Antifungal Susceptibility Test

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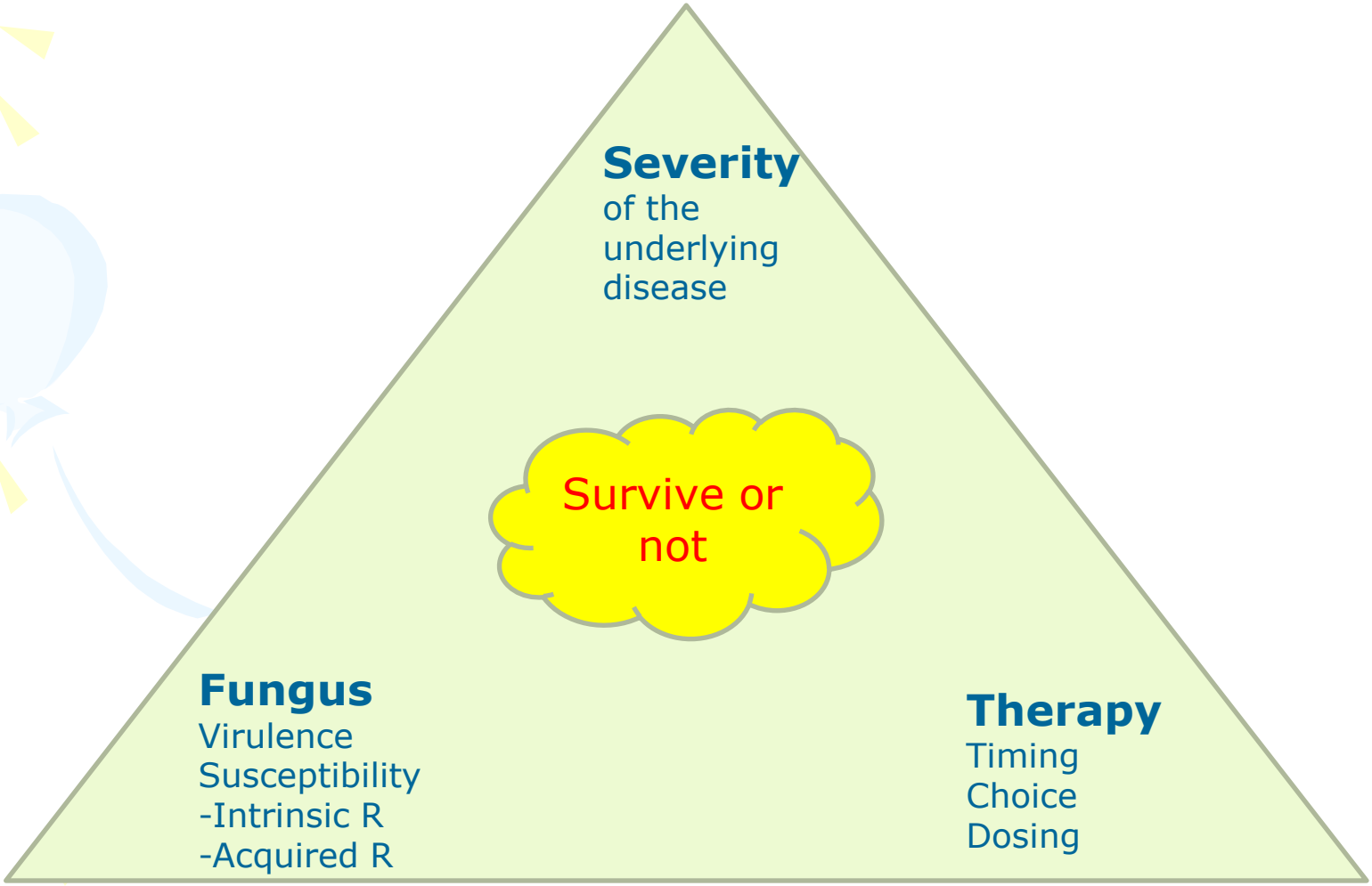
Outline

- Introduction
- Different antifungal susceptibility tests (AST)
  - Epidemiological cut-off value (ECV) and clinical breakpoint (CBP) in different antifungal agents
- Some tecknique problems
  - Trailing growth
  - Paradoxical growth

Outcome Triangle: Factors influencing outcome



Modified from Arendrup MC. Dan Med J 2013;60:B4698

Request to Clinical Lab

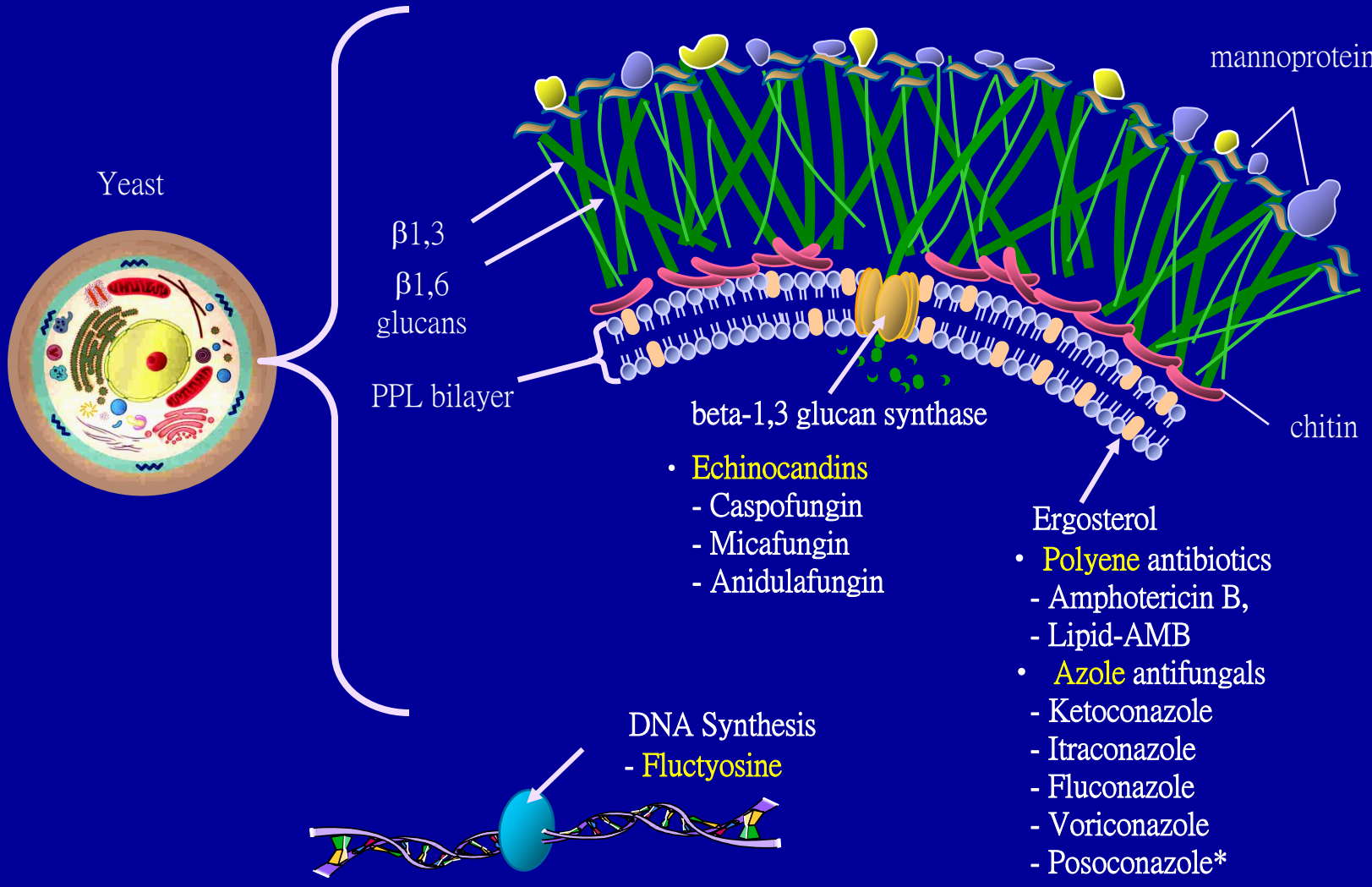
- Pathogens isolation and identification

- Susceptibility testing
  - Reproducibility
  - Agreement between different testing methods
  - Clinical correlation

Blood Culture Bottles

BACTECTM Myco/F Lytic Culture medium is a Middlebrook 7H9 and Brain Heart Infusion broth formulation

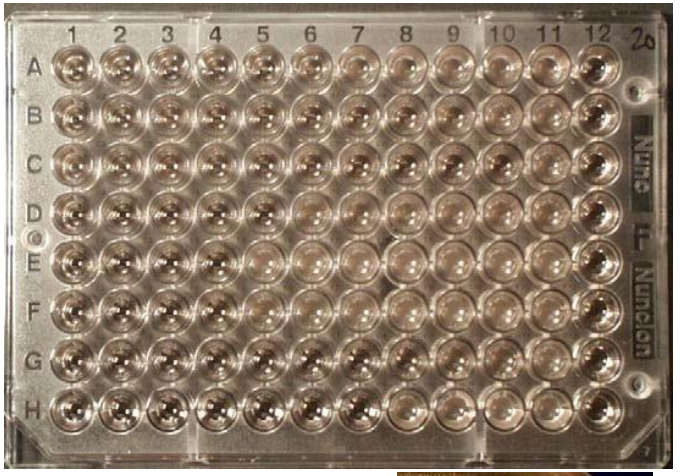
Targets of Antifungal Agents

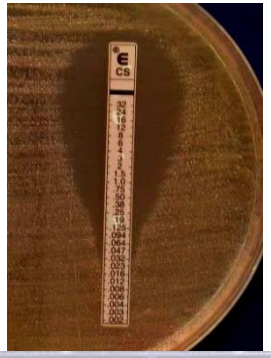


* Investigational

In vitro Antifungal Susceptibility Testing (AST)

- Broth-dilution
  - Macro-format
  - Micro-format
    - CLSI Reference method
    - EUCAST Reference method
  - Odds method
  - SensiTitre
  - ATB fungus 3
  - Vitek 2
- Disk / Etest diffusion
- Agar dilution





Modifiec From Arendrup.

Components in AST

- Standard powders preparation
- Inoculum preparation
- Media for tray and agar
- Quality control strains
- Reading criteria and methods

Solvents and Diluents for Preparation of Stock Solutions of Antifungal Agents

Antifungal agent	CLSI M27-S4	EUCAST Edef 7.2
Amphotericin B	DMSO	DMSO
Anidulafungin	DMSO	DMSO (Edef 7.1: water)
Caspofungin	Water->DMSO	DMSO (Edef 7.1: water)
Micafungin	Water->DMSO	DMSO
Flucytosine	Water	water
Fluconazole	Water or DMSO	DMSO
Itraconazole	DMSO	DMSO
Ketoconazole	DMSO	
Posaconazole	DMSO	DMSO
Ravuconazole	DMSO	
Voriconazole	DMSO	DMSO

DMSO: Dimethylsulphoxide

Clin Microbiol Infect 2012; 18: E246–7

Scheme for Preparing Dilutions of Water-Soluble Antifungal Agents to Be Used in Broth Dilution Susceptibility Tests in CLSI

Antimicrobial Solution								
Step	Concentration (µg/mL)	Source	Volume (mL)	+	Medium (mL)	=	Intermediate Concentration (µg/mL)	= Final Concentration at 1:10 (µg/mL)	Log_2
1	5120	Stock	1 mL		7		640 µg/mL	64	6
2	640	Step 1	1.0		1.0		320	32	5
3	640	Step 1	1.0		3.0		160	16	4
4	160	Step 3	1.0		1.0		80	8	3
5	160	Step 3	0.5		1.5		40	4	2
6	160	Step 3	0.5		3.5		20	2	1
7	20	Step 6	1.0		1.0		10	1	0
8	20	Step 6	0.5		1.5		5	0.5	-1
9	20	Step 6	0.5		3.5		2.5	0.25	-2
10	2.5	Step 9	1.0		1.0		1.25	0.125	-3
11	2.5	Step 9	0.5		1.5		0.625	0.0625	-4
12	2.5	Step 9	0.5		3.5		0.3125	0.03125	-5

Storage of stock

Scheme for Preparing Dilution Series of Water-Insoluble Antifungal Agents to Be Used in Broth Dilution Susceptibility Tests in CLSI

1% solvent

Antimicrobial Solution								
Step		Source	Volume (mL)	+	Solvent (mL) (eg, DMSO)*	= Intermediate Concentration (µg/mL)	= Final Concentration at 1:100 (µg/mL)	Log_2
1	1600	Stock				1600 µg/mL	16	4
2	1600	Stock	0.5		0.5	800	8.0	3
3	1600	Stock	0.5		1.5	400	4.0	2
4	1600	Stock	0.5		3.5	200	2.0	1
5	200	Step 4	0.5		0.5	100	1.0	0
6	200	Step 4	0.5		1.5	50	0.5	-1
7	200	Step 4	0.5		3.5	25	0.25	-2
8	25	Step 7	0.5		0.5	12.5	0.125	-3
9	25	Step 7	0.5		1.5	6.25	0.0625	-4
10	25	Step 7	0.5		3.5	3.13	0.0313	-5

Dispense small volume of sterile stock solution into sterile polypropene or polyethylene vials, sealed and store below <60C

Scheme for preparing dilution series of antifungal agents with a final concentration of 0.125–64 mg/ L

Step	Concentration (mg/L)	Source	Volume of antifungal (μL)	Volume of solvent[a] (μL)	Intermediate concentration (mg/L)	Concentration (mg/L) after 1:100 dilution with double-strength RPMI-1640 2% G[b]
1	12 800[c]	Stock	200	0	12 800	128
2	12 800	Stock	100	100	6400	64
3	12 800	Stock	50	150	3200	32
4	12 800	Stock	50	350	1600	16
5	1600	Step 4	100	100	800	8
6	1600	Step 4	50	150	400	4
7	1600	Step 4	50	350	200	2
8	200	Step 7	100	100	100	1
9	200	Step 7	50	150	50	0.5
10	200	Step 7	25	175	25	0.25

[a]Consult Table 3 for solvents required to make dilutions of antifungal agents.
[b]Dilution 1:1 with inoculum suspension gives final concentrations half those indicated.
[c]For dilution series with highest final concentrations of 16 mg/L or 8 mg/L, start with stock concentrations of 3200 mg/L and 1600 mg/L, respectively.

Storage of microdilution plates in EUCAST

- The plates can be sealed in plastic bags or aluminium foil and stored frozen at -70C or below for up to 6 months, or at -20C for not more than 1 month, without loss of drug potency.
- Once plates are defrosted, they must not be refrozen.
- Echinocandins are unstable, so the prepared trays must be stored at -70C or below for no more than 2 months.

Clin Microbiol Infect 2008; 14: 398–405

PREPARATION OF INOCULUM

- Culture all yeasts in ambient air at 35 ± 2C on non-selective nutritive agar medium (SDA or PDA) for 18–24 h* before testing.
- Prepare the inoculum by suspending 5 distinct colonies, >1 mm in diameter from 24 h cultures, in 5 mL of sterile distilled water.
- Evenly suspend the inoculum by vigorous shaking on a vortex mixer for 15 s. Adjust the cell density to the density of a 0.5X McFarland standard (Table 5) by measuring the absorbance in a spectrophotometer at a wavelength of 530 nm and adding sterile distilled water as required.

