Invasive Candidiasis and Antifungal Drug Resistance

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Candida species

- 4th leading cause of nosocomial bloodstream infections, 3rd most common cause of hospital-acquired UTI.
- Common cause of catheter-associated infections.
- Increased length of stay, attributable mortality, cost of care.
  - LOS increased by mean >21 days
  - Attributable mortality as high as 38 – 49%
  - Added cost of $34 – 44,000 per episode adult, $2 billion nationwide annually
- Higher crude mortality rate than Staphylococcus aureus or Pseudomonas bloodstream infection.

Fallas et al. Eur J Infect Dis 2006
Candida Epidemiology-Oncology Center

Causative Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>albicans</td>
<td>202</td>
<td>42%</td>
</tr>
<tr>
<td>tropicalis</td>
<td>85</td>
<td>18%</td>
</tr>
<tr>
<td>parapsilosis</td>
<td>84</td>
<td>17%</td>
</tr>
<tr>
<td>glabrata</td>
<td>53</td>
<td>11%</td>
</tr>
<tr>
<td>krusei</td>
<td>20</td>
<td>4%</td>
</tr>
<tr>
<td>lusitaniae</td>
<td>8</td>
<td>2%</td>
</tr>
<tr>
<td>guilliermondii</td>
<td>3</td>
<td>1%</td>
</tr>
<tr>
<td>Mixed</td>
<td>20</td>
<td>4%</td>
</tr>
<tr>
<td>Undetermined</td>
<td>4</td>
<td>1%</td>
</tr>
</tbody>
</table>

Figure 1. Episodes of hematogenous candidiasis per year of onset and causative Candida species. Data are from M. D. Anderson Cancer Center (1988 through 1992). Episodes due to C. parapsilosis, C. lusitaniae, C. guilliermondii and unidentified species are not included. Note that administration of fluconazole prophylaxis, introduced in 1989, became standard practice in 1990 for patients with leukemia.


Epidemiology of Candidemia
167 U.S. Centers (1992-2001)

C. tropicalis: 10%
C. parapsilosis: 13%
C. albicans: 54%
C. glabrata: 18%
Other Candida: 2%

3683 bloodstream isolates

Pfaller MA et al. Clin Microbiol Infect, 2004
Epidemiology of Candidemia
79 U.S. Centers (2008-9)

- C. albicans 50%
- C. glabrata 17.4%
- C. parapsilosis 17.4%
- C. tropicalis 9.8%
- Other Candida 3.6%
- C. krusei 1.8%

1239 bloodstream isolates

Pfaller et al. *Diagn Microbiol Infect Dis* 2010

Epidemiology of Candidemia
UNM/Tricore (2008)

- C. albicans 54%
- C. glabrata 20.7%
- C. parapsilosis 11.5%
- C. tropicalis 12.6%
- C. krusei 1.2%

87 bloodstream isolates

Unpublished data, Miceli et al. 2008
Candidemia--Underlying conditions

Table 2. Underlying medical conditions among all patients with candidemia (n = 837).

<table>
<thead>
<tr>
<th>Underlying condition</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic lung disease</td>
<td>122 (14.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>111 (13.3)</td>
</tr>
<tr>
<td>Chronic heart conditions</td>
<td>58 (6.9)</td>
</tr>
<tr>
<td>Stroke/neurological disorders</td>
<td>43 (5.1)</td>
</tr>
<tr>
<td>Other chronic conditions</td>
<td>37 (4.4)</td>
</tr>
<tr>
<td>History of alcoholism</td>
<td>32 (3.8)</td>
</tr>
<tr>
<td>Liver disease</td>
<td>22 (2.6)</td>
</tr>
<tr>
<td>Cancer</td>
<td>215 (25.6)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>21 (2.5)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>32 (3.8)</td>
</tr>
<tr>
<td>Other malignancies</td>
<td>165 (20)</td>
</tr>
<tr>
<td>Surgery</td>
<td>155 (18.5)</td>
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<tr>
<td>Abdominal surgery</td>
<td>113 (13.5)</td>
</tr>
<tr>
<td>Cardiothoracic surgery</td>
<td>26 (4.5)</td>
</tr>
<tr>
<td>Other surgeries</td>
<td>14 (1.7)</td>
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<tr>
<td>Rheumatologic/rheumatologic condition</td>
<td>73 (8.7)</td>
</tr>
<tr>
<td>Organ transplant</td>
<td>11 (1.3)</td>
</tr>
<tr>
<td>Rheumatologic disease</td>
<td>18 (2.2)</td>
</tr>
<tr>
<td>Steroid use</td>
<td>58 (6.9)</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>82 (9.8)</td>
</tr>
<tr>
<td>Prematurity</td>
<td>21 (2.5)</td>
</tr>
</tbody>
</table>

