

### Candida species: Antifungal susceptibility testing in 2013

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### Case

- Elderly man
  - Mitral valve replacement in 2011
- Repeat valve surgery in January 2012
  - Post-operative admission to ICU
- Candida parapsilosis* cultured from valve tissue obtained at surgery (= prosthetic valve fungal endocarditis)
  - Fluconazole MIC = 8 µg/ml (S\*)
  - Voriconazole MIC = 0.5 µg/ml (S\*)
  - Amphotericin B MIC = 0.5 µg/ml (no breakpoints)
- Severe renal impairment

\*CLSI M27-S3 2008

### Case

- IDSA recommendation for prosthetic valve IE
  - AmB ± flucytosine OR caspofungin
  - Valve replacement, if possible
  - Suppressive treatment with fluconazole (if isolate S) for minimum 6 weeks post-surgery or lifelong if surgery not possible

Pappas PG, et al. Clin Infect Dis 2009.

### Case

- Fluconazole 400 mg daily chosen because caspofungin was not available
  - Repeat blood cultures negative
  - Initial clinical resolution
- During prolonged ICU admission:
  - MRSA sepsis, treated with linezolid
  - Break-through *Candida parapsilosis* bloodstream infection
    - Fluconazole MIC = 16 µg/ml (SDD\*)
    - Voriconazole MIC = 4 µg/ml (R\*)
    - Amphotericin B MIC = 0.5 µg/ml (no breakpoints)
- Isolate submitted to reference lab for re-testing

\*CLSI M27-S3 2008

### Reference lab AST

	MIC (µg/ml)	Category*
Fluconazole	64	Resistant
Voriconazole	0.5	Intermediate
Anidulafungin	1	Susceptible
Micafungin	1	Susceptible
Caspofungin	0.5	Susceptible
Amphotericin B	0.047	No breakpoints
Flucytosine	0.25	Susceptible
Posaconazole	0.03	No breakpoints
Itraconazole	0.12	Susceptible

\*CLSI M27-S4 2013

### Case

- Repeat valve replacement and patient changed to caspofungin
- Patient died due to sepsis-related complications

### Outline

- CLSI vs. EUCAST
- Revised CLSI species-specific clinical breakpoints for:
  - Fluconazole
  - Voriconazole
  - Echinocandins
- Detection of resistance in species with no CBPs

### CLSI vs. EUCAST standards for AFST

	Different?	CLSI M27-A3 <sup>1</sup>	EUCAST EDef 7.1 <sup>2</sup>
<b>Test format</b>	No	Broth microdilution	Broth microdilution
<b>Wells</b>	Yes	Round-bottomed	Flat-bottomed
<b>Medium</b>	No	RPMI 1640 broth	RPMI 1640 broth
<b>Glucose content of medium</b>	Yes	0.2%	2.0%
<b>Inoculum density</b>	Yes	500 – 2 500 CFU/ml	50 000 – 200 000 CFU/ml
<b>Incubation temperature</b>	No	35°C	35°C
<b>MIC endpoint</b>	No	50% inhibition relative to growth control	50% inhibition relative to growth control
<b>Endpoint reading</b>	Yes	Visual	Spectrophotometric

1. CLSI M27-A4 2008.  
2. Rodriguez-Tudela JL et al. Clin Microbiol Infect 2008.

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### FLUCONAZOLE BREAKPOINTS

### Fluconazole PK/PD

- Dose range: 50 mg to 800 mg daily
  - Linear PK, i.e. direct correlation between dose and serum concentrations
- Very good distribution to CSF
- Time-dependent, concentration-independent fungistatic activity with prolonged *in-vivo* post-antifungal effect
  - AUC/ MIC is the predictive PD parameter for efficacy (target = 25)
  - AUC is almost exactly equal to daily dose in milligrams → dose/ MIC is a surrogate PD parameter

Pfaller MA, et al. Drug Resist Updates 2010.