This will give a yeast suspension of 1–5 x 10^6 CFU / mL. Prepare a working suspension from a 1 in 10 dilution of the standardised suspension in sterile distilled water to yield 1–5 x 10^5 CFU / mL. (CLSI: 1-5x10^3 CFU/mL)

Table 5. Preparation of 0.5× McFarland turbidity standard

Step	Procedure
1	Add 0.5 mL of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) to 99.5 mL of 0.18 mol/L (0.18 M) H_2SO_4 1% v/v and mix thoroughly
2	Check the density with a spectrophotometer having a 1-cm light path and matched cuvettes. The absorbance at 530 nm should be 0.12–0.15
3	Distribute in screw-cap tubes of the same size as those used for test inoculum adjustment
4	Store sealed standards in the dark at room temperature
5	Mix the standard thoroughly on a vortex mixer immediately before use
6	Renew standards or check their absorbance after storage for 3 months

* crypto: 48 h

Clin Microbiol Infect 2008; 14: 398–405

Steps of In Vitro MIC test—inoculation in microtiter plate in CLSI M27-A3

Microtiter tray

- Take 100 uL prepared inoculum and add to the well
- Final concentration: 0.5-2.5 x10^3 cell/mL (0.5-2.5x 10^5 cell/mL in EUCAST)
- Incubate in 35°C ambient air incubator for 24-48h, except *Cryptococcus* (70-74h)

CLSI M27-A, A2, A3 & EUCAST

	M27-A	M27-A2	M27-A3	EUCAST
Glu/inoculum conc.(/mL)	0.2% glu, 0.5-2.5 x 10^3			2% glu, 0.5-2.5 x 10^5
Plates & reading	Round bottom & visual			Flat & Spectrophotometer
Incub. time	48 h	24-48h	24 h	24 h
End points	80% inhib.	50% inhib.	50% inhib. (except AMB: clear)	50% inhib. except AMB (>90%)
ATCC 22019 (*C. parapsilosis*	2-8 ug/mL	0.5-4/1-4 ug/mL	0.5-4 ug/mL	0.5-2 ug/mL
ATCC 6258 (*C. krusei*)	16-64 ug/mL	8-64/16-128 ug/mL	8-64 ug/mL	16-64 ug/mL

Prominent inhibition (0-2)

Arendrup MC. Dan Med J 2013;60:B4698

Micafungin MIC for Candida in EUCAST version 8.0 footnote

- MICs for *C. tropicalis* are 1-2 two-fold dilution steps higher than for *C. albicans* and *C. glabrata* . In the clinical study successful outcome was numerically slightly lower for *C. tropicalis* than for *C. albicans* at both dosages (100 and 150 mg daily). However, the difference was not significant and whether it translates into a relevant clinical difference is unknown.
-
- MICs for *C. krusei* are approximately three two-fold dilution steps higher than those for *C. albicans* and, similarly, those for *C. guilliermondii* are approximately eight twofold dilutions higher. In addition, only a small number of cases involved these species in the clinical trials. This means there is insufficient evidence to indicate whether the wild-type population of these pathogens can be considered susceptible to micafungin.

CLSI Clinical Breakpoint (CBP) Susceptibility Cutoffs for *Candida* spp. (μg / mL) < M27-A3

	S	SDD	I	R	NS
5FC	≤4	--	8-16	≥32	--
FLU	≤ 8	16-32	--	≥64	--
ITRA	≤ 0.125	0.25-0.5	--	≥1	--
VORI	≤ 1	2	--	≥4	--
ANID	≤ 2	--	--	--	＞2
CAS	≤ 2	--	--	--	＞2
MICA	≤ 2	--	--	--	＞2

NS: non-susceptible; SDD: susceptible dose dependent

Summary of the pathogenicity endpoints for the three virulence groups of *Candida*

Group, *Candida* species	Mor-tality	No. pos. kidneys (%)		Log CFU count (median)		Mouse weight change (g, mean)	Kidney weight % of mouse weight	Inflam-mation score	Eye infec-tion (no. of mice)
		Day 2	Day 7	Day 2	Day 7				
I									
*albicans**	yes	100	100	5.64	6.24	-2.3	1.00	+++	1/3
tropicalis	yes	100	100	6.45	5.98	-2.1	0.92	++	2/3
II									
glabrata	no	100	100	4.42	6.04	0.2	0.79	+	0/3
lusitaniae	no	100	100	5.25	7.04	ND	ND	ND	ND
kefyr	no	100	100	5.2	6.41	ND	ND	ND	ND
III									
parapsilosis	no	100	69	4.5	3.72	ND	ND	ND	ND
krusei	no	100	38	3.65	n.y.d.	2.8	0.69	0-(+)	0/3
guilliermondii	no	50	6	4.00	n.y.d.	ND	ND	ND	ND
Uninfected control	no	0	0	ND	n.y.d	-0.1	0.73	0	0/3

* For *C. albicans* animals received an inoculum of 10^5 CFU (colony forming units) whereas animals challenged with the other species received 10^7 CFU. n.y.d: no yeast detected (detection level 10 CFU/g). ND: not done.

Arendrup et al. Infection 2002;30:286-91

Species-specific Clinical Breakpoints (CBP) against *Candida* spp.

CLSI M27-S4

Species	*Candida albicans* S (≤)	SDD	R (≥)	*Candida glabrata* S (≤)	SDD	R (≥)	*Candida krusei* S (≤)	SDD	R (≥)	*Candida parapsilosis* S (≤)	SDD	R (≥)	*Candida tropicalis* S (≤)	SDD	R (≥)	*guilliermondii* S (≤)	SDD	R (≥)
Azoles																		
FLU	2	4	8	—	≤32	64	—[a]	—	—	2	4	8	2	4	8	—	—	—
VOR	0.12	0.25–0.5	1	—	—	—	0.5	1	2	0.12	0.25–0.5	1	0.12	0.25–0.5	1	—	—	—

Species	*Candida albicans* S (≤)	I	R (≥)	*Candida glabrata* S (≤)	I	R (≥)	*Candida krusei* S (≤)	I	R (≥)	*Candida parapsilosis* S (≤)	I	R (≥)	*Candida tropicalis* S (≤)	I	R (≥)	*Candida guilliermondii* S (≤)	I	R (≥)
Echinocandins																		
AFG	0.25	0.5	1	0.12	0.25	0.5	0.25	0.5	1	2	4	8	0.25	0.5	1	2	4	8
CAS	0.25	0.5	1	0.12	0.25	0.5	0.25	0.5	1	2	4	8	0.25	0.5	1	2	4	8
MFG	0.25	0.5	1	0.06	0.12	0.25	0.25	0.5	1	2	4	8	0.25	0.5	1	2	4	8

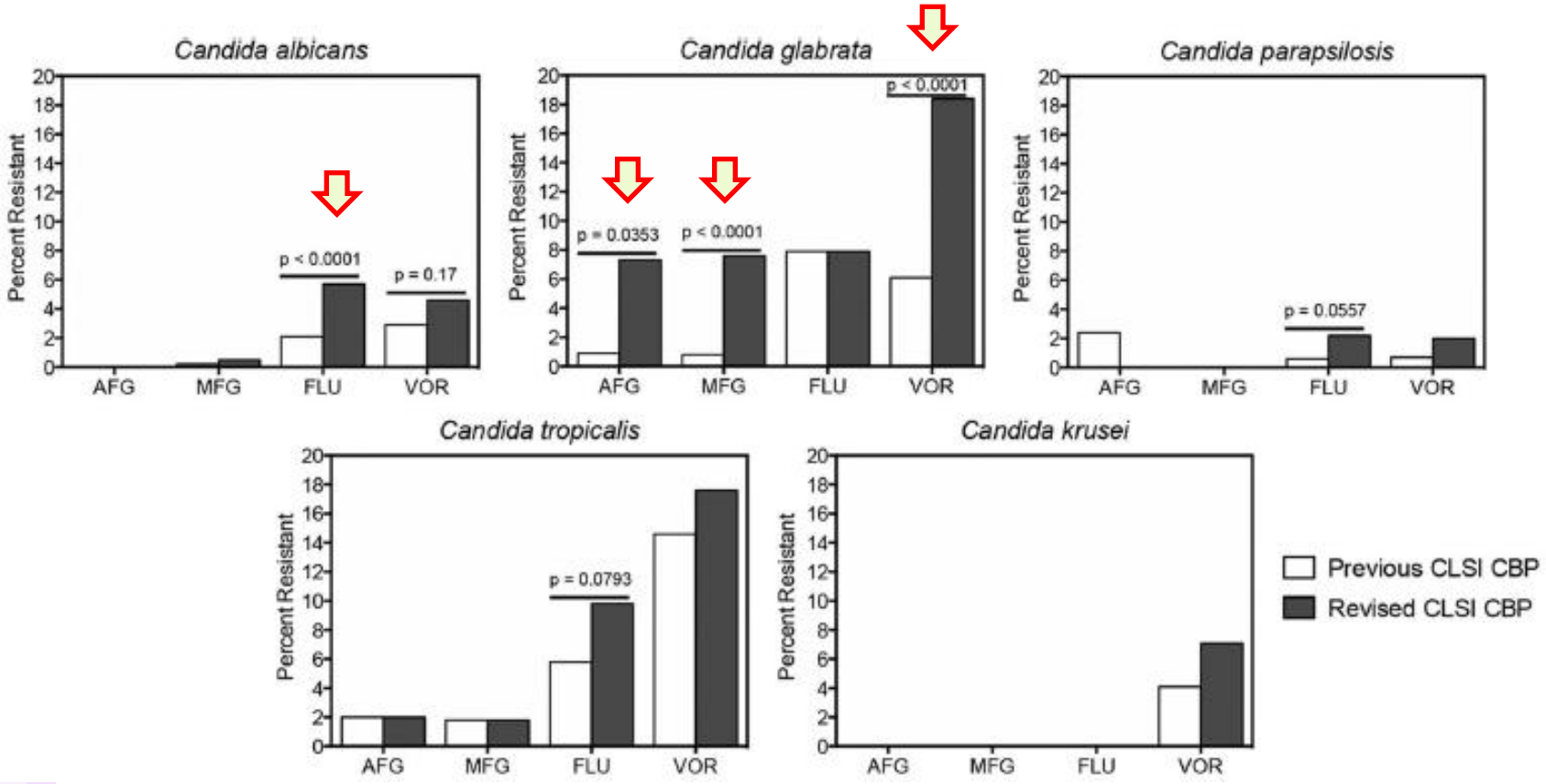
Species	*Candida albicans* S (≤)	R (>)	*Candida glabrata* S (≤)	R (>)	*Candida krusei* S (≤)	R (>)	*Candida parapsilosis* S (≤)	R (>)	*Candida tropicalis* S (≤)	R (>)	*Candida guilliermondii* S (≤)	R (>)
AMB	1	1	1	1	1	1	1	1	1	1	—	—
FLU	2	4	0.002	32	—	—	2	4	2	4	—	—
ITR	0.06	0.06	—	—	—	—	0.12	0.12	0.12	0.12	—	—
POS	0.06	0.06	—	—	—	—	0.06	0.06	0.06	0.06	—	—
VOR	0.12	0.12	—	—	—	—	0.12	0.12	0.12	0.12	—	—
AFG	0.03	0.03	0.06	0.06	0.06	0.06	0.002	4	0.06	0.06	—	—
MFG	0.016	0.016	0.03	0.03	—	—	0.002	2	—	—	—	—

EUCAST

Isavu: IE

CLSI M27-S4 supplement; EUCAST v.8

Impact of New CBP in CLSI: increase resistant rate



Fothergill, et al. *JCM* 2014;52:994-7

Disk diffusion (M-44A)

- For testing, 150-mm-diameter plates containing Mueller-Hinton agar supplemented with 2% glucose and methylene blue (0.5 g/ml) at a depth of 4.0 mm or RPMI-2G can be used.
- The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard (1-5x10^6 cell/mL).
- The plates were incubated in air at 35°C and read at 20-24 h.
- Zone diameter end points were read at 80% growth inhibition by using the BIOMIC image analysis plate reader system (version 5.9; Giles Scientific, Santa Barbara, CA).

CLSI M44-A2- S3

- Provides
  - Zone interpretive criteria for FLU, VORI & caspofungin
  - QC ranges for FLU,VORI, POSA, Caspo

Antifungal Agent	Disk Content	Zone Diameter, Nearest Whole (mm)				Equivalent MIC Breakpoints (µg/mL)			
		S*	S-DD*	R*	NS*	S*	S-DD*	R*	NS*
Caspofungin	**5 µg**	**≥11**	-		**≤10**	**≤2**	-	-	**>2**
Fluconazole‡	25 µg	≥19	15 - 18	≤14	-	≤8	16 - 32	≥64	-
Voriconazole	1 µg	≥17	14 - 16	≤13	-	≤1	2	≥4	-

E-test agar diffusion testing

- RPMI 1640–2G agar (AB Biodisk, Solna, Sweden)
- The agar surface was inoculated by using a nontoxic swab dipped in a cell suspension (530 nm, 0.5 McFarland standard.)
- After excess moisture was absorbed into the agar and the surface was completely dry (15 min at room temperature), the Etest strips were applied to each inoculated plate.
- The plates were incubated at 35°C and read at 24 h.
- The MIC was taken as the lowest concentration of antifungal agent at which the zone of inhibition intersected the strips.

E-test

Flucytosine

AMB

C. albicans and certain other *Candida spp.* can give diffuse end points, especially with azoles and on certain media. The following guidelines could be used to select the appropriate MIC end point.

Figure 1. Flucytosine: Inner zone of microcolonies. MIC 1 µg/ml.

Figure 2. Flucytosine: Macrocolonies in inhibition ellipse. MIC >32 µg/ml.

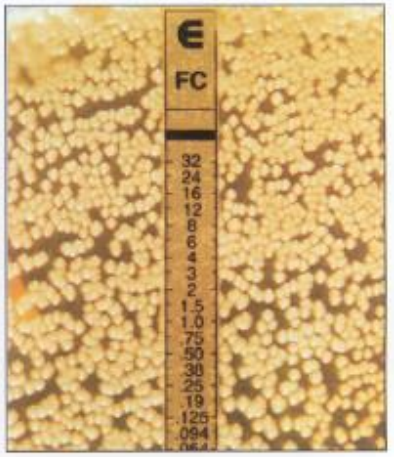


Figure 3. Flucytosine: Resistant strain. MIC >32 µg/ml.

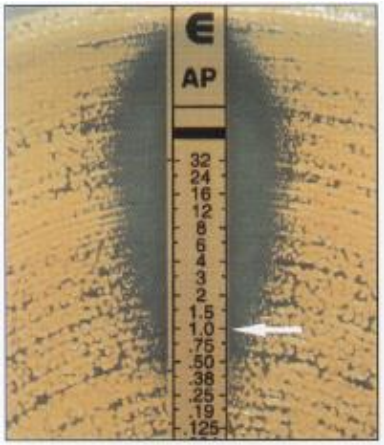


Figure 4. Amphotericin B: Microcolonies at the end point. MIC 1 µg/ml.

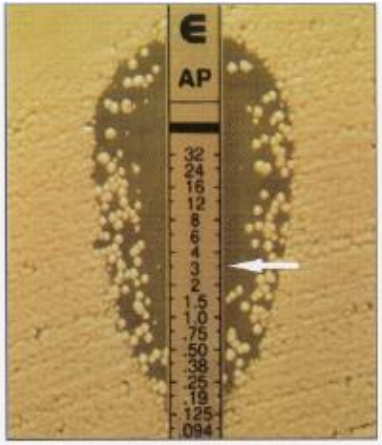


Figure 5. Amphotericin B: Macrocolonies in inhibition ellipse. MIC 3 µg/ml.

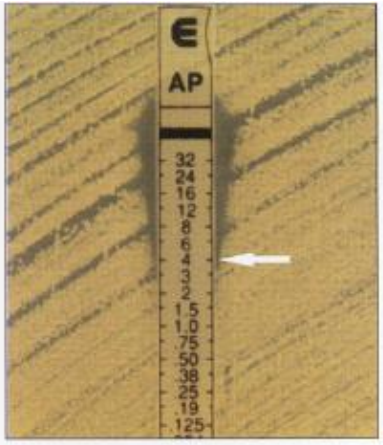


Figure 6. Amphotericin B: Very slim ellipse. MIC 4 µg/ml.

E-test

microcolony

C. *albicans* and certain other *Candida spp.* can give diffuse end points, especially with azoles and on certain media. The following guidelines could be used to select the appropriate MIC end point.

Itraconazole

Figure 10. Itraconazole: Sharp end point. MIC 0.125 µg/ml.

