Antifungal susceptibility testing: the EUCAST approach

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Research grants & Speaker: Astellas, Gilead, MSD & Pfizer;
Advisory board: MSD, Pcovery, Pfizer; Acted as consultant for: Alcimed, Astellas, Gilead & Pfizer
Chair(wo)man for EUCAST-AFST
Advisor for CLSI-AFST

Temperature check

Which method do you use when testing *Candida?*

1. EUCAST microdilution
2. CLSI microdilution
3. Commercially available method (e.g. Etest, Vitek, Sensititre)
4. We refer to a reference laboratory
5. Susceptibility testing of *Candida* is not necessary

Temperature check

Which method do you use when testing *Aspergillus* ?

1. EUCAST microdilution
2. CLSI microdilution
3. Commercially available method (e.g. Etest, Vitek, Sensititre)
4. We refer to a reference laboratory
5. Susceptibility testing of *Aspergillus* is not necessary

EUCAST Susceptibility Testing

Reference Methods

- Yeast
  - **E.DEF 7.2 (2012)**
  - **TN- E.DEF 7.2 (In press)**
  - E.DEF 7.1 (2008)
  - TN- E.DEF 7.1 (2008)
- Conidia forming moulds
  - E.DEF 9.1 (2008)
  - TN-E.DEF 9.1 (2008)

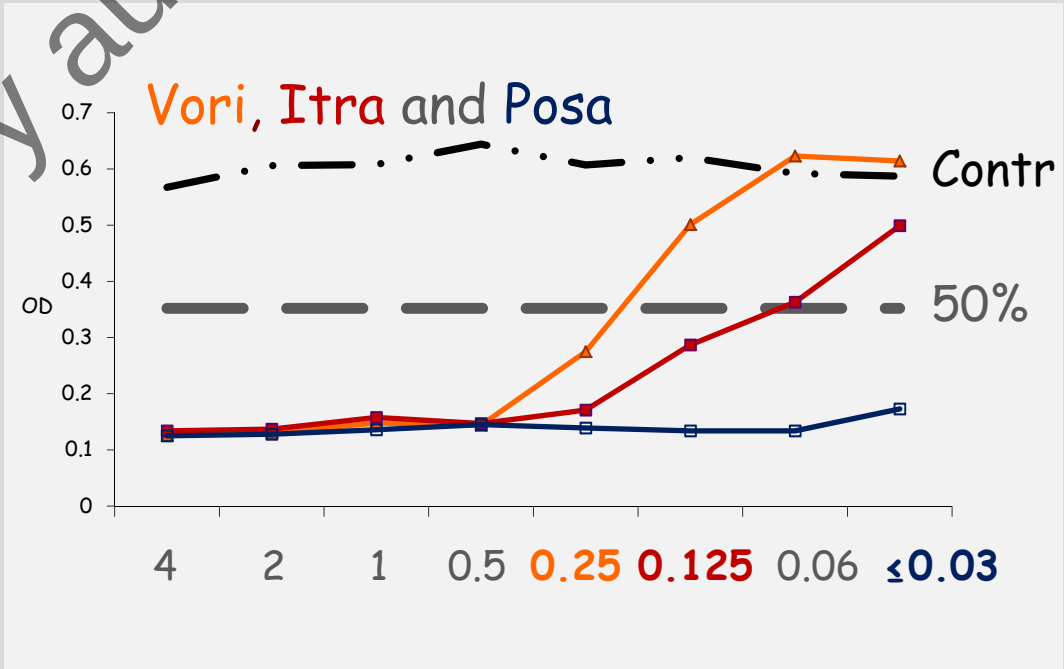
Breakpoints

Compound	*Candida*		*Aspergillus*	
	Rationale Doc	Techn. Note CMI	Rationale Doc	Techn. Note CMI
Amphotericin	2010	**2011**	**2012**	**Submitted ***
Anidulafungin	2010	**2011**		
Fluconazole	2007	2008	-	-
Itraconazole			**2012**	**Submitted ***
Posaconazole	2010	**2011**	**2012**	**Submitted ***
Voriconazole	2008	2008	**Discussion Doc.**	

Testing Yeast: EUCAST E.DEF 7.2

- 2 fold drug dilutions (0.1 ml/well)
- 1:10 dilution of a McFarland 1
  - 1-5 x 10^5 CFU/ml; 0.1 ml /well
- 24 h incubation