### Molecular mechanisms of azole resistance

Resistance mechanism	Molecular basis	Table 5 Impact of Resistance Mechanisms on in vitro Susceptibility of <i>Candida albicans</i> to Fluconazole and Voriconazole				
Efflux pump	CDR gene-encoded	Resistance Mechanisms			MIC (µg/ml)	
Efflux pump	MDR gene-encoded	Strain	CDR	ERG11	VRC	FLU
<b>Altered target</b>	<b>ERG11 mutations</b>	DSY294	Basal	WT/WT	0.008	0.25
Over-expression of target	ERG11 promoter mutations	DSY296	Increase	G464S/G464S	2	64
		DSY3083	Basal	G464S/G464S	0.13	4
Bypass pathway	ERG3 mutations	DSY3604	Basal	G464S/WT	0.06	2
		DSY3606	Increase	WT/WT	0.13	4
Respiratory deficiency with increased efflux pump expression	Mitochondrial DNA mutations	FLU = fluconazole; MIC = minimum inhibitory concentration; VRC = voriconazole; WT = wild-type. Adapted from MacCallum DM et al. 2010. <sup>44</sup>				

Pfaller MA. American J Med 2012.  
Pfaller MA, et al. Drug Resist Updates 2010.

### Fluconazole ECVs and CBPs

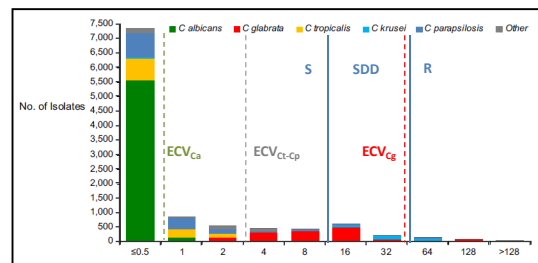


Figure 1 Fluconazole MIC distribution of 10,803 invasive *Candida* species using Clinical and Laboratory Standards Institute broth microdilution methods. Data compiled from Pfaller MA et al. 2010.<sup>20</sup>

Pfaller MA. American J Med 2012.

Ca-Ct-Cp: Fluconazole ECVs and CBPs

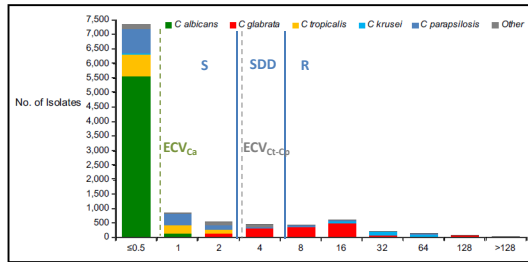


Figure 1 Fluconazole MIC distribution of 10,803 invasive *Candida* species using Clinical and Laboratory Standards Institute broth microdilution methods. Data compiled from Pfaller MA et al, 2010.<sup>20</sup>

Pfaller MA, *American J Med* 2012.

Cg: Fluconazole ECVs and CBPs

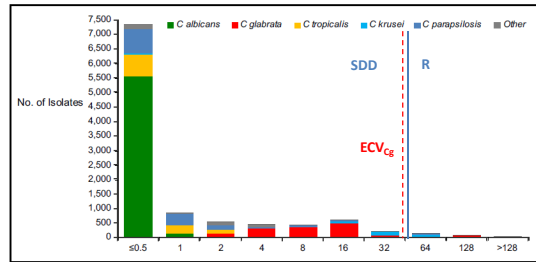


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Pfaller MA, *American J Med* 2012.

Dose/MIC vs. outcome

Table 16  
Relationship between dose/24-h MIC ratio and clinical response in fluconazole treatment of mucosal and invasive candidiasis.

Study	No. subjects	Dose/MIC breakpoint	% success or survival (n/N)
Baddley et al. (2008) <sup>a</sup>	84	$\ge 12.5$ $< 12.5$	74.0 (57/77) 42.9 (37/71)
Clancy et al. (2005)	32	$> 75$ $\le 75$	73.7 (14/19) 7.7 (1/13)
Pai et al. (2007) <sup>b</sup>	77	$> 15$ $\le 15$	94.0 (27/29) 72.9 (35/48)
Rex et al. (1997)	522	$\le 50$ $< 50$	92.4 (376/407) 66.9 (77/155)
Cuesta et al. (2010)	258	$> 75$ $\le 75$	93.9 (140/149) 14.7 (16/109)

<sup>a</sup> Outcome measure was mortality.