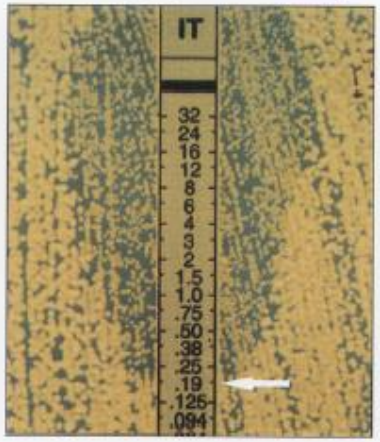


Figure 11. Itraconazole: Micro-colonies within a discernable ellipse. MIC 0.19 µg/ml.

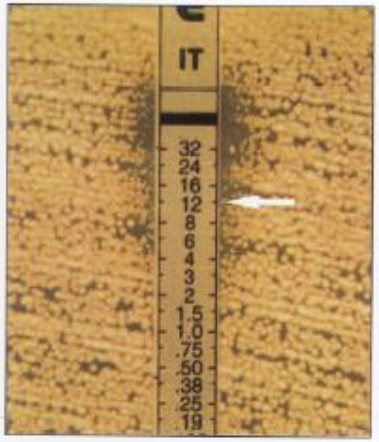


Figure 12. Itraconazole: Micro-colonies within a slim ellipse. MIC 12 µg/ml.

Fluconazole

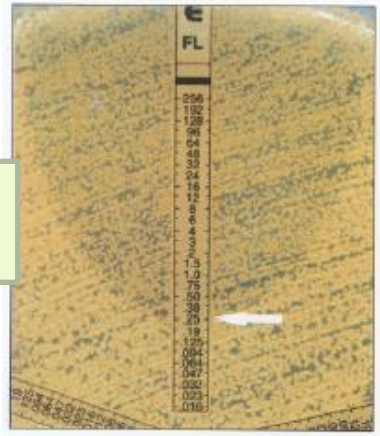


Figure 13. Fluconazole: Less pig-mented colonies within a discern-able ellipse. MIC 0.25 µg/ml.

Figure 14. Fluconazole: Micro-colonies and a double ellipse edge. MIC 2 µg/ml.

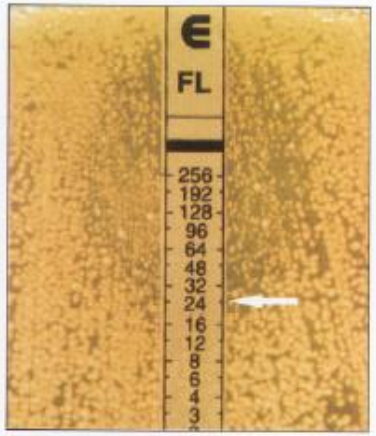
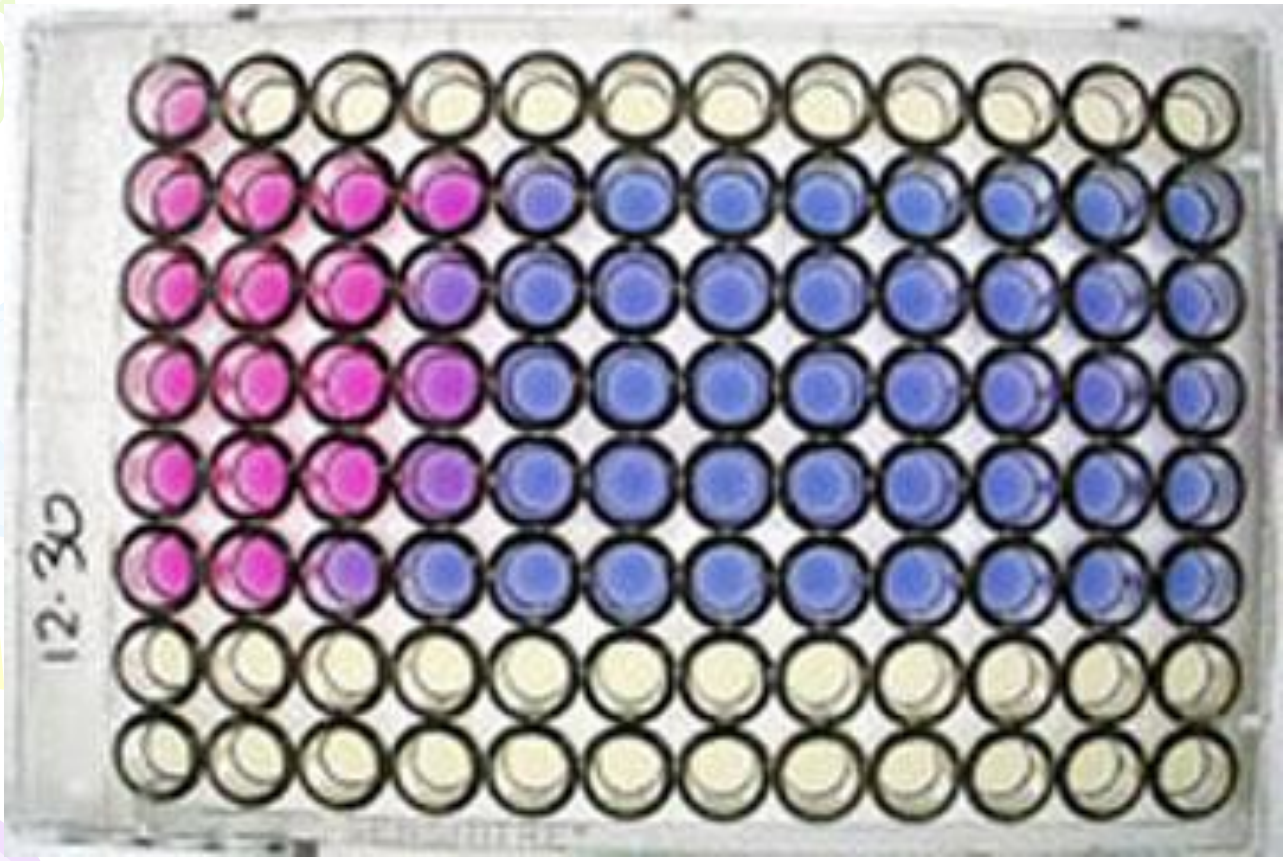


Figure 15. Fluconazole: Micro-colonies within a discernable ellipse. MIC 24 µg/ml.

Sensititre® YeastOne™ Test Panel



20 ul 0.5McF solution + 11 mL YST-1 broth

↓

1.5-8x10^3/mL

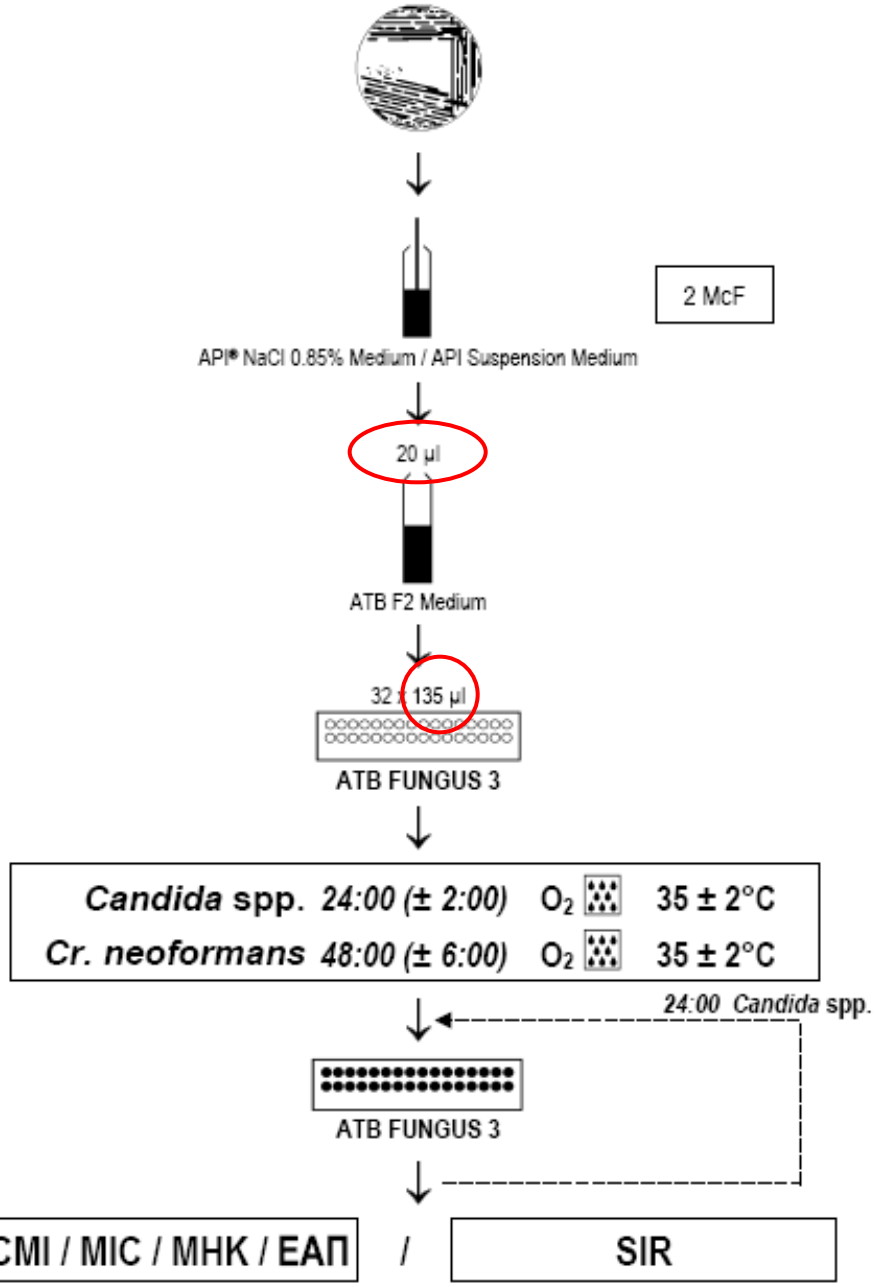
↓

100ul into plate

Yeast: 5-FC, AMB, 3 Candins, FCZ, Itra, Vori, Posa
Aspergillus: AMB, Itra, Vori, Posa
Excellent agreement was observed between BMD & SYO

Figure from Mycology online; ref: JCM 2004;42:4577; & 46:2155

ATB FUNGUS 3



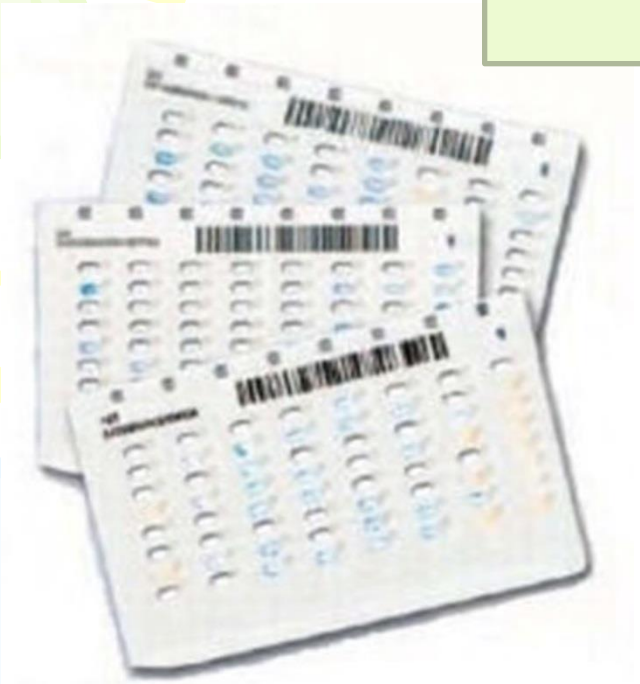
1). A calibrated suspension is prepared with the yeast to be tested *2 Mc Farland*

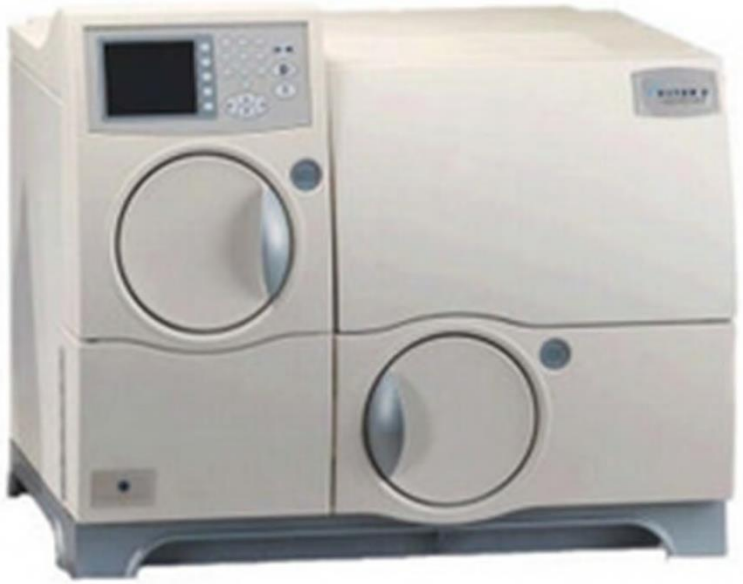
2). Then to be transferred (20 µl) into the culture medium (ATB Fungus 3 medium) 7ml and inoculated into the strip

3). Incubation 35°C / In a humid atmosphere

4). Reading of the growth in the cupules is performed either visually or using the ATB automated instrument

VITEK 2 System





- Species identification and in vitro susceptibility: up to 18 h.
- The Vitek 2 system was able to identify all but 2 of 59 investigated fluconazole-resistant organisms.
(Posteraro et al. 2009 JCM)

- The VITEK 2 system reliably detected FCZ, 5-FC, Vori, caspo, mica resistance among *Candida* spp. and demonstrated excellent quantitative and qualitative agreement with the reference BMD method (Pfaller et al. 2007, 2011 JCM)

Agreement between different tests

- Essential agreement (**EA**): no more than **2** $\log_2$ dilution discrepancies
- Categorical agreement (**CA**):
  - Very major error (VME): reference MIC:R but 24-h or compared method MIC:S
  - Major error (ME): reference MIC:S but 24-h or compared method MIC :R
  - Minor error: a single categorical shift between the two results

Espinel-Ingroff A, et al. ***JCM*** 2009;47:2766-71
Turnidge NP, et al. ***Clin Microbiol Infect*** 2006;12:418

In vitro susceptibility of Candida isolates to triazole as determined by 24-h CLSI & EUCAST BMD

Species (no. of isolates	Antifungal agent	Test method	MIC (ug/mL)		EA (%)
			Range	**Mode**	
C albicans (560)	FCZ	EUCAST	0.12-32	0.25	99.3
		CLSI	0.12-16	0.12	
	Posa	EUCAST	0.015-0.5	0.06	97.9
		CLSI	0.007-0.5	0.03	
	Voric	EUCAST	0.007-16	0.015	98.7
		CLSI	0.007-0.25	0.007	
Total (1056)	FCZ	EUCAST	0.12-128	0.25	98.6
		CLSI	0.12-256	0.12	
	Posa	EUCAST	0.015-16	0.015	97.6
		CLSI	0.007-16	0.03	
	Voric	EUCAST	0.007-16	0.015	96.9
		CLSI	0.007-8	0.007	

Pfaller et al. ***JCM*** 2011;49:845-50

Essential agreement of EUCAST to CLSI (24 h)

Species (no. of isolates)	Essential agreement (%)		
	Anidulafungin	Caspofungin	Micafungin
C albicans (32)	100	100	100
C glabrata (34)	97.1	91.2	97.1
C parap (25)	100	100	100
C tropicalis (12)	91.7	100	100
C krusei (11)	100	**63.6**	100
C guillier (19)	100	**63.2**	100

EA: within **2** dilution

EUCAST MIC tends to be <one 2-fold dilution **lower** than those determined by CLSI

Pfaller MA, et al. *JCM* 2010;48:1592-9

Essential agreement (EA) rates between results given by the Vitek 2 system, Sensititre YeastOne, and Etest and the reference procedures, by antifungal agent (149 isolates)

Antifungal agent	% EA between					
	Vitek and EUCAST	Vitek and CLSI24H	Etest and EUCAST	Etest and CLSI24H	SYOne and EUCAST	SYOne and CLSI24H
AMB	98.7	99.3	98.4	97.4	97.9	97.4
5FC	98.0	98.6	96.4	95.2	96.0	95.2
FLC	97.5	96.6	97.2	96.4	97.2	96.0
VRC	97.5	96.8	95.2	95.2	95.5	95.6
Total	**97.9**	**97.8**	**96.8**	**96.1**	**96.6**	**96.1**

Cuenca-Estrella et al. JCM2010; 48:1782-6

Numbers of errors and discrepancies between MICs obtained by the commercial techniques and the EUCAST reference procedure, by antifungal agent

Antifungal agent *a*	No. of errors or discrepancies between EUCAST and *b*:														
	Vitek					Etest					SYOne				
	VME	ME	MiE	SD	NSD	VME	ME	MiE	SD	NSD	VME	ME	MiE	SD	NSD
AMB				0	2				0	5				0	5
5FC				0	3				1	4				0	3
FLZ	8	0	23			7	0	24			7	0	20		
VOR	0	0	10			0	0	6			0	0	8		
Total	**8**	0	33	0	5	**7**	0	30	1	9	**7**	0	28	0	8

Etest, Etest method; SYOne, Sensititre YeastOne technique; VME, very major errors; ME, major errors; MiE, minor errors; SD, substantial discrepancies(>4 dilution difference); NSD, nonsubstantial discrepancies (3-4 dilution difference).