*NOTE.* HIV, human immunodeficiency virus.
*a* Patient may have had more than one underlying medical condition.
*b* Surgery within 2 months of fungal diagnosis.


Candidemia-Risk Factors

High Risk Patients
- Surgery
- Leukopenia
- Burns
- Premature infants

Exposures
- ICU stay ≥ 7 days
- Central Lines
- Antibiotics
- TPN

If Candidemia Develops...
- ~1/3rd die from candidemia
- ~1/3rd die with candidemia
- ~1/3rd survive
Clinical Epidemiology

- **C. albicans**
- **C. tropicalis**
  - Acute disseminated candidiasis in neutropenic patients, highly virulent
- **C. parapsilosis**
  - Nosocomial transmission (hands, catheters), pediatric patients
- **C. glabrata**
  - Up to half (R) to FLC, the other half are S-DD to FLC; increased MIC to AMB
- **C. krusei**
  - Intrinsic FLC resistance; increased MIC to AMB
- **Other Candida species**

Miceli and Lee, Lancet Inf Dis 2011

(Very) General Resistance Patterns in *Candida* species

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Ct</th>
<th>Cp</th>
<th>Cg</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLC</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>SDD-R</td>
<td>R</td>
</tr>
<tr>
<td>ITRA</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>SDD-R</td>
<td>SDD-R</td>
</tr>
<tr>
<td>VRC</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S-R</td>
<td>S-I</td>
</tr>
<tr>
<td>5FC</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I-R</td>
</tr>
<tr>
<td>AMB</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S-I</td>
<td>S-I</td>
</tr>
<tr>
<td>CANDINS*</td>
<td>S</td>
<td>S</td>
<td>S-R</td>
<td>S-R</td>
<td>S</td>
</tr>
</tbody>
</table>

*Full discussion to follow to reflect updated CLSI clinical breakpoints and clinical epidemiology of resistance.
Pathogenesis

- Colonization
- Adherence (mucosa, foreign body, etc.)
- Invasion
- Hematogenous spread
- Endothelial adherence and invasion
- Proliferation, local inflammation, microabscess formation

Images courtesy B. Wong, Oregon HSC; and CDC Public Health Image Library

Molecular Pathogenesis

Calderone and Fonzi. Trends Microbiol 2001
Gow and Hube. Curr Opin Microbiol 2012
Candida Biofilms

- Organized, complex microbial communities, encased in matrix of exopolymeric substance (EPS), attached to mucosa, plastic, or other surface.
- Complex 3D structure with spatial heterogeneity.
  - Facilitates influx of nutrients
  - Disposal of waste products
  - Establishment of micro-niches within the biofilm.
- Characterized by high levels of resistance to conventional antifungal therapy (AmB, FLU).
- Complex transcriptional network regulates formation.


Candida Biofilm Formation

Adhesion

Filamentation

Antifungal Resistance

Dispersion

Persistor Cells

Quorum Sensing


**Candida Biofilm Formation**

**Antimicrobial Resistance Within Biofilms**

*Candida* as a General Paradigm

- *Candida* biofilms are characterized by high levels of resistance to conventional antifungal therapy

- **Mechanisms include:**
  - Increased cell density
  - Decreased drug penetration through the EPS, binding of drug to beta-glucan
  - Alterations in ergosterol content
  - Decreased growth, nutrient limitation, amino acid starvation
  - Early increased expression of drug resistance genes
  - Development of a drug-resistant subpopulation of “persister” cells