- Spectrophotometer
  - Output in excel-format
  - Automated calculation of 50% endpoint
  - Objective MIC reading



Testing Moulds: EUCAST E.DEF 9.1

	EUCAST E.DEF 9.1
Glucose	2%
Inoculum size	0.5-1.25 x 10^5 (counted)
Plates & Reading	Flat bottom & Visual
Incubation time	48 (24-72) h
Endpoint	No growth (echinocandins MEC, macroscopic endpoint)

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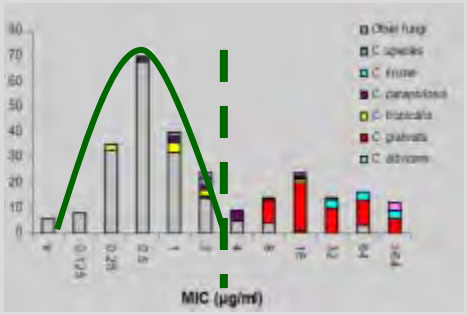
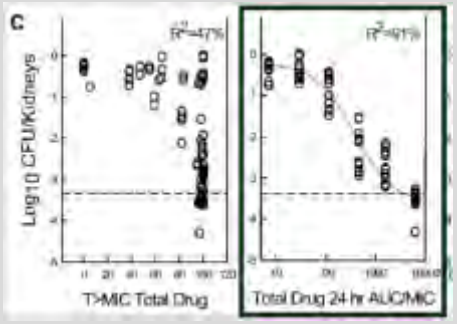
EUCAST BP establishing procedure

- MIC distributions
  - Per species
  - Several data sets
  - Epidemiological Cut Off Value (ECOFF)
- MIC-clinical outcome relationships
  - Per species
  - For wild type and non-wild type isolates
- PK/PD
  - BP never higher than ECOFF unless supported by data



MIC in mg/L	Candidaemia		OPC ≥ 100 mg/d		All
	No. cure/Total	% response	No. cure/Total	% response	% response
≤ 0.5	98/107	92	26/26	100	93
1	6/6	100	4/4	100	100
2	1/1	100	1/1	100	100
4	3/3	100	5/9	56	67
8	2/5	40	7/32	22	24
≥16	3/4	75	0/60	0	5



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Q 3: Breakpoints for *Candida*

Which set of breakpoints have you adopted for *Candida* interpretation?

1. EUCAST
2. CLSI M27S-3
3. Revised CLSI breakpoints as published in the scientific literature
4. National breakpoints different from the above
5. In house breakpoints

AMB & Azole breakpoints for *Candida* spp

Breakpoints (BPs): S: ≤X; R: >Y **Revised BPs**

	CLSI M27-S3	CLSI Revised 2010/11	EUCAST
AMB	≤1	≤1	≤1; >1
Fluco	≤8; >32	≤2; >4 (*alb, para, trop*) SDD ≤32; R >32 (*glab*) (*krus* poor target)	≤2; >4 (*albi, trop, para*) (*glab* IE) (*krus* poor target)
Itra	≤0.125; >0.5	≤0.125; >0.5	-
Posa	-	-	≤0.06; >0.06 (*alb, trop, para*) (*glab/krus* IE)
Vori	≤1; >2	≤0.125; >0.5 (*alb, para, trop*) ≤0.5; >1 (*krus*) (*glab* IE)	≤0.125; >0.125 (*alb, trop, para*) (*glab/krus* IE)

www.eucast.org; Pfaller Drug Resist Updat. 2010 & 2011; www.clsi.org

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EUCAST Fluconazole MIC & outcome

Fluconazole 258 cases
(128 candidaemia 58% *C. albicans*; 133 OPC all *C. albicans*)

MIC in mg/L	Candidaemia		OPC ≥ 100 mg/d		**All**	
	No. cure/Total	% response	No. cure/Total	% response	**% response**	
< 0.5	98/107	92	26/26	100	**93**	
1	6/6	100	4/4	100	**100**	S
2	1/1	100	1/1	100	**100**	
4	3/3	100	5/9	56	**67**	I/SDD
8	2/5	40	7/32	22	**24**	
≥16	3/4	75	0/60	0	**5**	R