Cuenca-Estrella et al. JCM2010; 48:1782-6

Numbers of errors and discrepancies between MICs obtained by commercial techniques and the CLSI reference procedure after 24 h of incubation, by antifungal agent

Antifungal agent[a]	No. of errors or discrepancies between CLSI24H and[b]:														
	Vitek					Etest					SYOne				
	VME	ME	MiE	SD	NSD	VME	ME	MiE	SD	NSD	VME	ME	MiE	SD	NSD
AMB				0	1				0	7				0	6
5FC	1	0	8			0	1	18			0	0	12		
FLZ	3	0	22			1	0	22			1	0	24		
VOR	2	2	3			0	1	5			0	2	4		
Total	6	2	33	0	1	1	2	45	0	7	1	2	40	0	6

VME, very major errors; ME, major errors; MiE, minor errors; SD, substantial discrepancies(>4 dilution difference); NSD, nonsubstantial discrepancies (3-4 dilution difference).

Cuenca-Estrella et al. JCM2010; 48:1782-6

Terminology: wild type

- A wild type (WT) organism is defined as a strain which does not harbor any acquired resistance to the particular antimicrobial agent being examined.
- The typical MIC distribution for WT organisms covers three to four 2-fold dilution steps surrounding the modal MIC.

Terminology ECV & CBP

- 'epidemiological cut-off value'(ECV) or wild-type (WT) cut-off value as WT ≤ X mg/L.
- ECVs can be considered to represent the most sensitive measure of the emergence of strains with decreased susceptibility to a given agent.
- Clinical interpretive breakpoints (CBPs) for *in vitro* antimicrobial susceptibility testing may be used to indicate those clinical isolates that are likely to respond to treatment with a given antimicrobial agent administered using the approved dosing regimen for that agent

Standard procedures to set interpretative breakpoints for AST by EUSAT & CLSI

Steps	EUCAST	CLSI
1	Identifying the most common dosage used in each European country	Examining available microbiological data
2	Defining the wild-type population for each target microorganism at the species level and determining the epidemiological cut-offs	Knowing resistance mechanisms and their relation to MIC values and *in vivo* outcomes
3	Describing the pharmacokinetics of the drug	Examining pertinent pharmacokinetic parameters
4	Examining the pharmacodynamics, including Monte Carlo simulations	Examining pharmacodynamic parameters
5	Exploring the correlation of MIC values with clinical outcome of patients treated with the drug	Analyzing clinical outcome data

*Cuenca-Estrella & Rodrigues-Tudela. Expert Rev. Anti Infect. Ther. 2010;*8:267–76

WT MIC distributions of posaconazole (Posa) and voriconazole (Vori) for 8 species of *Candida* obtained using CLSI BMD methods

Species	Antifungal agent	No. of isolates tested	No. of isolates with indicated MIC (μg/ml)										
			0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. a	Posa	8,619	927	4,093	2,561	899	89	32	15	2	1		
	Vori	8,616	8,100	355	72	43	17	15	10	4			
C. g	Posa	2,415	1		8	33	220	712	875	285	188	49	44
	Vori	2,415	8	45	275	1,016	670	135	35	58	95	67	11
C. p	Posa	2,278	22	175	621	815	551	79	14	1			
	Vori	2,279	1,258	665	144	98	63	27	15	6	1	2	
C. t	Posa	1,895	18	406	657	565	207	34	6	1	1		
	Vori	1,895	529	683	461	170	29	17	3	2		1	
C. k	Posa	508		4	7	34	149	258	51	5			
	Vori	507	1		9	79	282	118	15	2	1		
C. lu	Posa	205	2	39	72	65	18	7		2			
	Vori	205	166	28	4	1		4	1	1			
C. guil	Posa	177		3	16	22	75	47	12		2		
	Vori	177	2	23	71	61	9	8	3				
C. kef	Posa	93	1	5	25	29	28	5					
	Vori	93	77	16									

Pfaller MA, et al. ***JCM*** 2011;49:630

Wild type (WT) MIC distributions of anidulafungin, caspofungin and micafungin for eight species of *Candida, using CLSI BMD methods*

Species	Antifungal agent	No. of isolates tested	No. of isolates with MIC (μg/ml) of:										
			0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	>8
C. albicans	Anidulafungin	4,283	338	1,278	1,542	896	216	12		1			
	Caspofungin	4,283	92	1,181	2,037	898	68	6	1				
	Micafungin	4,283	608	2,952	625	90	5	1	1				
C. glabrata	Anidulafungin	1,236		7	161	715	320	26	2	2	2	1	
	Caspofungin	1,236		132	731	329	26	8	7	1			2
	Micafungin	1,236	208	935	71	12	4	2	1	2	1		
C. tropicalis	Anidulafungin	996	41	254	493	173	24	7	1		3		
	Caspofungin	996	17	318	482	161	12	4		1			1
	Micafungin	996	46	400	375	149	17	6	1	2			
C. krusei	Anidulafungin	270		4	159	91	14	1	1				
	Caspofungin	270		1		140	79	40	8	2			
	Micafungin	270		4	28	211	21	6					
C. kefyr	Anidulafungin	61		1	6	31	23						
	Caspofungin	61	8	47	6								
	Micafungin	61		4	27	30							
C. lusitaniae	Anidulafungin	99				5	14	33	43	4			
	Caspofungin	99			3	2	42	46	4	2			
	Micafungin	99	1		4	9	52	31	1	1			
C. parapsilosis	Anidulafungin	1,238		1	2	1	1	14	49	319	765	86	
	Caspofungin	1,238		2	5	31	126	545	399	113	16	1	
	Micafungin	1,238		2	2	1	10	66	261	676	220		
C. guilliermondii	Anidulafungin	88				1	5	7	5	31	32	7	
	Caspofungin	88			1	10	7	21	32	12	1		4
	Micafungin	88		2		5	8	16	31	23	2		1

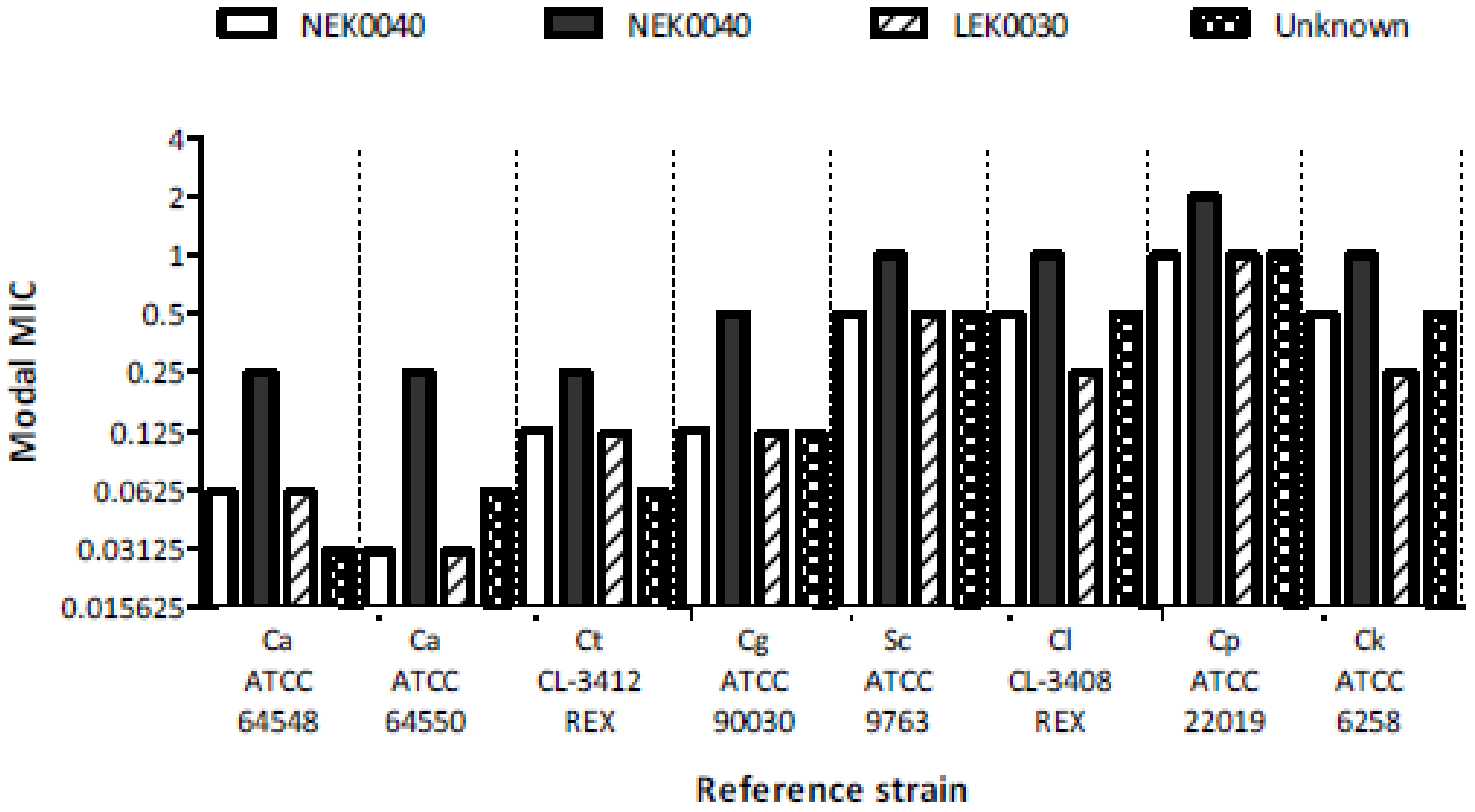
Pfaller MA, et al. ***JCM*** 2010;48:56

CLSI ECVs/ EUCAST ECOFF for systemically active antifungal agents and Candida spp. determined by 24-h broth microdilution methods

Antifungal agent	C alb	C glab	C parap	C trop	C krusei	
AMB	≤2	≤ 2	≤ 2	≤ 2	≤ 2	≤
5FC	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 32	≤
Anid	≤ 0.12/ 0.03	≤ 0.25/ 0.06	≤ 4/ 4	≤ 0.12/ 0.06	≤ 0.12/ 0.06	≤
Caspo	≤0.12	≤ 0.12	≤ 1	≤ 0.12	≤ 0.25	≤
Mica	≤ 0.03/ 0.015	≤ 0.03/ 0.03	≤ 4/ 2	≤ 0.12/ 0.06	≤ 0.12/ 0.25	≤
FCZ	≤ 0.5	≤ 32	≤ 2	≤ 2	≤ 64	≤
Itra	≤ 0.12	≤ 2	≤ 0.5	≤ 0.5	≤ 1	≤
Posa	≤ 0.06	≤ 2	≤ 0.25	≤ 0.12	≤ 0.5	≤
Vori	≤ 0.03	≤ 0.5	≤ 0.12	≤ 0.06	≤ 0.5	≤

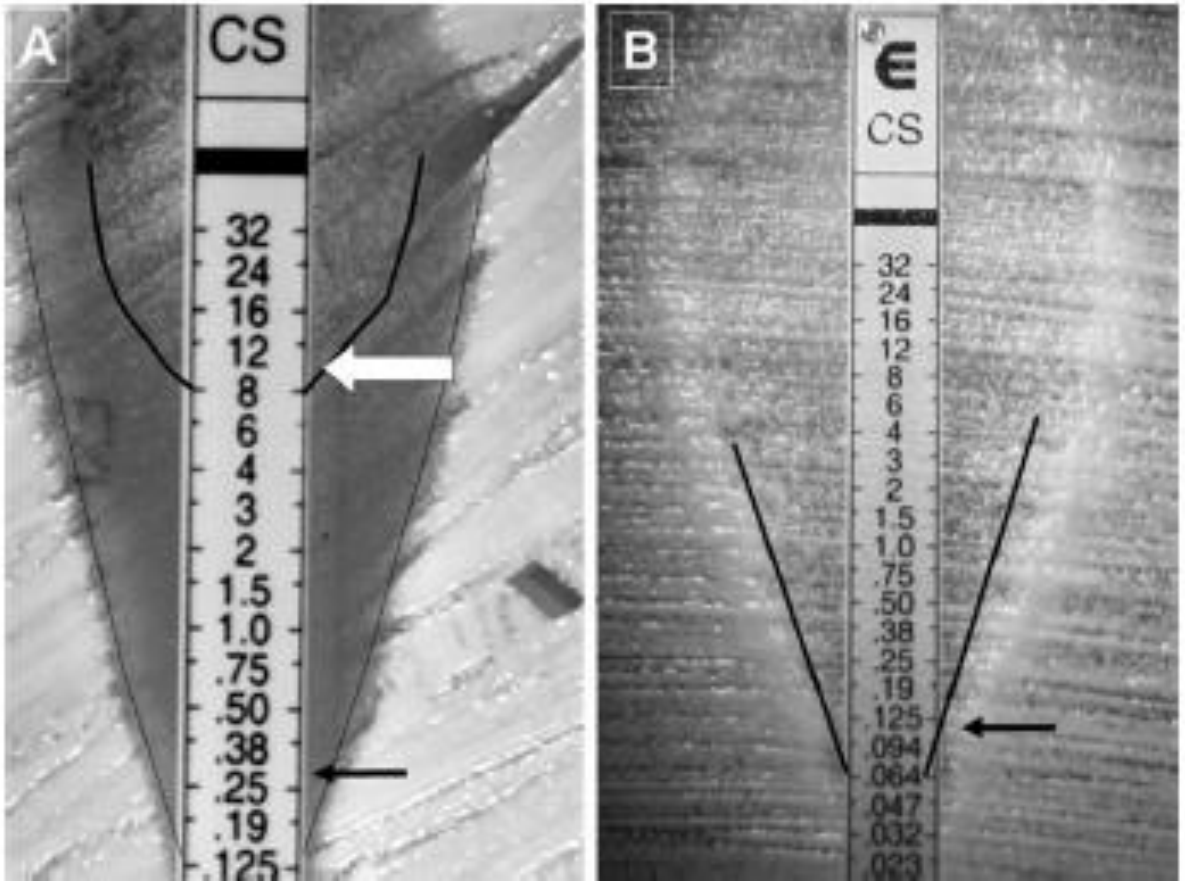
Pfaller MA, et al. DMID 2014;79:198-204; Arendrup et al. Drug Resistance Update 2013;16:81-95

Problem with Caspofungin-
Modal caspofungin MICs (MIC50) for 8 reference strains tested according to the EUCAST methodology and using the following four different lots of caspofungin