Ramage et al. *Eukaryot Cell* 2005

In vitro Studies

**Amphotericin B**

- Concentration of AMB (µg/ml)
- % Metabolic activity

**Fluconazole**

- Concentration of FLU (µg/ml)
- % Metabolic activity

Miceli et al. *Int J Antimicrob Ag* 2009a

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In vitro Effect of Anidulafungin, Caspofungin, and Micafungin Against *C. albicans* Biofilms

Miceli et al. *Int J Antimicrob Ab* 2009b
Clinical Syndromes

- Skin and mucosal infection
- Invasive
- Disseminated
- Chronic mucocutaneous candidiasis
Skin and Mucosal Infection

- Oropharyngeal (thrush)
- Esophagitis
- Vaginitis
- Cutaneous
  - Generalized
  - Intertriginous
  - Paronychia
  - Diaper rash
  - Balanitis
  - etc.

Oral candidiasis

- Pseudomembranous (Thrush): Creamy white, curd-like patches on tongue and mucosa.
- Scraping leaves raw, bleeding, painful surface.
- Dx by clinical appearance, can confirm by scraping/KOH smear.
- Culture not useful (Candida is normal colonizer).
Oral candidiasis

- Erythematous (acute – chronic)
  - Erythema, atrophic areas with mild burning/itching or asymptomatic, smooth tongue surface
- Hyperplastic (candidal leukoplakia)
  - Nodules or plaques on buccal mucosa or tongue with hyphae invading, and epithelial dysplasia
- Associated lesions:
  - Denture stomatitis - red, swollen underlying mucosa
  - Angular cheilitis – vit def, Staph
  - Median rhomboid glossitis – male, DM, smokers

Infectious Disease: Item 89

A 47-year-old male injection drug user with HIV infection develops difficulty swallowing associated with substernal chest pain and a dry mouth. He had received antiretroviral therapy for 2 years before resuming use of injection drugs, after which he was lost to follow-up. During treatment, his plasma HIV RNA viral load was induced but never became undetectable, and his highest CD4 cell count was 211/μL (0.211 x 10^9/L). He had thrush treated with fluconazole. However, the fluconazole was stopped when elevated serum amniontransferase levels were detected.

On physical examination, the patient appears dehydrated and ill. Temperature is 37.2 °C (98.9 °F), pulse rate is 106/min, respiration rate is 18/min, and blood pressure is 84/54 mm Hg. Arterial oxygen saturation is 97% by pulse oximetry with the patient breathing room air. The skin is taut and dry, and there is no perspiration. The mouth is also dry, and white patches are seen over the buccal mucosa bilaterally. Cardiopulmonary and abdominal examinations are normal.

<table>
<thead>
<tr>
<th>Laboratory Studies</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>12 g/dL (120 g/L)</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>3000/μL (3 x 10^9/L) with 90% neutrophils</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>45 mg/dL (16.97 mmol/L)</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>2 mg/dL (178.64 μmol/L)</td>
</tr>
</tbody>
</table>

Which of the following is most appropriate at this time?

A. Oral fluconazole
B. Intravenous micafungin
C. Intravenous amphotericin B by slow infusion
D. Nystatin swish and swallow
E. Oral acyclovir
Esophagitis

- Hematologic malignancy, HIV/AIDS
- Odynophagia
- Also, stomach, small and large bowel in cancer patients.
- Vaginitis in diabetes, abx, pregnancy.
Invasive Disease

- CNS-meningitis, microabscesses
- Pneumonia-bronchopneumonia rare
- Myocarditis, pericarditis, endocarditis
- Urinary tract (upper and lower)
- Osteomyelitis, arthritis
- Peritonitis-perit dialysis, GI surgery, perf
- Hepatosplenic-immunocompromised pts

Candiduria

- Candiduria is common—colonization v. infection
- Abx, Foley catheters are risk factors. Cystitis usually due to Foley.
- Upper tract infection caused by ascending infection or hematogenous.
- Kidney often involved in disseminated disease.
A 76-year-old man is hospitalized for complications related to chemotherapy for non-Hodgkin's lymphoma. His medications on admission were fluconazole, ciprofloxacin, and metoclopramide. A Hickman catheter is in place that was used to treat dehydration related to vomiting that persisted even after his leukocyte count normalized.