Rodriguez-Tudela AAC 2007

Outcome Triangle

FUNGUS

- Virulence
- Susceptibility

HOST

- Immune status
- Severity/duration of infection at the start of therapy

DRUG PK/PD & Timing

- C_{max}, $T\frac{1}{2}$, AUC
- Concentration at site

S → higher likelihood of success
Some pts will die despite appropriate treatment

R → higher likelihood for failure
Some pts will get well despite inappropriate treatment

Outcome Triangle

FUNGUS

- Virulence
- Susceptibility

HOST

- Immune status
- Severity/duration of infection at the start of therapy

DRUG PK/PD & Timing

- C_{max}, $T\frac{1}{2}$, AUC
- Concentration at site

S → **S**imilar to other pts

IR → **I**ncreased **R**isk
Intelligence **R**equired !!!

Q 4: echinocandin susceptibility

My *C. glabrata* is classified as "S" (susceptible) if

1. The anidulafungin MIC is ≤0.06 mg/L
2. The caspofungin MIC is ≤2 mg/L
3. The micafungin MIC is ≤0.25 mg/L
4. None of these

Echinocandin breakpoints for *Candida* spp

Breakpoints (BPs): S: ≤X; R: >Y

	CLSI M27-S3	CLSI Revised 2011 (Revised BPs)	EUCAST
ANF	≤2	≤0.25; >0.5 (*alb, krus, trop*) ≤0.125; >0.25 (*glab*)	≤0.032; >0.032 (*alb*) ≤0.06; >0.06 (*glab, krus, trop*) (*para* not a good target) (*guillier* IE)
CSF	≤2	≤2; >4 (*para, guillier*)	-
MFG	≤2	≤0.25; >0.5 (*alb, krus, trop*) ≤0.06; >0.125 (*glab*) ≤2; >4 (*para, guillier*)	-

www.eucast.org; Pfaller Drug Resist Updat. 2010 & 2011; www.clsi.org

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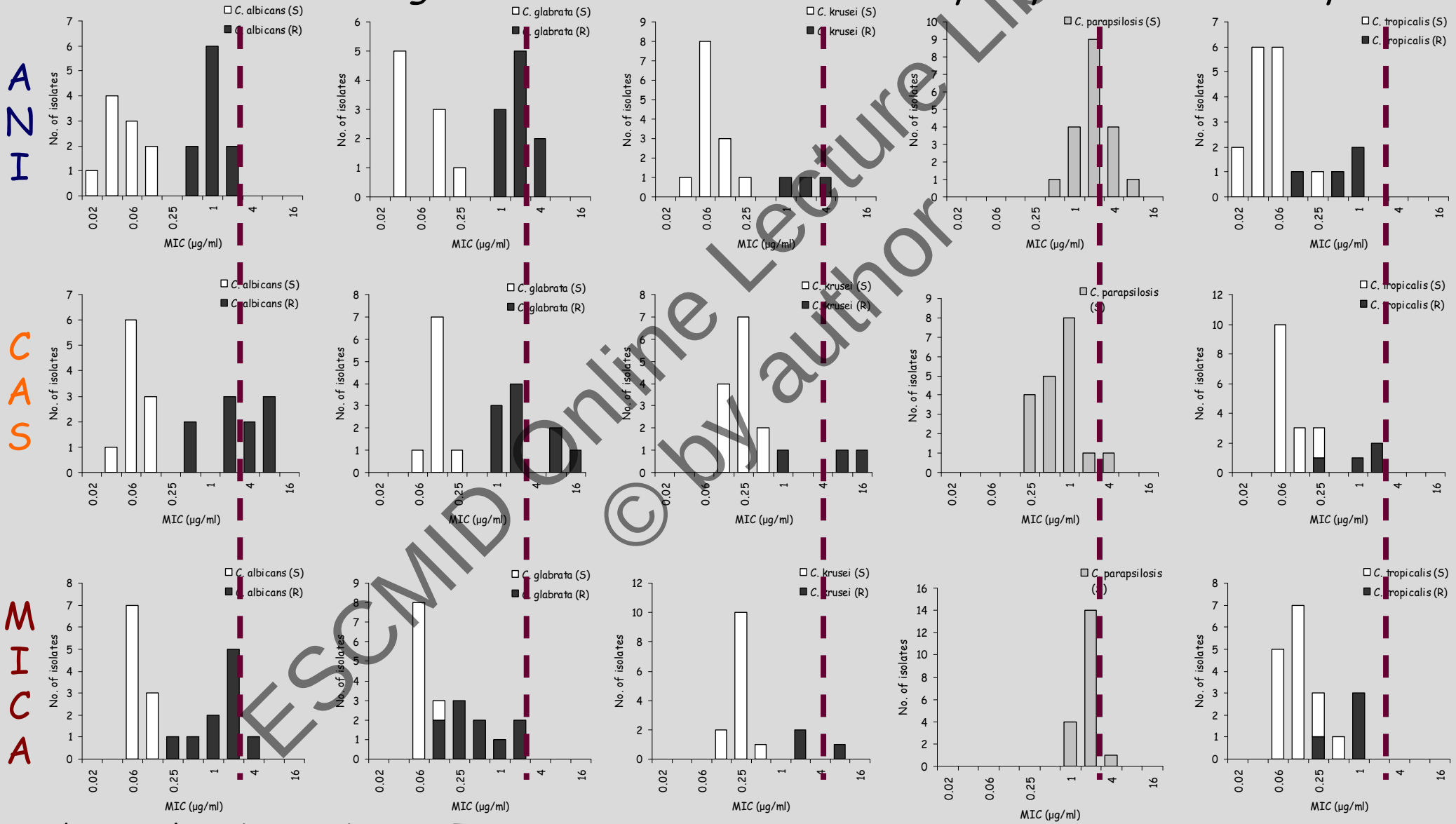
CLSI echinocandin MICs "M27-S3 BP"