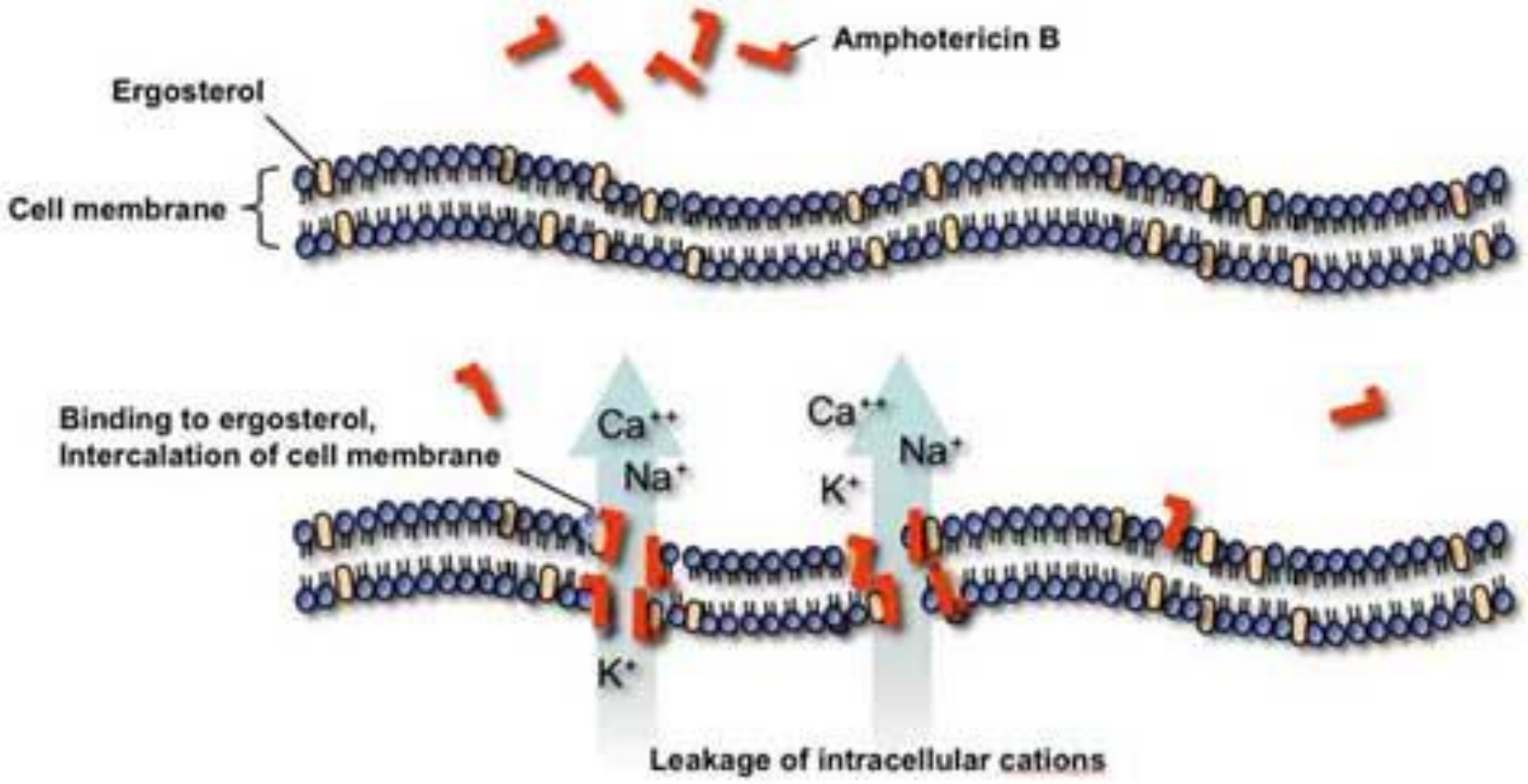
Ca, *C. albicans*; Ct, *C. tropicalis*;
Cg, *C. glabrata*; Sc, *S. cerevisiae*;
Cl, *C. lusitaniae*; Cp, *C. parapsilosis*;
Ck, *C. krusei*.

Trailing Growth and Paradoxical Growth in C albicans with Caspofungin



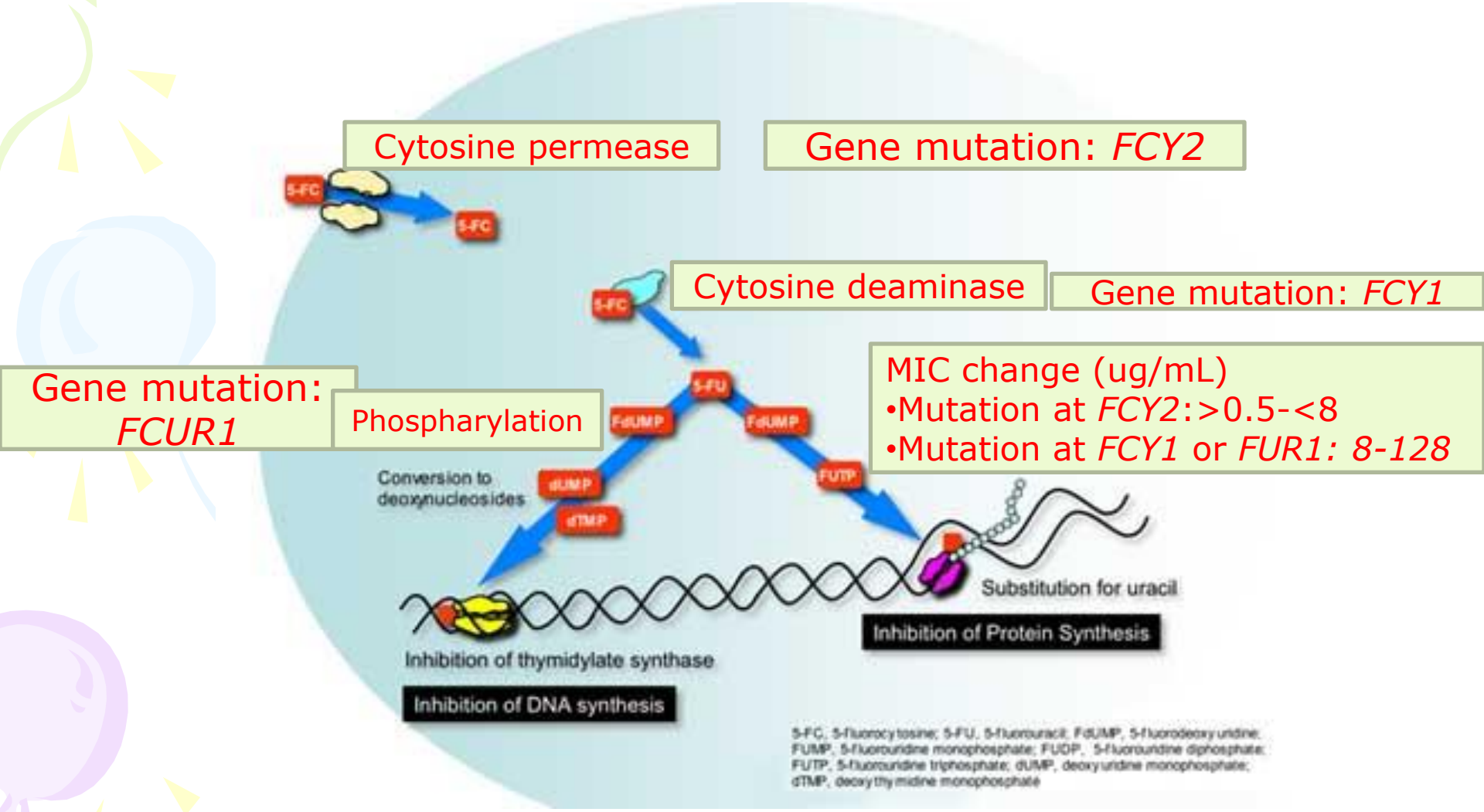
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY2010;54:1365–8

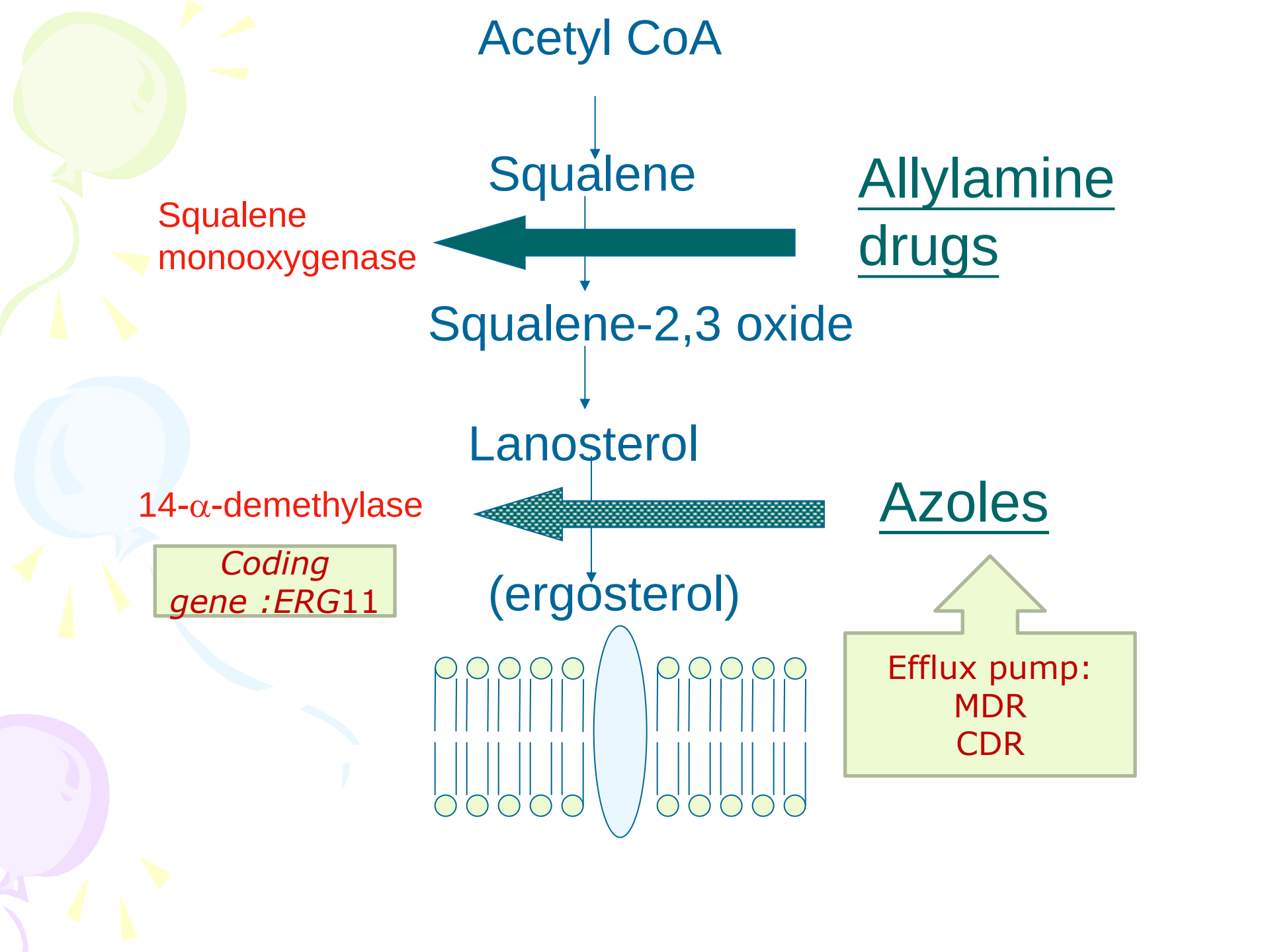
Structure and antifungal mechanism of Amphotericin B



Resistance: change in target lipid or decrease in ergosterol amount

Mechanism of antifungal activity and Resistance mechanism for 5-FC

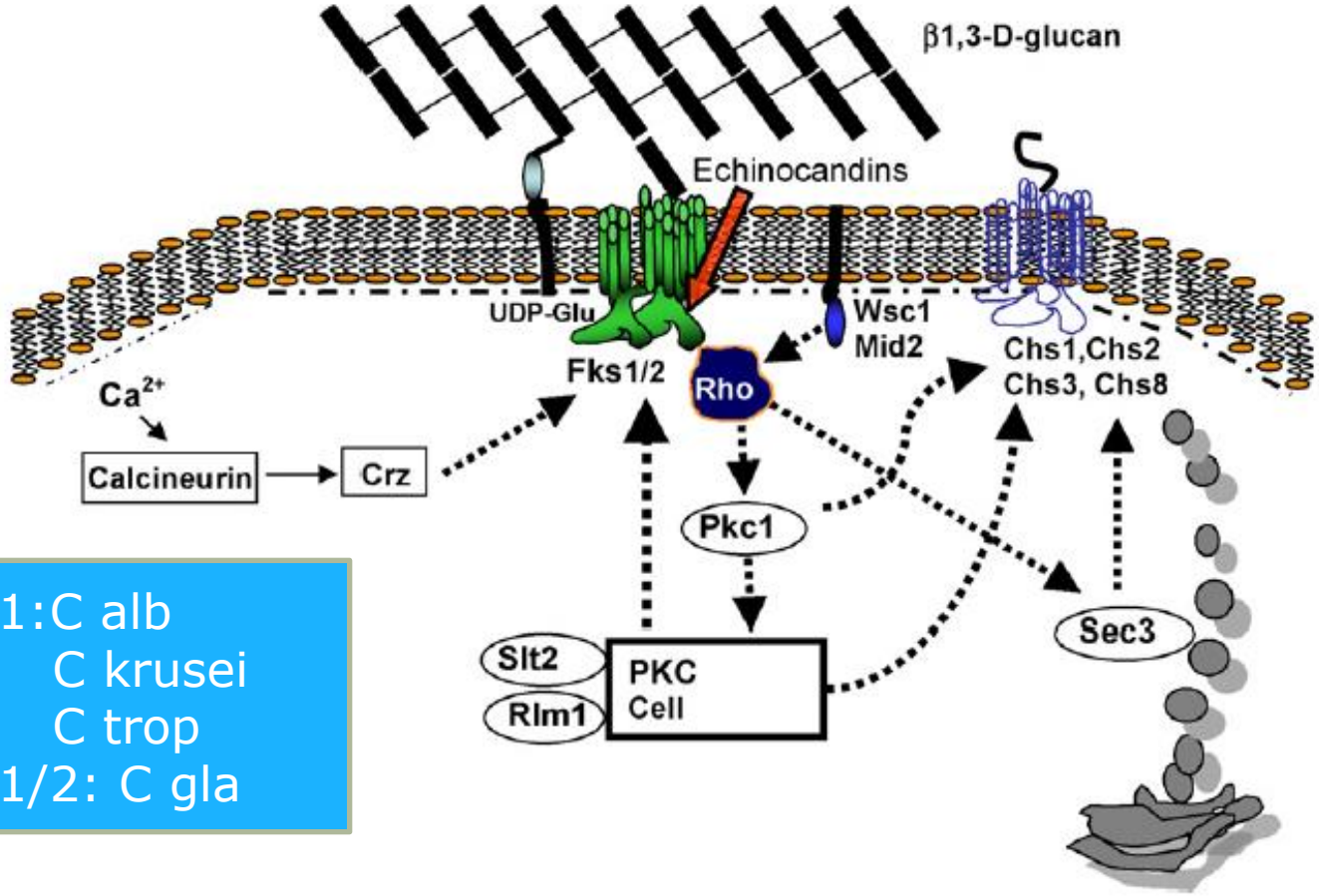



Acetyl CoA
Squalene
Squalene monooxygenase
Allylamine drugs
Squalene-2,3 oxide
Lanosterol
14-α-demethylase
Azoles
Coding gene :ERG11
(ergosterol)
Efflux pump:
MDR
CDR

MIC result of series Oral swab Isolates from an HIV-infected Patient with ATB FUNGUS 3 testing and (Yeastone testing)