On admission, oral medications are stopped, and saline, 3 liters intravenously, is given through the Hickman catheter. The patient improves. However, on the third hospital day, he develops fever, slight confusion, and loss of appetite. On physical examination, he appears ill. Temperature is 38.7°C (101.7°F), pulse rate is 112/min, respiration rate is 18/min, and blood pressure is 110/70 mm Hg. General examination is normal. There is no redness or tenderness over the site of the Hickman catheter.

<table>
<thead>
<tr>
<th>Laboratory Studies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>11 g/dL (110 g/L)</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>40000/µL (4 x 10⁹/L)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>94,000/µL (94 x 10⁹/L)</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>0.9 mg/dL (75.88 umol/L)</td>
</tr>
</tbody>
</table>

After blood culture samples are drawn, broad-spectrum intravenous antibiotic therapy is begun for suspected bacteremia. The next day, the laboratory reports that two culture bottles are growing what appears to be a yeast.

**In addition to removing the Hickman catheter and continuing supportive care, which of the following is most appropriate at this time?**

1. A  Monitor temperature for 24 hours before beginning specific therapy
2. B  Await blood culture species identification before beginning specific therapy
3. C  Begin oral fluconazole now
4. D  Begin intravenous caspofungin now
5. E  Begin intravenous fluconazole now

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**Answer and Critique (Correct Answer = D)**

The presence of a bloodstream fungal infection has serious implications for patients. Although removal of any catheters is desirable (when possible), treatment with an active anti-Candida medication prevents both distant metastatic complications and early mortality.

This patient should be started on therapy now with an echinocandin such as caspofungin, micafungin, or anidulafungin. These agents are fungicidal against Candida species and are relatively easy to administer in once-daily infusions with few side effects and very few drug interactions.

The choice of agent for treating candidemia is not always clear because clinical trials with conventional-dose fluconazole (400 mg/d), high-dose fluconazole (>400 mg/d), voriconazole, amphotericin B, and various echinocandins all show good results. Differences in efficacy among these agents are difficult to document in comparative trials. Fluconazole has variable activity against Candida, but this agent is never used alone because of the possibility of emergence of drug resistance. Fluconazole provides effective therapy once an isolate is known to be a species for which this drug is usually active (i.e., C. albicans or C. tropicalis). However, about 50% of Candida strains found in patients with nosocomial infections are not C. albicans, and many of these strains may be resistant to fluconazole. Some clinicians are therefore reluctant to use fluconazole alone to initiate therapy. In addition, this patient's previous use of fluconazole makes this drug less attractive than caspofungin for empiric therapy.
Disseminated Infection

- Candidemia from IV catheter infection
- Endophthalmitis
- Acute Disseminated-multiple organs, kidney, brain, myocardium, eye.
  - acute leukemia
  - postoperative i.e. organ transplant, heart surgery, GI tract surgery
  - burns

Skin Lesions in Disseminated Candidiasis
Skin Lesions (Histology)

Endophthalmitis

- Any eye structure
- Direct inoculation, hematogenous spread
- Risk for blindness
- Usually indicates disseminated disease
Endocarditis

- Most common cause of fungal endocarditis.
- Difficult to treat, usually requires surgical valve replacement.
- Also myocarditis, pericarditis.

