

Macrodilution  Microdilution

EUCAST modification - extra glucose
- flat-bottom plates
- read at 24 hours
MIC testing

MIC 1.0 mg/L or 0.25 mg/L depending on concentration range
Reading of microtitre plates

Microtitre plate

Reading on a spectrophotometer is possible if flat bottom plates are used - this produces a non-subjective reading

Reading mirror
NCCLS method for caspofungin against mould (MEC)

64 mg/L 0.25 mg/L Control
Minimum Lethal (Fungicidal) Concentration (MLC/MFC)

- MLC
- MIC

mg/L: 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125

GROWTH CONTROL
NEGATIVE CONTROL
Yeast species implicated in infection
(number referred to the Mycology Reference Laboratory for susceptibility testing 2004 and 2005)

**C. albicans (3420)**
- *C. glabrata* (1460)
- *C. parapsilosis* (734)
- *C. tropicalis* (368)
- *C. krusei* (144)
- *C. lusitaniae* (123)
- *C. guilliermondii* (73)
- *C. dubliniensis* (40)
- *C. inconspicua* (29)
  - *C. famata* (23)
  - *C. kefyr* (21)
  - *C. chiropterum*
  - *C. ciferrii*
  - *C. haemulonii*
  - *C. humicola*
  - *C. lipolytica*
- *C. norvegensis*
- *C. pelliculosa*
- *C. pintolopesii*
- *C. pulcherrima*
- *C. rugosa*
- *C. utilis*
- *C. blankii*
- *C. zeylanoides*

**Pichia anomola**
(= *Candida pelliculosa*) (8)

**Saccharomyces cerevisiae** (80)

**Trichosporon spp.** (25)

**Rhodotorula spp.** (52)

**Malassezia spp.** (36)
*Rhodotorula mucilaginosa* (99 strains)

- Amphotericin B: susceptible
- Flucytosine: susceptible
- Fluconazole: susceptible
- Itraconazole: susceptible
- Voriconazole: susceptible
- Caspofungin: susceptible

Colors:
- Green: susceptible
- Yellow: intermediate/SDD
- Red: resistant
### Spectrum of activity against yeast species

<table>
<thead>
<tr>
<th></th>
<th>Candida albicans</th>
<th>Candida glabrata</th>
<th>Candida parapsilosis</th>
<th>Cryp. neoformans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida tropicalis</td>
<td>Candida krusei</td>
<td>Candida lusitaniae</td>
<td>Trichosporon spp.</td>
<td></td>
</tr>
</tbody>
</table>

- **Amphotericin B**
- **Flucytosine**
- **Caspofungin**
- **Itraconazole**
- **Fluconazole**
- **Voriconazole**

- Green: Susceptible
- Yellow: Intermediate/SDD
- Red: Resistant
Antifungal drug resistance

Intrinsic (primary) resistance:

The strain was resistant to the drug before treatment started

Acquired (emergent) resistance:

The strain became resistant during treatment
### CLSI (NCCLS) reference method: breakpoints

<table>
<thead>
<tr>
<th></th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Flucytosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>≤ 8</td>
<td>≤ 0.125</td>
<td>≤ 4.0</td>
</tr>
<tr>
<td>Intermediate*</td>
<td>16 - 32</td>
<td>0.25 - 0.5</td>
<td>8.0 - 16</td>
</tr>
<tr>
<td>Resistant</td>
<td>≥ 64</td>
<td>≥ 1</td>
<td>≥ 32</td>
</tr>
</tbody>
</table>

* susceptible - dose dependent

1,295 patient-episode-isolate events: 692 mucosal, 603 invasive candidosis
12 published clinical studies - 13.3% resistance

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Flucytosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 8</td>
<td>85%</td>
<td>67%</td>
<td>42%</td>
</tr>
<tr>
<td>16 - 32</td>
<td>(841/993)</td>
<td>(87/130)</td>
<td>(72/172)</td>
</tr>
<tr>
<td>≥ 64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Strong relationship between MIC, fluconazole dose and outcome

Pfaller et al. 2006 CMR 17:2:435-447
The 90 - 60 Rule

Applies with azole drugs against yeasts

S isolates respond ~90% of the time
R isolates respond ~60% of the time

Correlation of MIC with outcome for *Candida* species tested against voriconazole: analysis and proposal for interpretive breakpoints

Pfaller *et al.* 2006 JCM 44:3 819-826

S = \leq 1  
R = \geq 4.0

8,702 clinical isolates  
\( \text{MIC}_{90} \leq 0.25 \text{ mg/L} \)  
\( \text{MIC}_{99} \leq 1.0 \text{ mg/L} \)

249 patients from 6 phase III clinical trials  
statistically significant \( (P = 0.021) \)  
correlation between MIC and investigator end-of-treatment assessment of outcome
Activity of voriconazole, itraconazole, fluconazole and amphotericin B in vitro against 1763 yeast from 472 patients in the voriconazole phase III clinical studies

Johnson et al. 2008 IJAA 32: 511-514

109 isolates had voriconazole MIC > 4.0 mg/L

*Candida albicans*
*Candida glabrata*
*Candida tropicalis*

All cross-resistant to itraconazole most to fluconazole

Resistant isolates (34): 56% response to voriconazole
Susceptible isolates (261): 71% response to voriconazole

23 base-line resistant, 8 developed on treatment, 3 different species
Correlation of caspofungin MICs to overall outcomes for patients with invasive candidiasis (MITT population)

Kartsonis N et al, AAC 2005 49:3616-3623
Turn-around times for local laboratory (final verification of results) compared to reference laboratory turn-around.