Table 17  
Correlation of dose/24-h MIC ratios versus outcome for patients with candidemia or oropharyngeal candidiasis (OPC) due to *C. glabrata*.<sup>a</sup>

Dose/MIC	Successful outcome (no. cured/total) by dose/MIC ratio		OPC		All	
	n/N	%	n/N	%	n/N	%
400->1000	3/3	100.0	7/7	100.0	10/10	100.0
150->200	2/4	50.0	3/3	100.0	5/7	71.4
100	4/7	57.1	8/9	88.9	12/16	75.0
50	0/1	0.0	7/9	77.8	7/10	70.0
25	2/2	100.0	6/6	100.0	8/8	100.0
12.5	3/5	60.0	3/5	60.0	6/10	60.0
6.25	1/2	50.0	0/1	0.0	1/2	50.0
$\le 3.125$	3/7	42.9	3/3	100.0	6/10	60.0

<sup>a</sup> Data compiled from Rex et al. (1997, 2001).

Pfaller MA, et al. *Drug Resist Updates* 2010.

Validation of CBPs

Table 2 Summary Analysis of Clinical Fluconazole Minimum Inhibitory Concentration (MIC) Ranges in Patients with Candidemia and Mucosal Candidiasis

MIC ( $\mu\text{g/mL}$ )	Outcome at MIC			
	24-Hour <sup>a</sup>		48-Hour <sup>b</sup>	
	No. of Events	% Success	No. of Events	% Success
$\le 2$	550	92	366	92
4	52	83	30	90
$\ge 8$	212	37	155	67

Adapted from Pfaller MA et al, 2010.<sup>20</sup>  
<sup>a</sup>Data compiled from Clancy CJ et al, 2005<sup>34</sup>, Rex JH et al, 1997,<sup>35</sup>  
 Rodriguez-Tudela JL et al, 2007.<sup>36</sup>  
<sup>b</sup>Data compiled from Clancy CJ et al, 2005,<sup>34</sup> Rex JH et al, 1997.<sup>35</sup>

Pfaller MA, *American J Med* 2012.

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VORICONAZOLE BREAKPOINTS

Voriconazole PK/PD

- Two loading doses followed by 4mg/kg IV or 100-200 mg PO q12h
  - Non-linear PK, i.e. no direct correlation between dose and serum concentrations
- Reasonable distribution to CSF
- Time-dependent, concentration-independent fungistatic activity with prolonged *in-vivo* post-antifungal effect
  - AUC/ MIC is the predictive PD parameter for efficacy (target  $\approx 25$ )

Pfaller MA, et al. *Diagnost Microbiol Infect Dis* 2011.

### Molecular mechanisms of azole resistance

Resistance mechanism	Molecular basis	Table 5 Impact of Resistance Mechanisms on in vitro Susceptibility of <i>Candida albicans</i> to Fluconazole and Voriconazole
<b>Efflux pump</b>	<b>CDR gene-encoded</b>	
Efflux pump	MDR gene-encoded	
<b>Altered target</b>	<b>ERG11 mutations</b>	
Over-expression of target	ERG11 promoter mutations	
Bypass pathway	ERG3 mutations	
Respiratory deficiency with increased efflux pump expression	Mitochondrial DNA mutations	

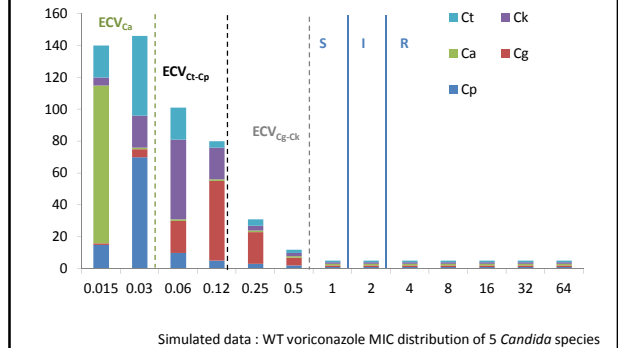
  

Strain	Resistance Mechanisms			MIC ( $\mu\text{g/mL}$ )	
	CDR	ERG11	VRC	FLU	
DSY294	Basal	WT/WT	0.008	0.25	
DSY296	Increase	G464S/G464S	2	64	
DSY3083	Basal	G464S/G464S	0.13	4	
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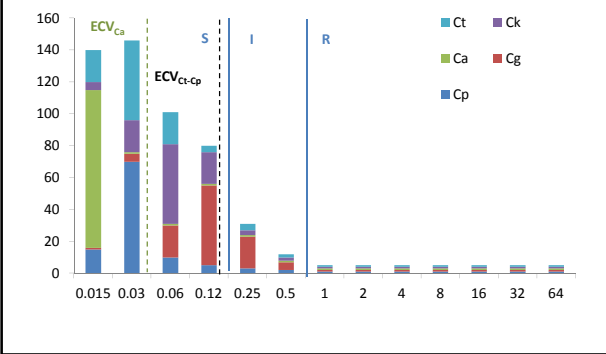
FLU – fluconazole; MIC – minimum inhibitory concentration; VRC – voriconazole; WT – wild-type.  
Adapted from MacCallum DM et al, 2010.<sup>44</sup>