Candida myocarditis
Diagnosis

- Culture
- Histopathology
- Beta-glucan
- T2 MR: PCR–nanoparticle conjugation–NMR detection (species-specific)

Investigational methods:
- Anti-fungal Antibodies
- Antigen: Histoplasma, Cryptococcus, Aspergillus
- Candida: i.e. enolase, Hsp70
- PCR and other molecular methods
- Metabolites (D-arabinitol, D-mannitol)
- Mass Spec/MALDI-TOF

(1→3)-β-D-Glucan Structure

- Homo-polymer of glucose, typically poorly water-soluble
- Beta-linkages provide sterically-constrained structure
- Often branched and cross-linked
- Subject to synthetic and degradative processes in fungi
G test for beta-glucan

G test (beta-glucan Limulus assay)

Limulus test reacts to both endotoxin and (1→3)β-D-glucan, whereas G test reacts to the glucan only.
Current IDSA Guidelines for Candidemia (2009)

- **Intravenous catheter removal is strongly recommended** for non-neutropenic patients with candidemia
  - Shorter duration of candidemia
  - Reduced mortality in adults
  - Reduced mortality in neonates
- For neutropenic patients, consideration of venous catheter removal (including removal of tunneled catheters) who have persistent candidemia and in whom it is logistically feasible.
- The role for antifungal lock solutions is not well defined.
- **Wouldn’t it be great if we didn’t have to take the catheter out?**

Pappas et al. *Clin Infect Dis* 2009

Major changes from 2004 IDSA Guidelines

- Emphasis on fluconazole and echinocandins as the ‘preferred choices’ for proven/suspected invasive disease
- De-emphasis on amphotericin B and lipid-associated amphotericin B under most circumstances
- Concept of step down therapy is strongly encouraged
  - Voriconazole generally advised as step down therapy for selected isolates (most notably, *C. krusei*)
- There is little distinction made among the echinocandins
- Resource-limited environments are acknowledged

Slide adapted from John Rex, 2008
Candidemia, non-neutropenic

- If species is unknown, either fluconazole (800 mg or 12 mg/kg loading dose, 400 mg or 6 mg/kg daily dose) or an echinocandin is appropriate initial therapy for most adult patients (AI)
- An echinocandin is favored if
  - Moderately severe to severe illness,
  - Recent azole use for treatment or prophylaxis (AIII), or
  - Isolate is known to be C. glabrata or C. krusei (BIII)
- Fluconazole for patients who are
  - Less critically ill, and
  - Who have no recent azole exposure (AIII).
- Move from candid to fluconazole when isolates likely susceptible to fluconazole (e.g., C. albicans) and patient is clinically stable (AIII)
- Remove or exchange intravenous catheters
- Treat for two weeks after clearance of bloodstream

Slide adapted from John Rex, 2008

Other settings

- Thinking of non-neutropenia as the start point
  - The less you know or
  - The more the patient scares you
- The more the guidelines point to
  - An echinocandin
  - A lipid-associated amphotericin B
- But, for resource constrained settings...
  - The guidelines do note that classic AmB works

Slide adapted from John Rex, 2008
Echinocandins: Mechanism of Action

- Non-competitive inhibitor of 1,3 B-D-glucan synthase
- Present in fungal, but not mammalian cell membrane
- Glucan essential for cell wall integrity


Micafungin

- Non-inferior to CAS in candidemia/IC
  - Treatment success: 76.4% MFG 100 mg vs. 71.4% MFG 150 mg vs. 72.3% CAS
  - Pappas *et al.*, *Clin Infect Dis* 2007

- Non-inferior to L-AmB in candidemia/IC
  - 181 (89.6%) vs. 170 (89.5%) treatment success
Micafungin versus Caspofungin for Treatment of Candidemia and Other Forms of Invasive Candidiasis


Anidulafungin versus Fluconazole for Invasive Candidiasis


International, randomized, double-blind trial in adults with invasive candidiasis
Micafungin (100 mg) v micafungin (150 mg) v caspofungin (70 mg then 50 mg daily)
Micafungin non-inferior to caspofungin

Randomized, double-blind, international, multicenter study.
Anidulafungin non-inferior to fluconazole in the treatment of invasive candidiasis
Comparison of Caspofungin and Amphotericin B for Invasive Candidiasis

- Double-blind trial caspofungin vs. amphotericin B deoxycholate
- 224 patients with invasive candidiasis
- Successful outcome in 73.4% with caspofungin and 61.7% with amphotericin B
- Less nephrotoxicity with caspofungin
- Caspofungin appears at least as effective as amphotericin B
- Caspofungin has considerably less toxicity
- Few neutropenic patients in trial - further evaluation needed in this group