Isolates collected date	Fluconazole	Iraconazole	Voriconazole
Break point	≤ 2	≤ 0.12	≤ 0.12
2013/11/18	128	<0.12	0.5
2014/1/6	128 (32)	2 (0.25)	1 (0.12)
2014/3/8	64	1	0.5
2014/5/13	64	0.5	0.5

Resistance Mechanism to Echinocandin

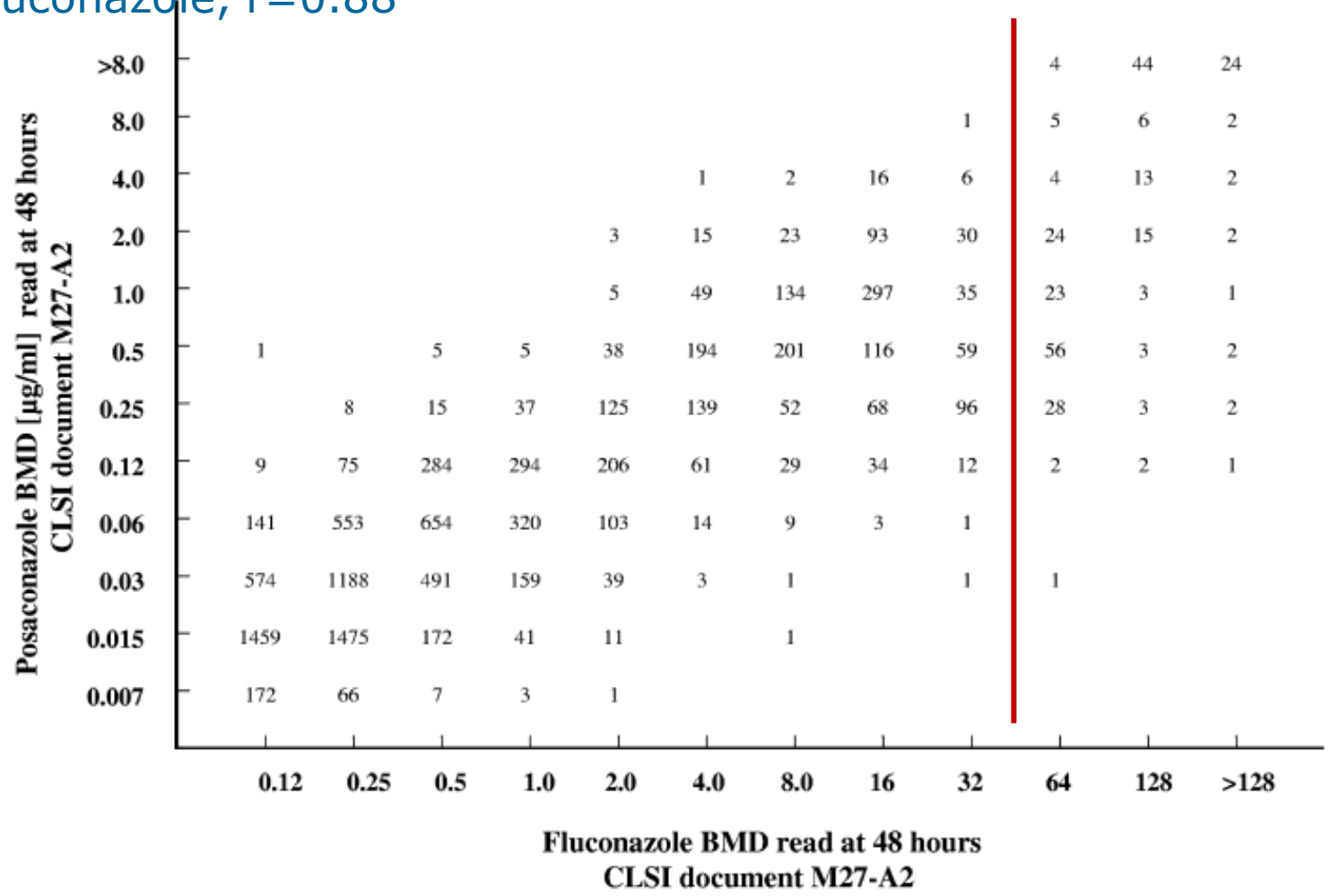


Fks1:C alb
C krusei
C trop
Fks1/2: C gla

Perlin DS. Drug Resist Update 2007;10:121-30

Cross-Resistance: Fluconazole and Posaconazole

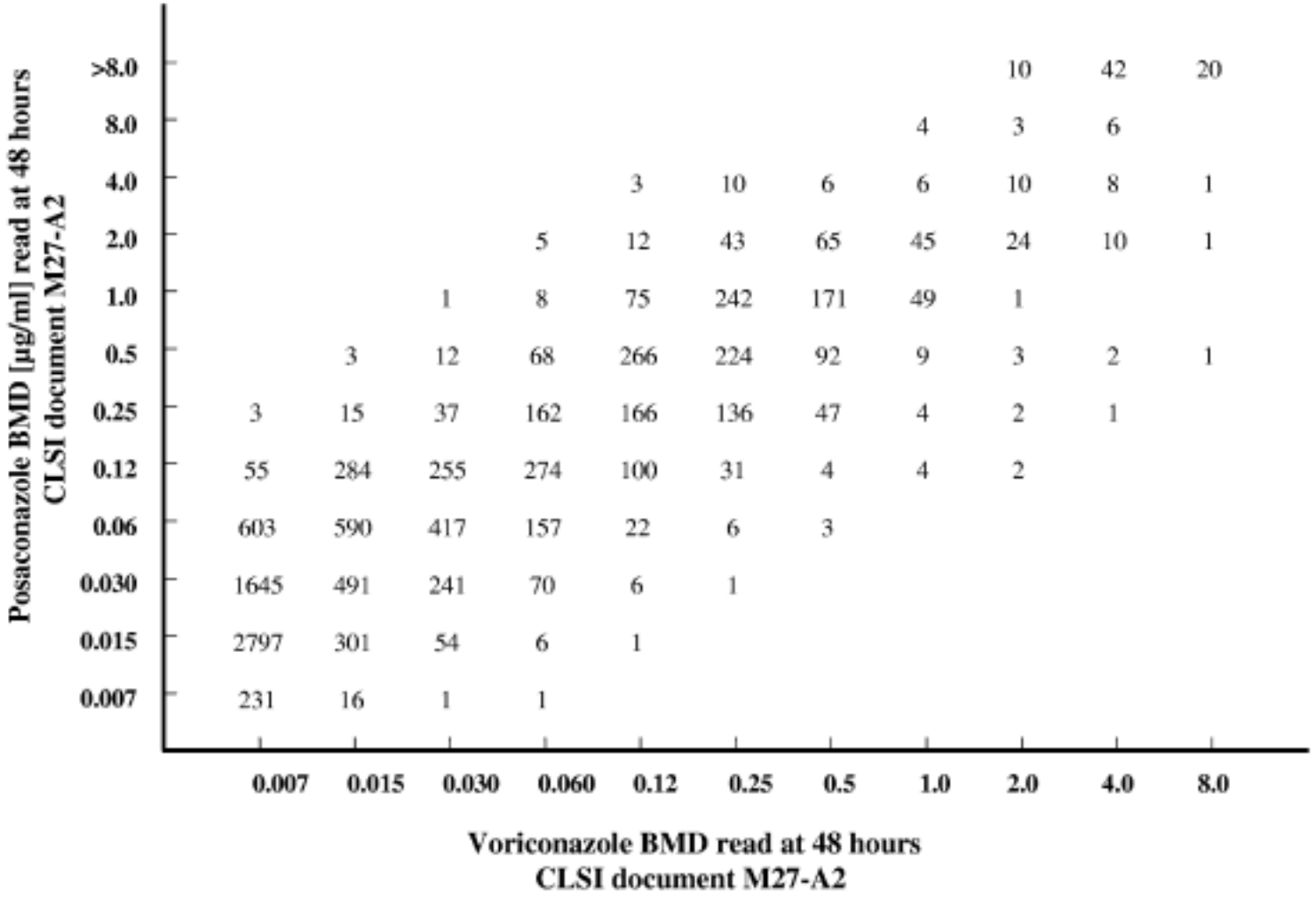
MIC of 10,807 strains of *Candida* spp. posaconaozle vs fluconazole, r=0.88



Pfaller MA, et al. *JCM* 2008;46:551

Cross-Resistance: voriconazole and Posaconazole

MIC of 10,803 strains of *Candida* spp. posaconaozle vs voriconazole, r=0.89



Posaconazole BMD	0.007	0.015	0.030	0.060	0.12	0.25	0.5	1.0	2.0	4.0	8.0
>8.0									10	42	20
8.0								4	3	6	
4.0					3	10	6	6	10	8	1
2.0				5	12	43	65	45	24	10	1
1.0			1	8	75	242	171	49	1		
0.5		3	12	68	266	224	92	9	3	2	1
0.25	3	15	37	162	166	136	47	4	2	1	
0.12	55	284	255	274	100	31	4	4	2		
0.06	603	590	417	157	22	6	3				
0.030	1645	491	241	70	6	1					
0.015	2797	301	54	6	1						
0.007	231	16	1	1							

Pfaller MA, et al. *JCM* 2008;46:551

Limitation of In Vitro testing

- the *in vitro susceptibility* of an infecting organism to the administered antimicrobial agent is only one of the factors that may influence the likelihood that therapy for an infection will be successful.

- Factors related to the **host immune response, severity of underlying disease, drug pharmacokinetics** and **pharmacodynamics, drug interactions, and proper patient management** and factors related to the **virulence of the infecting organism** and its interaction with both the host and the antimicrobial agent all influence the outcome of treatment of an infectious episode.

Pfaller & Diekema. ***JCM*** 2012;50:2846-56

Recommendations for use of antifungal susceptibility testing of *Candida spp.* in the clinical laboratory---Routine part

- Species-level identification of all *Candida* isolates from deep sites (e.g., blood, normally sterile body fluids, tissues, abscesses)
- Routine antifungal testing of fluconazole, voriconazole and an echinocandin against *C. glabrata* from deep sites or blood
- Mucosal candidiasis that is unresponsive to usual antifungal regimen
- Use CBPs or ECVs to interpret results as appropriate
- Clinical failure in patients with invasive diseases caused by species with significant rates of acquired resistance
- Invasive disease caused by unusual fungal species for which antifungal susceptibility patterns have not been well established or are unpredictable

Pfaller & Diekema. ***JCM*** 2012;50:2846-56; UpToDate 2016 Jul 2

Recommendations for use of antifungal susceptibility testing of *Candida spp* with high rates of intrinsic or acquired resistance

- Susceptibility testing not necessary when intrinsic resistance is known
  - *C. lusitaniae* and amphotericin
  - *C. krusei* and fluconazole, flucytosine
  - *C. guilliermondii* and echinocandins (?)

- With high rates of acquired resistance, monitor closely for signs of failure and perform susceptibility testing
  - *C. glabrata* and FCZ, AMB, and echinocandins
  - *C. krusei* and AMB
  - *C. guilliermondii* and AMB
  - *C. rugosa* and AMB, FCZ, and echinocandins

Pfaller & Diekema. ***JCM*** 2012;50:2846-56

Thanks for Your Attention

ECV for Amphotericin B in *Candida* species

	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤2	>2				
C. glab	≤2	>2				
C. parap	≤2	>2				
C. trop	≤2	>2				
C. krus	≤2	>2				
C. lusit	≤2	>2				
C. guill	≤2	>2				
C. dubl	≤2	>2				

- No available CBP
- PK/PD showed maximall effect: Cmax/MIC=2

Pfaller MA, et al. ***JCM*** 2012;50:2846–56

ECV for Flucytosine (5-FC) in *Candida* species

	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.5	>0.5				
C. glab	≤0.5	>0.5				
C. parap	≤0.5	>0.5				
C. trop	≤0.5	>0.5				
C. krus	**≤32**	**>32**				
C. lusit	≤0.5	>0.5				
C. guill	≤1	>1				
C. dubl	≤0.5	>0.5				

CLSI CBP ≤4 (ug/ml), I=8-16, R≥32
(too high to detect resistance)

ECV & CBP for Fluconazole in *Candida* species

	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.5	>0.5	≤2	4		≥8
C. glab	≤32	>32		≤32		>32
C. parap	≤2	>2	≤2	4		≥8
C. trop	≤2	>2	≤2	4		≥8
C. krus	≤64	>64				
C. lusit	≤2	>2				
C. guill	≤8	>8				
C. dubl	≤0.5	>0.5				
C. kefyr	≤1	>1				
C. orthop	≤2	>2				
C. pelli	≤4	>4				

Pfaller MA, et al. ***JCM*** 2012;50:2846–56

ECV & CBP for Itraconanazole in *Candida* species

	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.12	>0.12	<0.12	0.25-0.5		≥1
C. glab	≤2	>2				
C. parap	≤0.5	>0.5				
C. trop	≤0.5	>0.5				
C. krus	≤1	>1				
C. lusit	≤0.5	>0.5				
C. guill	≤1	>1				
C. dubl	≤0.25	>0.25				

Pfaller MA, et al. ***JCM*** 2012;50:2846–56

ECV & CBP for Voriconazole in *Candida* species

	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.03	>0.03	≤0.12		0.25-0.5	≥1
C. glab	≤0.5	>0.5				
C. parap	≤0.12	>0.12	≤0.12		0.25-0.5	≥1
C. trop	≤0.06	>0.06	≤0.12		0.25-0.5	≥1
C. krus	≤0.5	>0.5	≤0.5		1	≥2
C. lusit	≤0.03	>0.03				
C. guill	≤0.25	>0.25				
C. dubl	≤0.03	>0.03				
C. kefyr	≤0.015	>0.015				
C. ortho	≤0.06	>0.06				
C. pelli	≤0.25	>0.25				

Pfaller MA, et al. ***JCM*** 2012;50:2846–56

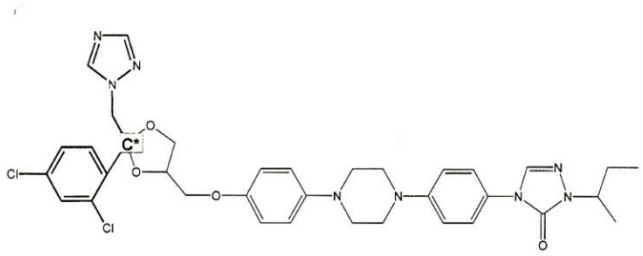
ECV for Posaconazole in *Candida* species

	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.06	>0.06				
C. glab	≤2	>2				
C. parap	≤0.25	>0.25				
C. trop	≤0.12	>0.12				
C. krus	≤0.5	>0.5				
C. lusit	≤0.12	>0.12				
C. guill	<0.5	>0.5				
C. dubl	<0.12	>0.12				
C. kefyr	≤0.25	>0.25				
C. ortho	≤0.25	>0.25				
C. pelli	≤2	>2				

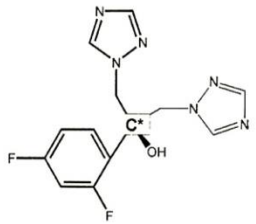
No CBP

Pfaller MA, et al. ***JCM*** 2012;50:2846–56

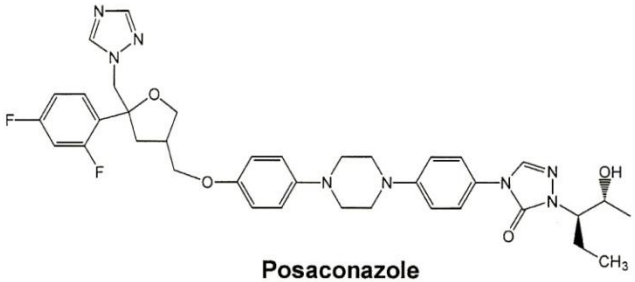
Structural formulas of systemic antifungal triazoles



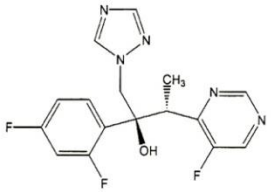
Itraconazole



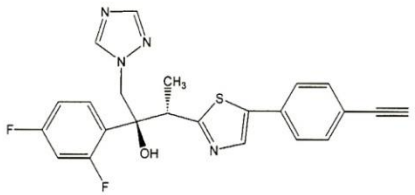
Fluconazole



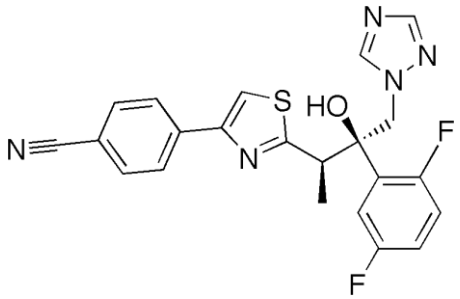
Posaconazole



Voriconazole



Ravuconazole



isavuconazole

Azole resistance mechanisms as they relate to MIC in serial isolates of *C. albicans* from an HIV-infected patient with recurrent oropharyngeal candidiasis*a*

C. albicans isolate(s)	MIC (μg/ml)		Molecular change(s)
	FLC	ITR	
1	0.25	0.06	None (WT)
3	8	0.06	Increase in *MDR1* mRNA
12–15	16–32	0.12–0.25	Mutation in *ERG 11* gene, loss of heterozygosity in *ERG 11*, increase in *ERG11* mRNA
16, 17	64–128	4–8	Increase in CDR mRNA

Clin. Infect. Dis. **18:240 –242**
Antimicrob. Agents Chemother. **41:1482–7 &1488 –94**
Clin. Microbiol. Rev. **11:382– 402**

Impact of resistance mechanisms on the *in vitro* susceptibility of *C. albicans* to voriconazole (VRC) and posaconazole (PSC)

	Resistance mechanism(s)		MIC (μg/ml)	
Strain	CDR[b]	ERG 11[c]	VRC	PSC
DSY294	Basal	WT/WT	0.007	0.03
DSY296	Increase	G464S/G464S	2	0.25
DSY3083	Basal	G464S/G464S	0.12	0.03
DSY3604	Basal	G464S/WT	0.06	0.03
DSY3606	Increase	WT/WT	0.12	0.25

[b] Data represent the level of expression of *CDR1/CDR2* efflux pumps
[c] Data represent wild type or a mutation (G464S) in either or both of two *ERG 11* alleles.