Pfaller MA. *American J Med* 2012.  
Pfaller MA, et al. *Drug Resist Updates* 2010.

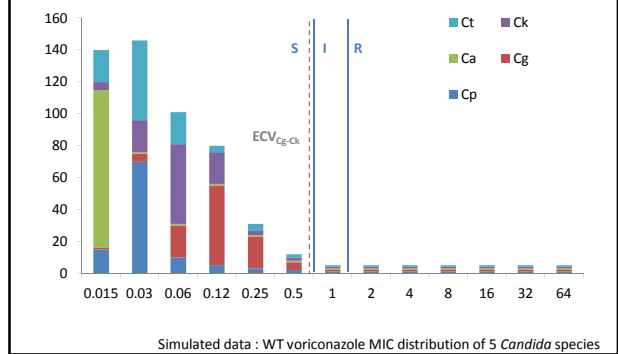
### Voriconazole ECVs and CBPs



### Ca-Ct-Cp: Voriconazole ECVs and CBPs



### Ck: Voriconazole ECVs and CBPs



### Validation of CBPs

**Table 3** Summary Analysis of Clinical Voriconazole Minimum Inhibitory Concentration (MIC) Ranges in Patients with Candidemia and Invasive Candidiasis

MIC ( $\mu\text{g/mL}$ )	Outcome at MIC			
	24-hr		48-hr	
	No. of Events	% Success	No. of Events	% Success
$\leq 0.125$	173	75.7	159	78.6
0.25-0.5	9	100.0	15	60.0
$\geq 1$	8	37.5	16	56.3

Adapted from Pfaller MA et al, 2011.<sup>21</sup>

Pfaller MA. *American J Med* 2012.

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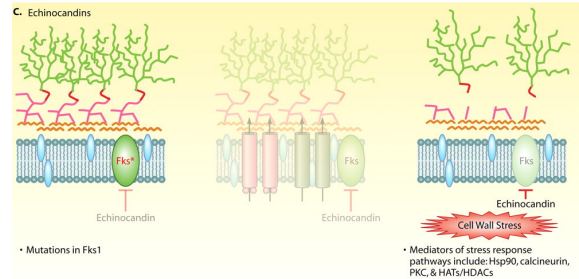
### ECHINOCANDIN BREAKPOINTS

### Echinocandin PK/PD

- One loading dose followed by 50-100 mg IV daily
  - Linear PK, i.e. direct correlation between dose and serum concentrations
- Poor distribution to urine, CSF and eye
- Concentration-dependent fungicidal activity with prolonged *in-vivo* post-antifungal effect
  - AUC/MIC is the predictive PD parameter for efficacy (target  $\approx$  10-20)

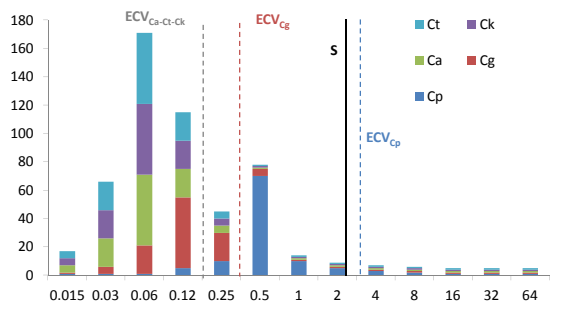
Pfaller MA, et al. *Drug Resistance Updates* 2011.