Arendrup et al AAC 2010, CLSI M27-S3

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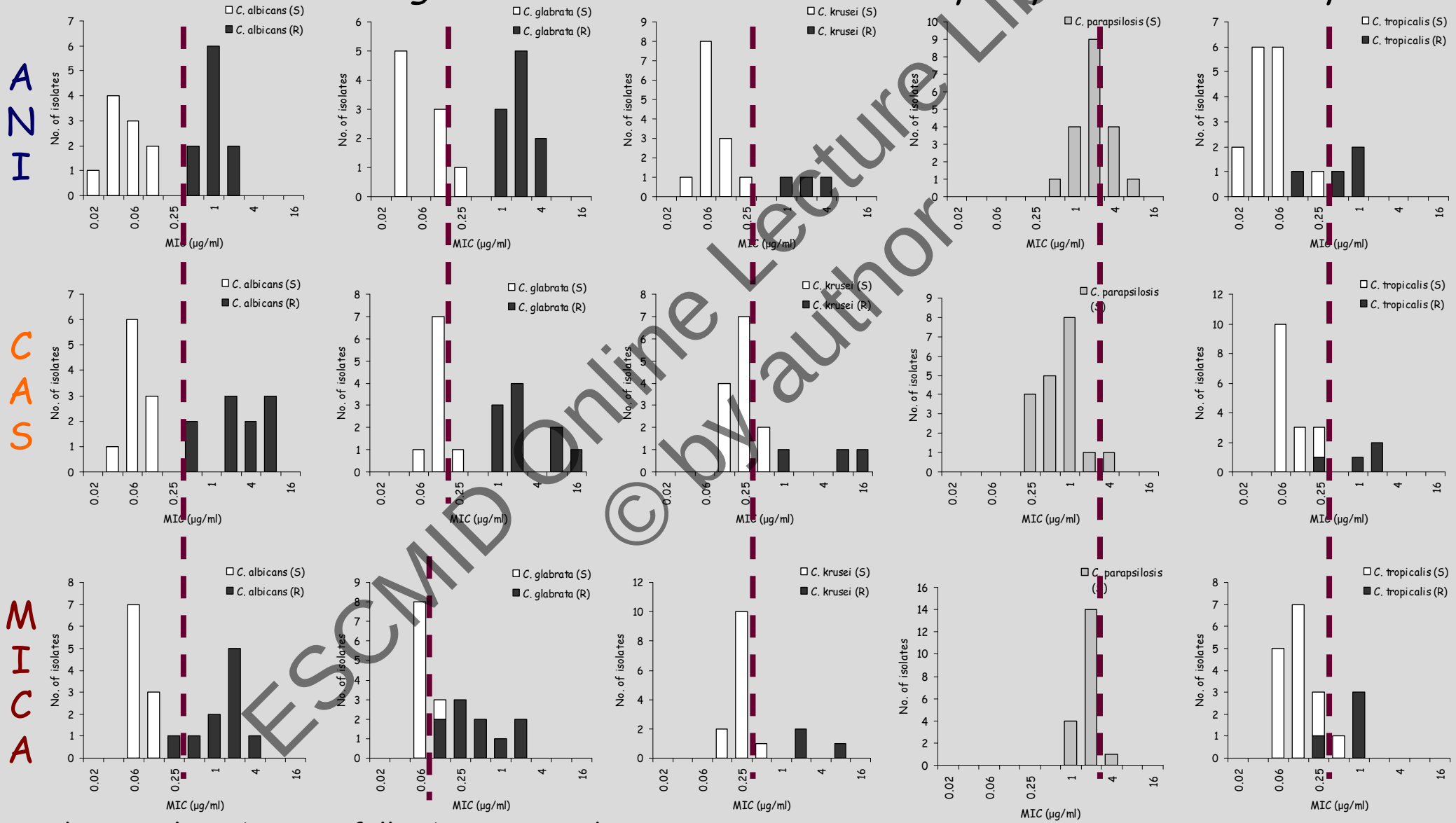
CLSI echinocandin testing – revised BP