Antimicrob. Agents Chemother. 2010;**54:1476 –1483**

ECV & CBP for Anidulafungin in *Candida* species

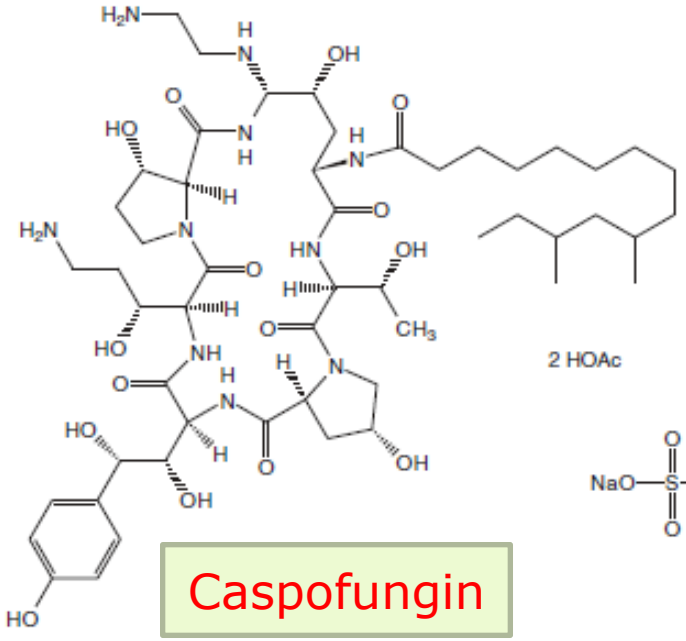
	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.12	>0.12	≤0.25		0.5	≥1
C. glab	≤0.25	>0.25	**≤0.12**		**0.25**	**≥0.5**
C. parap	≤4	>4	≤2		4	≥8
C. trop	≤0.12	>0.12	≤0.25		0.5	≥1
C. krus	≤0.12	>0.12	≤0.25		0.5	≥1
C. lusit	≤2	>2				
C. guill	≤4	>4				
C. dubl	≤0.12	>0.12				
C. kefyr	≤0.25	>0.25				
C. ortho	≤2	>2				
C. pelli	≤0.12	>0.12				

ECV & CBP for Caspofungin in *Candida* species

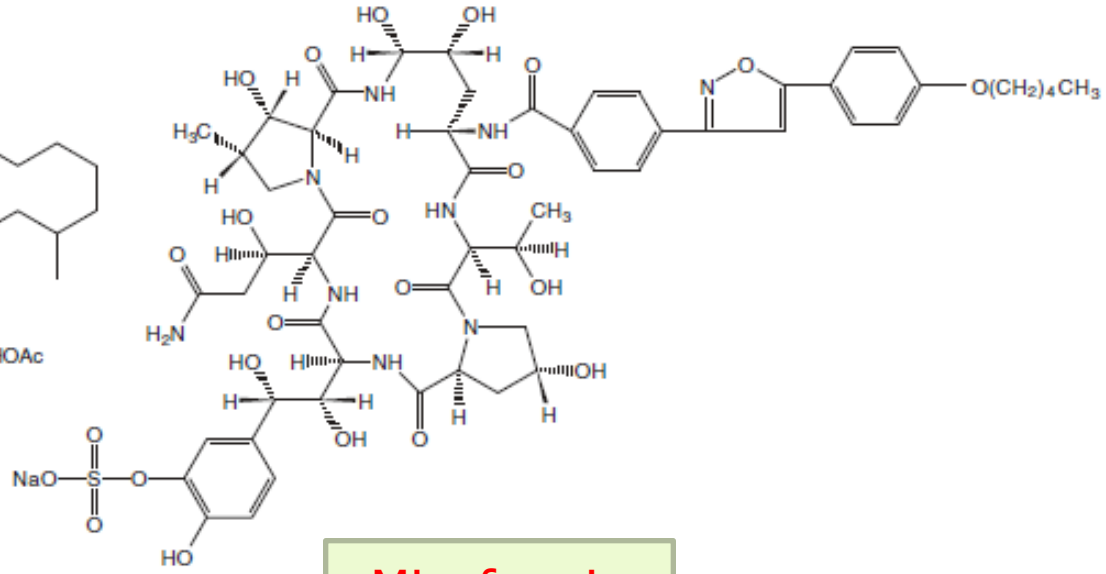
	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.12	>0.12	≤0.25		0.5	≥1
C. glab	≤0.12	>0.12	≤0.12		0.25	≥0.5
C. parap	≤1	>1	≤2		4	≥8
C. trop	≤0.12	>0.12	≤0.25		0.5	≥1
C. krus	≤0.25	>0.25	≤0.25		0.5	≥1
C. lusit	≤1	>1				
C. guill	≤2	>2				
C. dubl	≤0.12	>0.12				
C. kefyr	≤0.03	>0.03				
C. ortho	≤0.5	>0.5				
C. pelli	≤0.12	>0.12				

ECV & CBP for Micafungin in *Candida* species

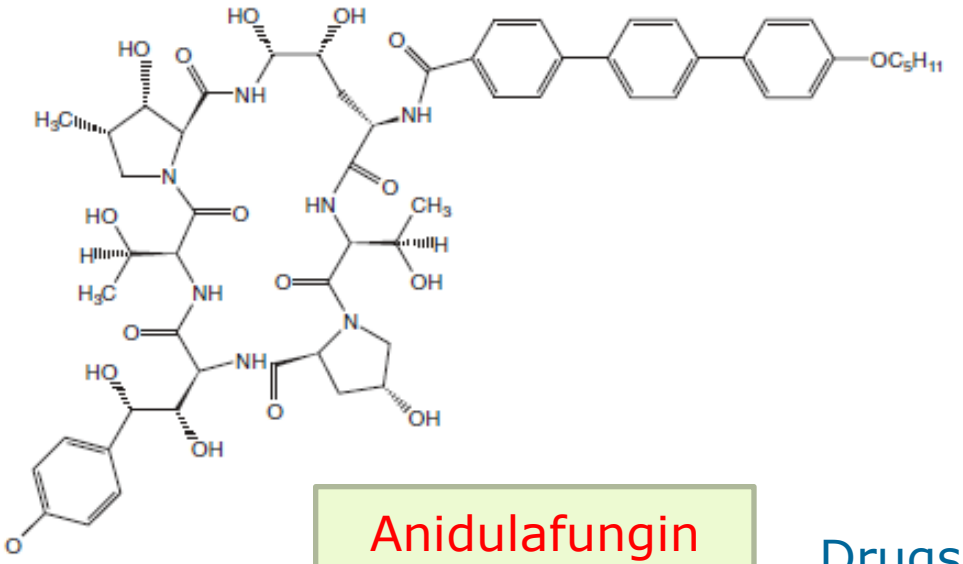
	ECV (ug/ml)		CBP(ug/ml)			
Organism	WT	Non-WT	S	SDD	I	R
C. alb	≤0.03	>0.03	≤0.25		0.5	≥1
C. glab	≤0.03	>0.03	≤0.06		0.12	≥0.25
C. parap	≤4	>4	≤2		4	≥8
C. trop	≤0.12	>0.12	≤0.25		0.5	≥1
C. krus	≤0.12	>0.12	≤0.25		0.5	≥1
C. lusit	≤0.5	>0.5				
C. guill	≤2	>2	≤2		4	≥8
C. dubl	≤0.12	>0.12				
C. kefyr	≤0.12	>0.12				
C. ortho	≤1	>1				



Caspofungin



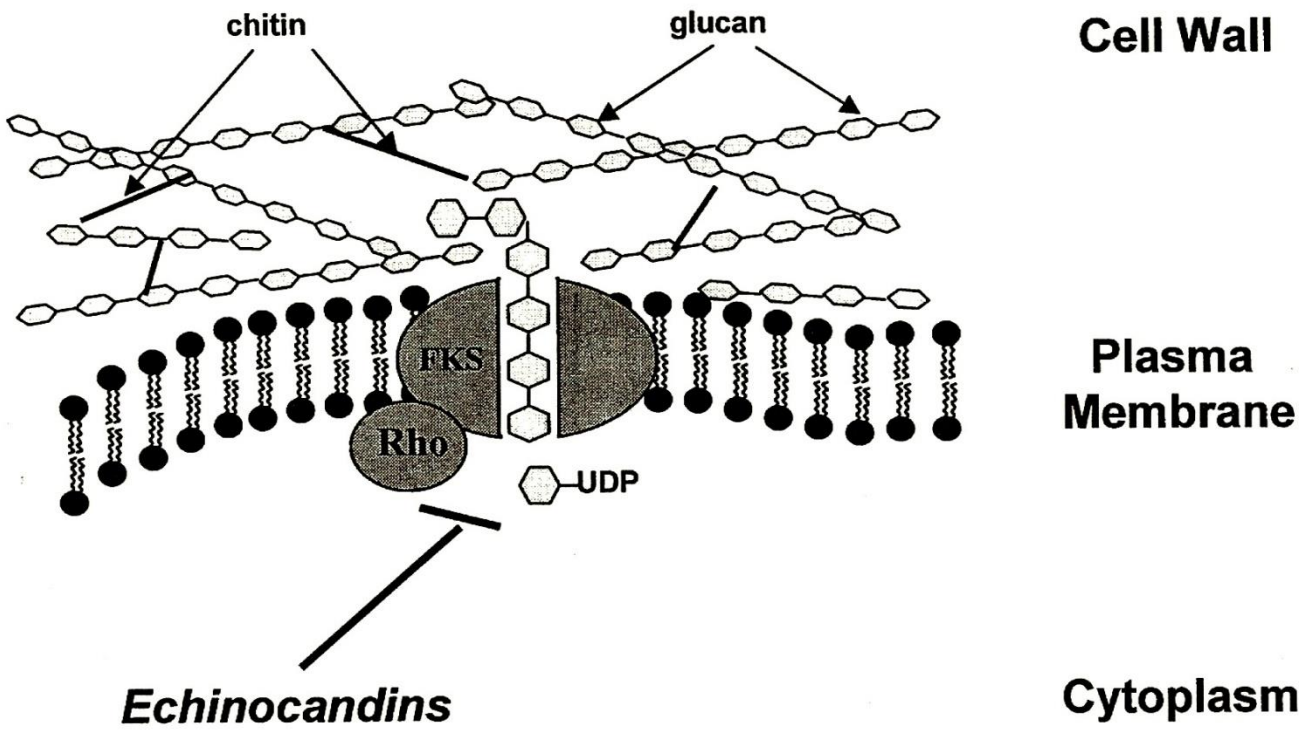
Micafungin



Anidulafungin

Drugs 2011;71:11

Mechanism of antifungal activity and Resistance mechanism for Echinocandin



Published Cases of Candida spp Infection with Increased MICs of Echinocandin

Ref.	Organism	Infection	Isolate	MIC (μg/mL)			FKS mutation	Comments (mutation)
				CAS	MFG	AFG		
Dodgson	*C glabrata*	Fungemia	Blood	>8	>8	>8	Yes	S663P
Hakki	*C krusei*	Fungemia	Blood	2	0.5	0.25	None	
			Throat	8	4	4	Yes	F655C
Cleary	*C glabrata*	Fungemia	Blood	>4	>4	>4	Yes	
			Blood	>4	>4	>4	Yes	D632E

Pfaller MA, et al. JCM 2008;46:2620-9

Antimicrobial Susceptibility Testing

- The“ 90-60 Rule ”（expected correlation）
- General rules to guide interpretation of results
  - Infections due to susceptible isolates respond to appropriate therapy ~ 90 % of the time
  - Infections due to resistant isolates（or infections treated with inappropriate therapy）respond at ~ 60 % of the time

From Fothergill AW

Clinical success for patient-episode-isolate events treated with fluconazole, voriconazole, or itraconazole by their respective CLSI MIC interpretive categories for *Candida spp.*

Antifungal agent	MIC breakpoint (ug/ml)	No. of events	% success
Fluconazole	Susceptible (≤2)	550	**92**
	Resistant (≥8)	212	**37**
Voriconazole	Susceptible (≤0.12)	173	**76**
	Resistant (≥1)	8	**38**
Itraconazole	Susceptible (≤0.12)	193	**88**
	Resistant (≥1)	6	**67**

Drug Resist. Updat. **13:180 –195.**
Diagn. Microbiol. Infect. Dis. **70:330 –343**
Clin. Infect. Dis. **24:235–247.**

Frequency of Trailing Growth by Drug and *Candida* spp.