Changes in CLSI Clinical Breakpoints

- In 2010 and 2011, CLSI established new species-specific clinical breakpoints for FLC, VRC, and candins.
- Established for *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. guilliermondii*
- FLC – Ca, Ct, Cp same breakpoints; Cg much higher (S-DD ≤32, but use 12 mg/kg/d).
- VRC – Ca, Ct, Cp same breakpoints; Ck slightly higher; Cg no breakpoint due to suboptimal response/correlation
- Candins – Ca, Ct, Ck similar breakpoints; Cp higher; vary by candidin for Cg
Changes in CLSI Clinical Breakpoints

<table>
<thead>
<tr>
<th>Table 1. Previous and recently revised CLSI antifungal clinical breakpoints (CBP) for resistance for fluconazole, voriconazole, anidulafungin, and micafungin against Candida species. Breakpoints are in μg/mL. The epidemiologic cut-off value was used for voriconazole against C. glabrata.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous or revised CBP</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Anidulafungin</td>
</tr>
<tr>
<td>Previous</td>
</tr>
<tr>
<td>Revised</td>
</tr>
<tr>
<td>Micafungin</td>
</tr>
<tr>
<td>Previous</td>
</tr>
<tr>
<td>Revised</td>
</tr>
<tr>
<td>Fluconazole</td>
</tr>
<tr>
<td>Previous</td>
</tr>
<tr>
<td>Revised</td>
</tr>
<tr>
<td>Voriconazole</td>
</tr>
<tr>
<td>Previous</td>
</tr>
<tr>
<td>Revised</td>
</tr>
</tbody>
</table>

Candida Epidemiology

**SENTRY Antimicrobial Surveillance**

- Echinocandin and triazole antifungal susceptibility patterns for 3,418 contemporary clinical isolates of yeasts and molds; 98 laboratories in 34 countries during 2010 - 2011.
- Among Candida spp., resistance to the candins was low (0.0 to 1.7%). *C. albicans* and *C. glabrata* that were resistant to anidulafungin, caspofungin, or micafungin were shown to have fks mutations.
- Resistance to FLC was low for *C. albicans* (0.4%), *C. tropicalis* (1.3%), and *C. parapsilosis* (2.1%); however, 8.8% of *C. glabrata* isolates were resistant to fluconazole.
- Among echinocandin-resistant *C. glabrata* isolates from 2011, 38% were FLC resistant. VRC was active against all Candida spp. except *C. glabrata* (10.5% non-WT), whereas POSA showed decreased activity against *C. albicans* (4.4%) and *C. krusei* (15.2% non-WT).
- Overall, echinocandin and triazole resistance rates were low; however, the FLC and candin coreistance among *C. glabrata* strains warrants continued close surveillance.
Updated Epidemiology of Candida Resistance to FLC

Fungus Testing Laboratory

Orasch et al. Clin Microbiol Infect 2013

Futhermore, it is important to note the resistance patterns of different Candida species to fluconazol (FLC) as shown in the graphs. The updated epidemiology suggests a significant increase in resistance among certain species, indicating the need for further research and adaptation of treatment strategies. This information is crucial for hospitals and clinics in managing fungal infections effectively.
Cross-Resistance in *Candida* spp.

- Azole cross-resistance can occur due to overexpression of *Candida Drug Resistance* (CDR) efflux pumps (e.g., *C. glabrata*), or to a lesser extent overexpression and/or mutation of lanosterol 14-α-demethylase.

- Strong positive correlation between *fluconazole* MICs and those of *itraconazole*, *voriconazole*, and *posaconazole*, indicating considerable cross-resistance. Thus, resistance to FLC may serve as a surrogate marker in predicting resistance.

- *Candida* spp for which FLC MICs are ≥64 mcg/mL (resistant) tend to be less susceptible to *itraconazole*, *voriconazole* and *posaconazole*. FLC MICs of ≤32 mcg/mL predict susceptibility, and MICs of ≥64 mcg/mL predict resistance of *Candida* spp to VRC and POSA.