Arendrup et al AAC 2010, Pfaller Drug res updates 2011

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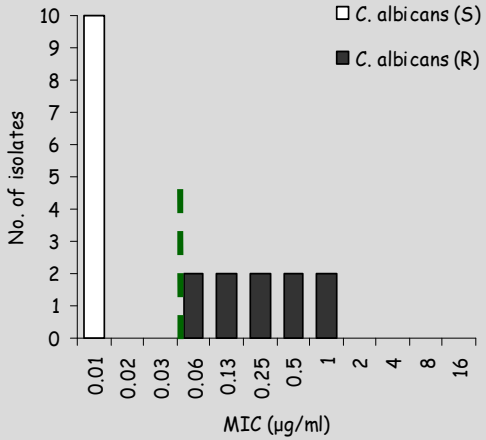
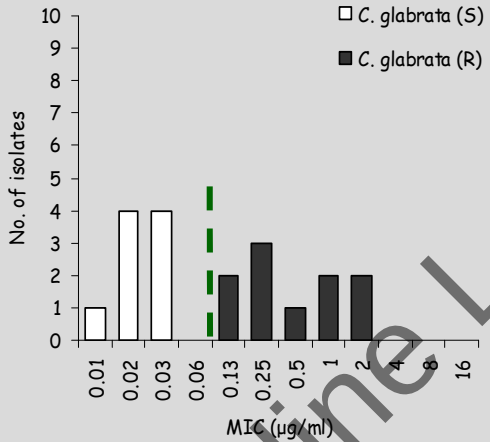
EUCAST Anidulafungin WT vs. resistant