	Frequency (%) of trailing with				
Species	5FC	FLU	ITR	POS	VOR
C albicans (733)	9	12	13	6	6
C glabrata (458)	1	14	29	30	32
C krusei (50)	18	14	25	20	14
Cparapsilosis (391)	3	7	11	4	2
C tropicalis (307)	4	22	19	17	22

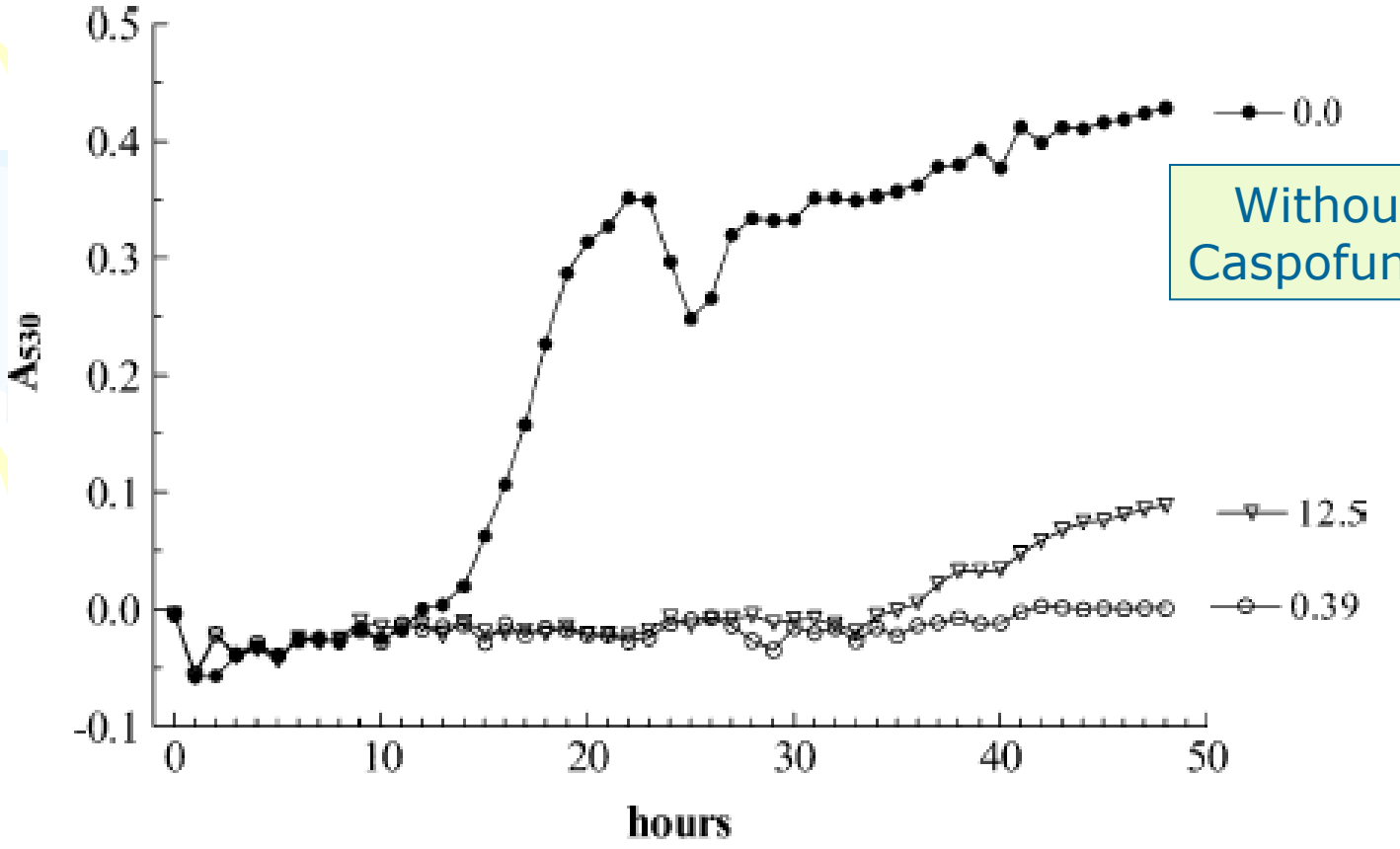
Ostrosky-Zeichner L, et al. *AAC* 2003;47:3149-54

Frequency of "Trailing Growth" by Drug and *Candida* spp.

	Frequency (%) of trailing with		
Species	Anidulafungin	Caspofungin	Micafungin
C albicans (733)	1	3	2
C glabrata (458)	2	2	2
C krusei (50)	2	6	4
Cparapsilosis (391)	16	12	11
C tropicalis (307)	1	3	2

Ostrosky-Zeichner L, et al. *AAC* 2003;47:3149-54

Hourly Spectrophotometer of *Candida* Growth with "Paradoxical Growth"



Stevens DA, et al. Diag Microbiol Infect Dis 2005;51:173

Paradoxical Effect of Echinocandin Against Different *Candida* Species

Candida spp (no. of isolate)	Paradoxical effect (%)			Median paradoxical growth start/end point (ug/mL)		
	CAS	MICA	ANID	CAS	MICA	ANID
CA (20)	60	0	40	8/32	NA	
CP (10)	90	0	0	8/64	NA	NA
CT (10)	40	70	20	16/48	12/48	0.125/1.0
CK (10)	10	60	0	8/32-64	0.125/0.5	NA
CG (10)	0	0	0	NA	NA	NA

Paradoxical effect is echinocandin-specific, species-related and medium-related (seen only in RPMI)

Chamilos G, et al. *AAC* 2007;51:2257-9

Pai MP, et al. *Diag Microbiol Infect Dis* 2007;58:129-32

M27-A3 Breakpoints (24H reading)
Candida albicans

	S	SDD	I	R	WT	Non-WT
AMB	---	---	---	---	≤2	>2
ANID	≤0.25	---	0.5	>1	≤0.125	>0.125
CAS	≤0.25	---	0.5	>1	≤0.125	>0.125
MICA	≤0.25	---	0.5	>1	≤0.03	>0.03
FLU	≤2	4	---	≥8	≤0.5	>0.5
POS	---	---	---	---	≤0.06	>0.06
VORI	<0.125	---	0.25-0.5	>1	≤0.03	>0.03
ITRA	<0.125	0.25-0.5		>1	≤0.125	>0.125
5FC	---	---	---	---	≤0.25	>0.25

M27-A3 Breakpoints (24h) *Candida glabrata*

	S	SDD	I	R	WT	Non-WT
AMB	---	---	---	---	≤2	>2
ANID	≤0.125	---	0.25	≥0.5	≤0.125	>0.125
CAS	≤0.125	---	0.25	≥0.5	≤0.125	>0.125
MICA	<0.06	---	0.125	0.25	≤0.03	>0.p3
FLU	---	≤32	---	≥64	≤32	>32
POS	---	---	---	---	≤2	>2
VORI	---	---	---	---	≤0.5	>0.5
ITRA	---	---	---	---	≤2	>2
5FC	---	---	---	---	≤0.5	>0.5

M27-A3 Breakpoints *Candida tropicalis*

	S	SDD	I	R	WT	Non-WT
AMB	---	---	---	---	≤2	>2
ANID	≤0.25	---	0.5	≥1	≤0.125	>0.125
CAS	≤0.25	---	0.5	≥1	≤0.125	>0.125
MICA	≤0.25	---	0.5	≥1	≤0.125	>0.125
FLU	≤2	4		≥8	≤2	>2
POS	---	---	---	---	≤0.125	>0.125
VORI	<0.125	0.25-0.5	---	≥1	≤0.06	>0.06
ITRA	---	---		>1	≤0.5	>0.5
5FC	---	---	---	---	≤0.25	>0.25

M27-A3 Breakpoints (24h) *Candida parapsilosis*

	S	SDD	I	R	WT	Non-WT
AMB	---	---	---	---	≤2	>2
ANID	<2	---	4	>8	≤4	>4
CAS	<2	---	4	>8	≤1	>1
MICA	<2	---	4	>8	≤4	>4
FLU	≤2	4	---	≥8	≤2	>2
POS	---	---	---	---	≤0.25	>0.25
VORI	≤0.125		0.25-0.5	≥1	≤0.125	>0.125
ITRA	---	---	---	---	≤0.5	>0.5
5FC	---	---	---	---	≤0.5	>0.5

M27-A3 Breakpoints (24h) *Candida krusei*

	S	SDD	I	R	WT	Non-WT
AMB	---	---	---	---	≤2	>2
ANID	≤0.25	---	0.5	≥1	≤0.125	>0.125
CAS	≤0.25	---	0.5	≥1	≤0.25	>0.25
MICA	≤0.25	---	0.5	≥1	≤0.125	>0.125
FLU	---	---	---	---	≤64	>64
POS	---	---	---	---	≤0.5	>0.5
VORI	<0.5	---	1	≥2	≤0.5	>0.5
ITRA	---	---	---	---	≤1	>1
5FC	---	---	---	---	≤32	>32

M27-A3 Breakpoints (24h) *Candida guilliermondii*

	S	SDD	I	R	WT	Non-WT
AMB	---	---	---	---	≤2	>2
ANID	<2	---	4	>8	≤4	>4
CAS	<2	---	4	>8	≤1	>1
MICA	<2	---	4	>8	≤4	>4
FLU	---	---	---	---	≤8	>8
POS	---	---	---	---	≤0.5	>0.5
VORI	---	---	---	---	≤0.25	>0.25
ITRA	---	---	---	---	≤1	>1
5FC	---	---	---	---	≤1	>1

M27-A3 Breakpoints (24h) *Candida dubliniensis*

	S	SDD	I	R	WT	Non-WT
AMB	---	---	---	---	≤2	>2
ANID	---	---	---	---	≤0.12	>0.12
CAS	---	---	---	---	≤0.12	>0.12
MICA	---	---	---	---	≤0.12	>0.12
FLU	---	---	---	---	≤0.5	>0.5
POS	---	---	---	---	≤0.12	>0.12
VORI	---	---	---	---	≤0.03	>0.03
ITRA	---	---	---	---	≤0.25	>0.25
5FC	---	---	---	---	≤0.5	>0.5

M27-A3 Breakpoints (24h) *Candida kefyr*

	S	SDD	I	R	WT	Non-WT
ANID	---	---	---	---	≤0.25	>0.25
CAS	---	---	---	---	≤0.03	>0.03
MICA	---	---	---	---	≤0.12	>0.12
FLU	---	---	---	---	≤1	>1
POS	---	---	---	---	≤0.25	>0.25
VORI	---	---	---	---	≤0.015	>0.015

M27-A3 Breakpoints (24h) *Candida orthopsilosis*

	S	SDD	I	R	WT	Non-WT
ANID	---	---	---	---	≤2	>2
CAS	---	---	---	---	≤0.5	>0.5
MICA	---	---	---	---	≤1	>1
FLU	---	---	---	---	≤2	>2
POS	---	---	---	---	<0.25	>0.25
VORI	---	---	---	---	≤0.06	>0.06

M27-A3 Breakpoints (24h) *Candida pelliculosa*

	S	SDD	I	R	WT	Non-WT
CAS	---	---	---	---	≤0.12	>0.12
FLU	---	---	---	---	≤4	>4
POS	---	---	---	---	≤2	>2
VORI	---	---	---	---	≤0.25	>0.25

M27-A3 Proposed ECVs (72h) *Crytococcus neoformans*

	S	SDD	I	R	WT	Non-WT
FLU	---	---	---	---	≤8	>8
POS	---	---	---	---	≤0.25	>0.25
5FC (48h)	---	---	---	---	≤0.125	>0.125

Absolute categorical agreement and error rates when the azole surrogate fluconazole result was used to predict voriconazole susceptibility of *Candida spp*

Organism(s) tested	No. of isolates	%[a]: Agreement	VME	ME	Minor errors
All *Candida*	13,338	91.6	0.0	1.4	7.0
All *Candida* minus *C. krusei*	13,026	93.6 (97.7)	0.0 (0.1)	0.5 (1.4)	5.9 (0.8)
C. albicans	7,725	99.1	0.0	0.3	0.6
C. glabrata	1,966	66.1 (93.9)	0.0 (0.1)	1.5 (1.5)	32.4 (4.5)
C. parapsilosis	1,623	97.4	0.0	0.3	2.3
C. tropicalis	1,253	99.1	0.0	0.2	0.7
C. krusei	312	3.2	0.0	39.7	57.1
C. lusitaniae	134	97.8	0.0	0.8	1.4
C. dubliniensis	103	93.2	0.0	1.0	5.8
C. guilliermondii	92	91.3	0.0	0.0	8.7
C. pelliculosa	34	100	0.0	0.0	0.0
C. kefyr	33	100	0.0	0.0	0.0
C. famata	19	73.7 (100)	0.0	0.0	26.3 (0.0)
C. rugosa	19	100	0.0	0.0	0.0

[a] Values in parentheses are categorical agreement and error rates obtained using the following categories for fluconazole: susceptible, MIC of ≤32 μg/ml (S and SDD combined); resistant, MIC of ≥64 μg/ml.

Pfaller MA, et al. JCM 2007;45:70-5

M38-A2 (BMD for Mould)

- Inoculum 530 nm wavelength 0.5-4 x 10^4
- 80-82% T (OD 0.09-0.13): *Aspergillus spp., Paecilomyces spp, Exophiala dermatitidis, Sporothrix spp.*
- 68-70% T (OD 0.15-0.17)*: Fusarium spp,. Scedosporium spp., zygomycetes*
- 50-55% T (OD 0.25-0.3): *Bipolaris spp., Alternaria spp., Curvularia spp.*
- Dermatophytes – 1-3 x 10^3
- Conidia counted in hemocytometer

M38-A2

- Incubation - 35°C
  - Echinocandins – 24 hours despite species
  - If growth is not apparent at 24 then read at 48
  - Zygomycetes – 24 hours for all drugs
  - *Aspergillus spp., Fusarium spp. etc. – all non-candin drugs – 48 hours*
  - Species with insufficient growth @ 48 should be read @ 72 hours
  - Dermatophytes – 96 hours

M38-A2

- End Points
  - Non-dermatophytes AMB, ITRA, POSA, VORI
    - 100 % Inhibition
  - Dermatophytes ITRA, POSA, VORI
    - 80% Inhibition
  - Candins
    - MEC (minimum effective concentration)
  - FLU, KETO, 5FC
    - 50% Inhibition

Reason of Antifungal susceptibility testing

- To provide a reliable estimate of the relative activities of two or more antimicrobial agents against the pathogen of interest
- To correlate with *in vivo* activity and to predict the likely outcome of therapy
- To provide a quantitative means by which to survey the development of resistance among members of a normally susceptible population of organisms
- To predict the therapeutic potential and spectrum of activity of newly developed investigational agents.

CLSI vs EUCAST breakpoint (BP) establishing procedure

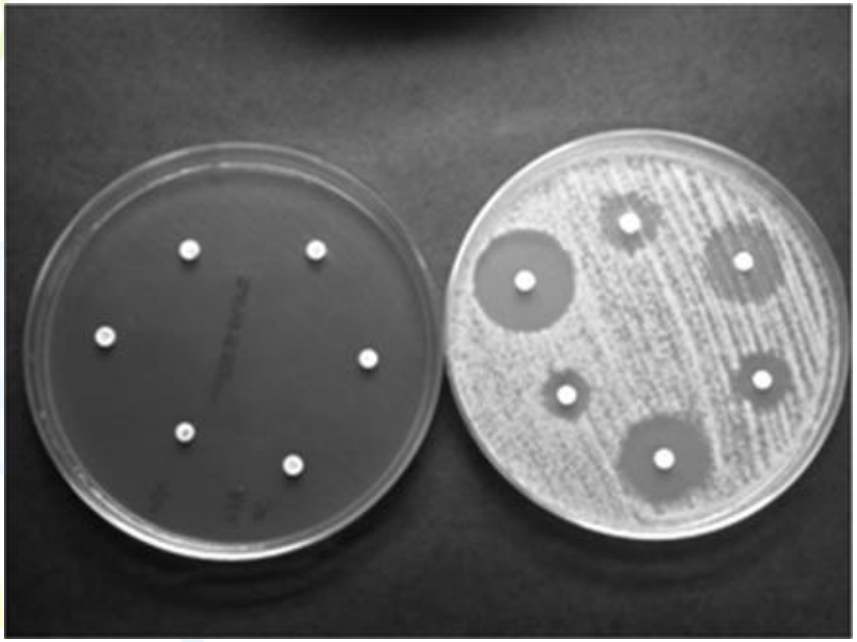
	CLSI	EUCAST
MIC distributions	Cumulative % per species	Several data sets Epidemiological cutoffs (ECV) per species
PK/PD	From animal models or humans	Target validated by means of **Montecarlo simulations**
MIC/Clinical outcome relationships	Rule "90-60"	•Data mining •BP never higher than **ECVs** unless supported by clinical data

From Arendrup.

Steps of In Vitro MIC test—Inoculum preparation in CLSI method

- Use the overnight cultured colony
- Adjust the turbidity to 90±1 (blank,100; 85=0.5McFarland)
- Take 10 uL and add to 5 mL RPMI 1640 medium
- Final conc.: 1-5 $x10^3$ cell/mL

BIOMIC reader system



Criteria for Fluconazole
S: zone diameter of **19** mm;
SDD: zone diameter of **15** to **18** mm;
R: zone diameter of **14** mm