- Certain *Candida* species have predictable cross-resistance.
  - None of the triazoles exhibit meaningful activity against *fluconazole*-resistant isolates of *C. glabrata*.
  - *Voriconazole* and *posaconazole* are active against the intrinsically *fluconazole*-resistant *C. krusei*.

Co-Resistance in *C. glabrata*

- Some *C. glabrata* bloodstream isolates with resistance to *fluconazole* and *voriconazole* are also resistant to the echinocandins.

- Surveillance of the in vitro susceptibility of 1669 *C. glabrata* bloodstream isolates in the USA between 2006 – 2010:
  - 162 isolates (9.7 percent) were resistant to *fluconazole*, of which 98.8 percent also not susceptible to *voriconazole* (MIC >0.5 mcg/mL), and 9.3, 9.3, and 8.0 percent were resistant to *anidulafungin*, *caspofungin*, and *micafungin*, respectively.
  - Of the 162 isolates that were resistant to *fluconazole*, 18 (11.1 percent) were resistant to one or more of the echinocandins; all of these isolates contained an *FKS1* or *FKS2* mutation.

- No echinocandin-resistant strains detected among 110 *fluconazole*-resistant *C. glabrata* isolates tested between 2001 - 2004, years during which only caspofungin was available and echinocandins were used sparingly.

- In a study of *C. glabrata* bloodstream infections at a single medical center in the USA between 2001 - 2010, among 78 *fluconazole*-resistant isolates, 11 (14.1 percent) were resistant to one or more echinocandins and 8 (10.3 percent) were resistant to all echinocandins.

  Pfaffer et al. *J Clin Microbiol* 2013
  Alexander et al. *Clin Infect Dis* 2013
When to Do Antifungal Susceptibility Testing

- *C. glabrata* – FLC, VRC, candins
- Mucosal candidiasis unresponsive to Rx
- IC unresponsive to initial Rx
- Clinical failure after appropriate Rx
- IFI due to unusual species with unknown/unpredictable resistance
- Species with predictable resistance do not need susceptibility testing (i.e. *C. krusei* and FLC; *Cryptococcus*, *Trichosporon*, and *Rhodotorula* to candins).

Antifungal Susceptibility Testing Methods for *Candida*

- CLSI Broth macrodilution or microdilution
- CLSI Disk Diffusion
  - FLC, VRC, POS, MICA, CAS
- E Test
  - FDA-approved: FLC, ITRA, 5FC, VRC
- SensiTitre YeastOne
  - Colorimetric broth microdilution, FDA-approved: FLC, ITRA, 5FC, VRC
- Vitek2
  - Automated spectrophotometric, FDA-approved: FLC, VRC, CAS
Role of ASP in IC

- AST - decreased time to effective Rx (median 13.5 vs 1.3 hours, \( p = 0.04 \)); increased appropriate Rx [67 (88%) vs. 80 (99%), \( p = 0.008 \)]. Quasi-study, single tertiary care hospital (OSU).
- AST - 100% antifungal Rx within 24h, 95% appropriate initial Rx, 95% appropriate duration of Rx, 85% CVC removal within 48h. Quasi-prospective observational study, single tertiary care hospital (Nice UH, France).
- Caspofungin bundle – reduction in median days of CAS (4.00 vs. 2.00 days, \( p = 0.001 \)). Prospective bundle cohort/matched retrospective controls, tertiary care ICU (West Virginia U).
- Candidemia bundle – adherence (78.0% vs 40.5%, \( p=0.0016 \)), ophthalmologic examination (97.6% vs. 75.7%, \( p=0.0108 \)), appropriate antifungal Rx (100% vs 86.5%, \( p=0.0488 \)), appropriate duration (97.6% vs 67.7%, \( p=0.0012 \)). Quasi-study, single tertiary care hospital (U Michigan).