C. albicans



C. glabrata

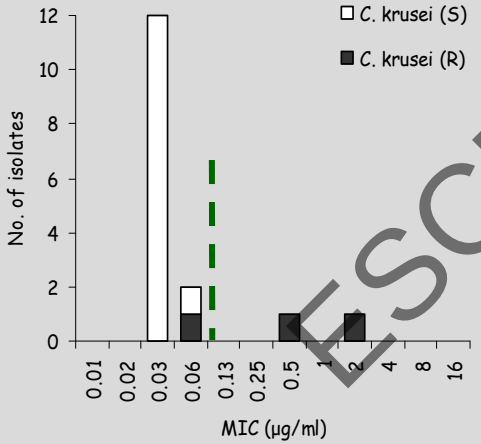


Wild type isolates

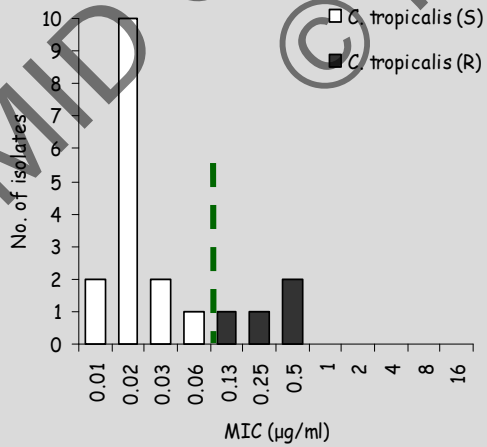
Resistant isolates

EUCAST BP

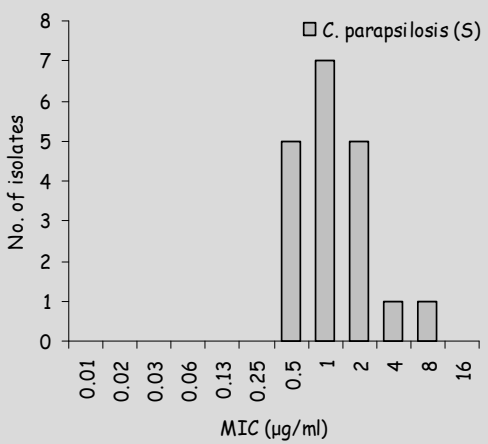
C. krusei



C. tropicalis



C. parapsilosis



Arendrup et al AAC 2010 and EUCAST-AFST

EUCAST *Aspergillus* BPs 2012

BPs indicated as S ≤x / R >y

AF compound	*Aspergillus*				
	flavus	*fumigatus*	*nidulans*	*niger*	*terreus*
Amphotericin	IE*	1/2	Note	1/2	Poor Target
Itraconazole	1/2	1/2	1/2	IE*	1/2
Posaconazole	IE*	0.125/0.25**	IE*	IE*	Note
Voriconazole	Note	1/2	Note	Note	Note

* MICs are higher than for *A. fumigatus*

** provided sufficient levels can be achieved

Note: the MICs are similar to *A. fumigatus* but insufficient clinical data for BP setting