Aliyu et al. 2006 Q J Med

50% isolates reported

Days

Local laboratory turn around times

Reference laboratory turn around times
## Susceptibility testing methods

### Yeast
- **CLSI (NCCLS) M27-A2**
- **CLSI (NCCLS) M44-P disc diffusion**
- **EUCAST**
- Disc diffusion / neosensitabs
- **Yeast one (sensititre)**
- **ATB Fungus**
- **Fungitest**
- **E-test**

### Mould
- **CLSI (NCCLS) M38-A**
- **Agar dilution**
- **E-test**
Fungitest - Diagnostics Pasteur

- Low and high concentration of each of 6 antifungal agents
- Concentrations do not correspond with all accepted breakpoints
- One test organism per strip
- Reasonable correlation with NCCLS method except with Candida glabrata

Davey et al. 1998 JCM 36:926-30
Yeast One (TREK)

- Same format as CLSI (NCCLS) microtitre method
- Chromogenic substrate for ease of reading
- Different drug in each row therefore one test organism per plate. Voriconazole and caspofungin now CE marked
- Good correlation with CLSI (NCCLS) method

Davey et al. 1998 JAC 42:439-444
Etest for yeasts

- Strips impregnated with a concentration gradient of antifungal agent
- MIC value rather than just susceptibility category
- Good correlation with CLSI (NCCLS) method
- Some false resistance due to interpretation of background growth

Warnock et al. 1998 JAC 42:321-331
NEQAS antifungal susceptibility testing

- Yeast isolates only
- Three distributions each year - two strains
- Strains selected and tested against antifungal panel
- Strains lyophilised and then 4 ampoules re-tested in Bristol, Manchester (+ EUCAST) and at QAL (Etest)
- Susceptibility categories agreed
- Strains distributed
- Results analysed and reported
UK NEQAS susceptibility testing methods

Fluconazole resistant
*Candida albicans*

- CLSI
- ATB Fungus
- Disc diffusion
- Fungitext
- Yeast one
- Etest

Fluconazole susceptible
*Cryptococcus neoformans*

- CLSI
- ATB Fungus
- Disc diffusion
- Fungitext
- Yeast one
- Etest

Colors indicate:
- **susceptible**
- **intermediate**
- **resistant**
Itraconazole MIC testing
Aspergillus fumigatus

<table>
<thead>
<tr>
<th>Aspergillus fumigatus strain number</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar incorporation</td>
<td>1 2 3 4 5 6</td>
</tr>
<tr>
<td>Broth microdilution</td>
<td>1 2 3 4 5 6</td>
</tr>
</tbody>
</table>

Denning et al. 1997 JAC
CLSI method for mould MIC
Etest for moulds
Survival curves in a neutropenic mouse model infected with an itraconazole susceptible
*A. fumigatus* strain (MIC 0.25 mg/L)

Denning *et al.* 1997 JAC
Survival curves in a neutropenic mouse model infected with an itraconazole resistant
*A. fumigatus* strain (MIC >64 mg/L)

Denning *et al.* 1997 JAC
Etest result with *Scedosporium apiospermum*

- **Amphotericin B**
- **Itraconazole**
Comparison of E-test result for amphotericin B with broth microdilution result for 10 strains of *Scedosporium apiospermum*
Antifungal drug resistance

Innate resistance
Most common
Predictable if organism speciated (sometimes only a percentage)
Defines an antifungal agents spectrum of activity

Emergent resistance
Currently rare
Rare in invasive disease
Very rare with moulds

Issues
Multi-resistant organisms (esp. *Scedosporium prolificans*)
Over the counter drugs (esp. azoles)
Susceptibility testing

**HOW?**
CLSI (NCCLS) method or CLSI validated method
Standardized methods for yeasts and moulds

**WHEN?**
Identification is crucial - predictable innate resistance
*Candida krusei, C. glabrata* and *C. tropicalis*
Yeasts from recalcitrant infections (mucosal and deep)
All moulds from deep infections
Should not be used as the sole criterion for selecting therapy

**WHY?**
Evidence for *in vitro-in vivo* correlation not perfect but at least as good as for bacteria
Emergent resistance in some yeasts
New agents - therapeutic choices
Emerging pathogens