Reed et al. Diagn Microbiol Infect Dis 2014
Mondain et al. Infection 2013
Antworth et al. Pharmacotherapy 2013

Role of ASP and Antifungal Susceptibility Testing

- Objective: Determine role of antifungal susceptibility in changing therapy of candidemia.
- Methods: Retrospective study, 161 patients, 2006-9. St. Luke’s Hospital, Houston, TX. Vitek 2 susceptibility testing for all Candida BSI.
- Results:
  - Ages 59 ± 16 years (male, 54%; Caucasian, 52%; APACHE II score ≥ 15, 48%; and ICU, 50%) were identified
  - 130 (81%) had FLC-susceptible candidemia. (19% FLC-resistant candidemia).
  - 58 patients (36%) were initiated on FLC, 103 (64%) on a candin.
  - Mean time from culture to the susceptibility report was 5 ± 2 d.
  - Prior to susceptibility report, 20 FLC-initiated patients (34%) were switched to a candin; 14 echinocandin-initiated patients (14%) were switched to FLC.
  - Once susceptibility report available, 35 of 89 (39%) patients with FLC-susceptible candidemia on a candin were de-escalated to FLC.
  - 11 patients on FLC just prior to a susceptibility report were identified with a fluconazole-resistant *Candida* species.

Role of ASP and Antifungal Susceptibility Testing

- Improvement in timing of initiation of empiric antifungal Rx still needed.
- Improvement in de-escalation of candin to FLC is needed.
- 11 of 52 (21%) of FLC-treated patients had FLC-resistant *Candida* species.
- Routine antifungal susceptibility testing may assist in guiding appropriate acute Rx and de-escalation.


Approach to Yeast in Blood Culture

- Is the yeast *Candida*?
  - *Cryptococcus neoformans* – India Ink?
  - *C. gattii*
  - Other emerging yeasts (*Trichosporon, Rhodotorula, Geotrichum, Hansenula, Malassezia, Saccharomyces*)

- Is the yeast *Candida albicans* or non-albicans?
  - Germ tube test?

- *C. glabrata?* *C. krusei?* Or other species?
A Simplified Approach to Candidemia

Question 1: Is the patient hemodynamically unstable or neutropenic?

- Yes: Strongly consider avoiding use of fluconazole; use of polyaene, echinocandin, or voriconazole preferred

- No: Question 2: Is C. glabrata or C. krusei likely to be the cause?*

  - Yes: Strongly consider avoiding use of fluconazole; use of polyaene or echinocandin preferred; use of voriconazole is an alternative option

  - No: Strongly consider use of fluconazole


Approach to Yeast in Blood Culture

- Antifungal therapy for IC:
  - If sick, start echinocandin (alternative L-Amb)
  - If C. albicans confirmed, pt. stable, uncomplicated catheter-associated, use Fluconazole 6-10 mg/kg/d iv (or more)

- Remove vascular catheter whenever feasible

- Ophthalmoscopy and Abd CT (if neutropenic, do so on marrow recovery)

- Antifungal susceptibility testing
Known Gaps in Antifungal Coverage

• Fluconazole  
  *C. glabrata* (SDD - R);  
  *C. krusei; Candida* biofilms; all moulds

• Amphotericin B  
  *C. lusitaniae, Aspergillus terreus,  
  Fusarium, Scedosporium*

• Echinocandins*  
  *Cryptococcus, non-Aspergillus moulds*,  
  endemic fungi, some emerging yeasts

• Voriconazole  
  *Zygomycetes*

*Limited data except for *Candida* and *Aspergillus*.

Conclusions

• Do not underestimate the ability of infectious pathogens to develop resistance!
  o The spectre of antifungal resistance should not and can not be ignored.

• NEW ANTIFUNGAL DRUGS AND DIAGNOSTICS ARE NEEDED!
  o Understanding molecular pathogenesis can contribute to the identification of rational drug targets.
  o High-throughput screening approaches to identify novel and repurposed therapeutic agents are of substantial interest.

• Antifungal lock therapy (AfLT) is a promising therapeutic strategy worthy of further investigation.
  o Combinations of agents may prove effective for prevention or treatment of CRBSI due to *Candida* spp. and mixed infections.
Chernobyl – Hundreds of Mold Species Resistant to Lethal Dose Radiation

Zhdanova et al. *Mycol Res* 2004

Some final “Food for thought”

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“Your gut is the largest biofilm ever” Henry Lin MD
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