### Molecular mechanisms of resistance



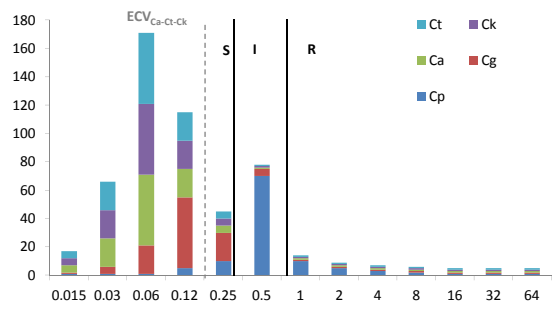
Shapiro R S et al. *Microbiol. Mol. Biol. Rev.* 2011;75:213-267

### Echinocandin ECVs and CBPs



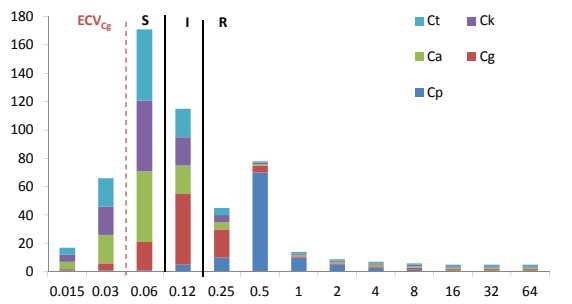
Simulated data : WT echinocandin MIC distribution of 5 *Candida* species

### Ca-Ct-Ck: Echinocandin ECVs and CBPs



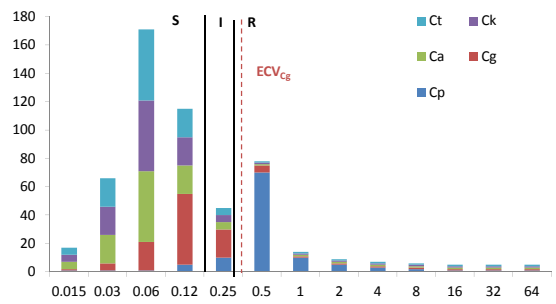
Simulated data : WT echinocandin MIC distribution of 5 *Candida* species

### Cg: Micafungin ECVs and CBPs

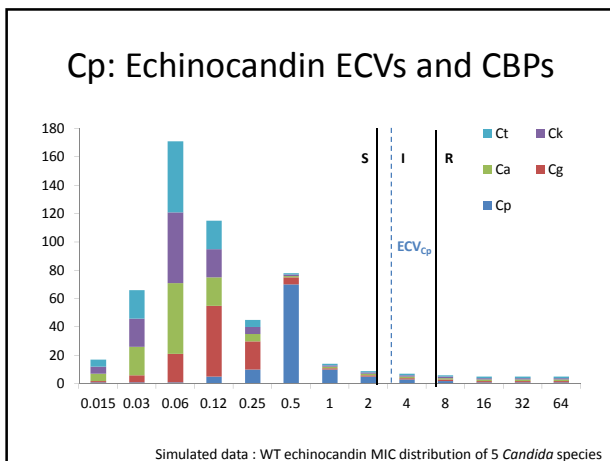


Simulated data : WT echinocandin MIC distribution of 5 *Candida* species

### Cg: Caspofungin and Anidulafungin ECVs and CBPs



Simulated data : WT echinocandin MIC distribution of 5 *Candida* species



### Validation of CBPs

- Only three phase III studies (invasive candidiasis)
- Smaller patient numbers

Pfaller MA, et al. *Drug Resistance Updates* 2011.

### What do when there are no CBPs

- Use ECVs to determine if the isolate is non-WT and less likely to respond to antifungal treatment, e.g. amphotericin B ECV = 2 µg/ml

### Case (revisited)

- *Candida parapsilosis* cultured from valve tissue obtained at surgery (= prosthetic valve fungal endocarditis)
  - Fluconazole MIC = 8 µg/ml (R)
  - Voriconazole MIC = 0.5 µg/ml (I)
  - Amphotericin B MIC = 0.5 µg/ml (probably WT strain)
- Avoid azoles
  - Based on revised CBPs (CLSI M27-S4)
  - *Fungistatic drug not ideal for IE*

### Summary

- CLSI and EUCAST methods are different but yield similar 24-h MICs
- CLSI and EUCAST CBPs have been harmonised to a large extent by using a similar evidence base
  - Species-specific CBPs for common species
  - Drug-specific CBPs in some instances
- ECVs have been determined for *Candida* species (including many uncommon species) → can use these cut-offs to detect potential mutants and for epidemiologic surveillance