Rationale documents available at www.eucast.org

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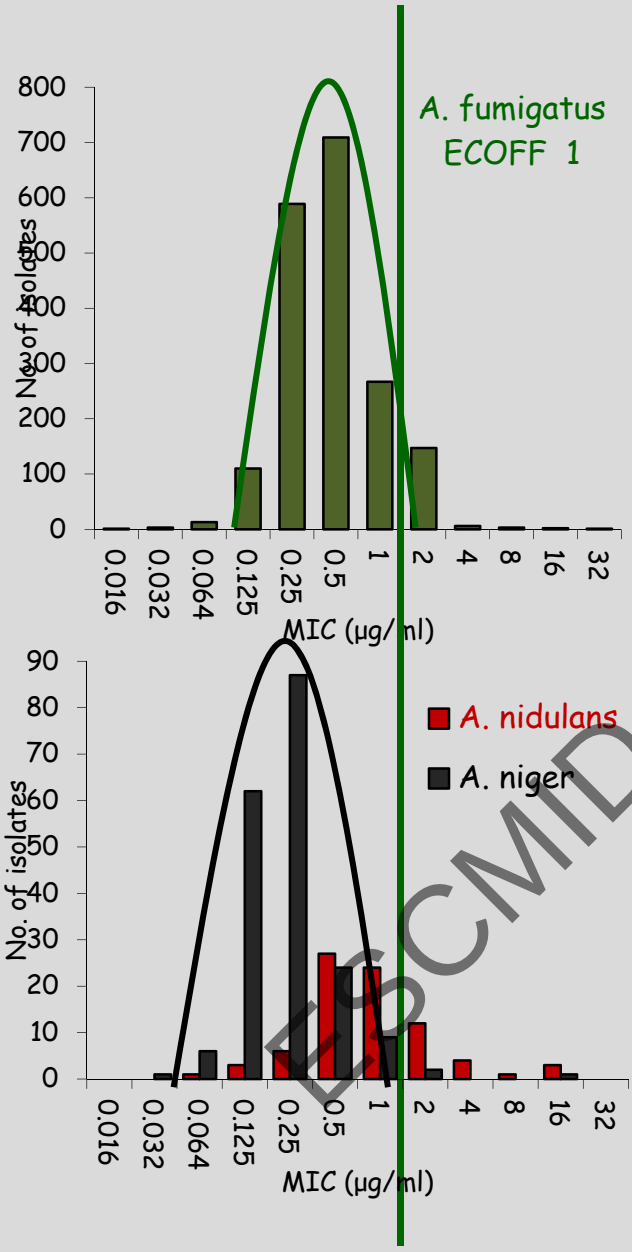
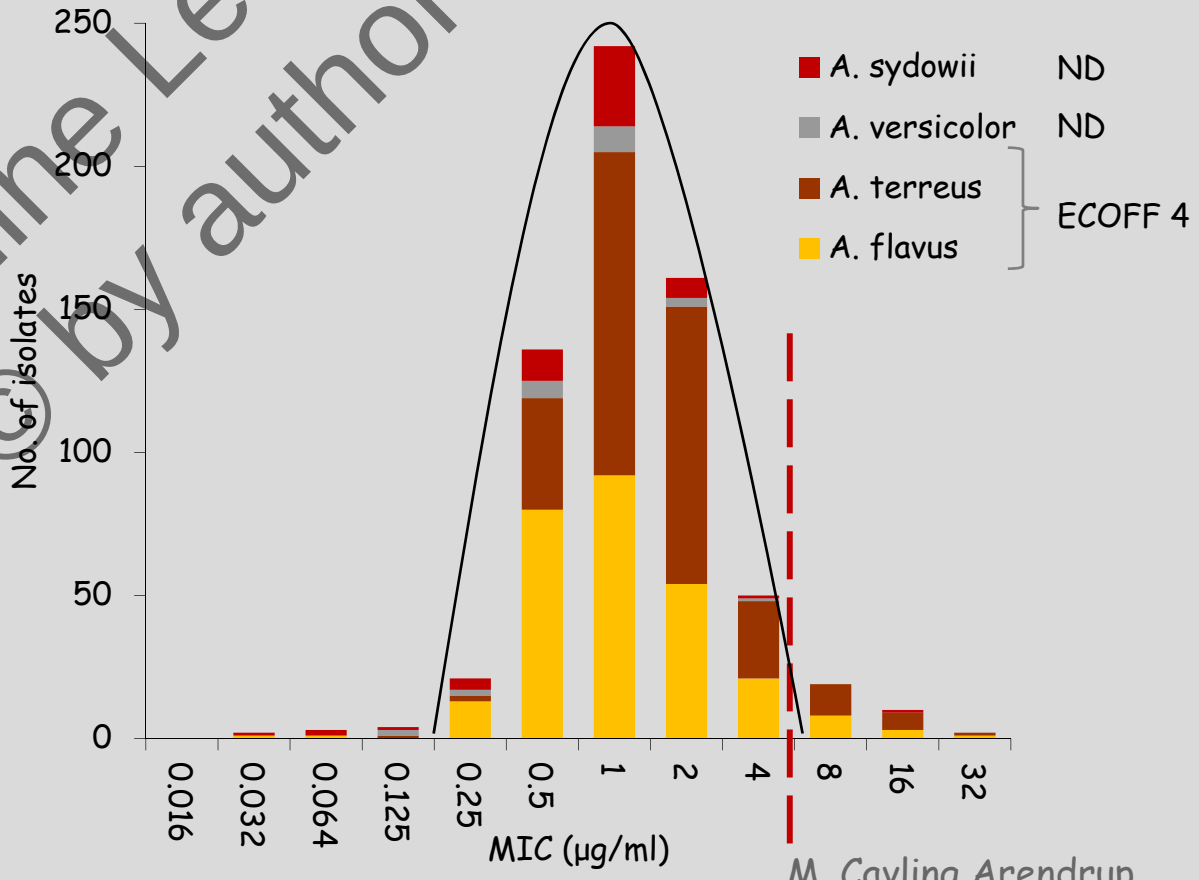
Aspergillus and Amphotericin B



- *Aspergillus* good target (except *A. terreus*), but most experience is with *A. fumigatus*.
- No MIC - clinical outcome or animal PK/PD data



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EUCAST *Aspergillus* BPs 2012

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Itraconazole	1/2	1/2	1/2	IE*	1/2
Posaconazole	IE*	0.125/0.25**	IE*	IE*	Note
Voriconazole	Note	1/2	Note	Note	Note

* MICs are higher than for *A. fumigatus*

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Rationale documents available at www.eucast.org

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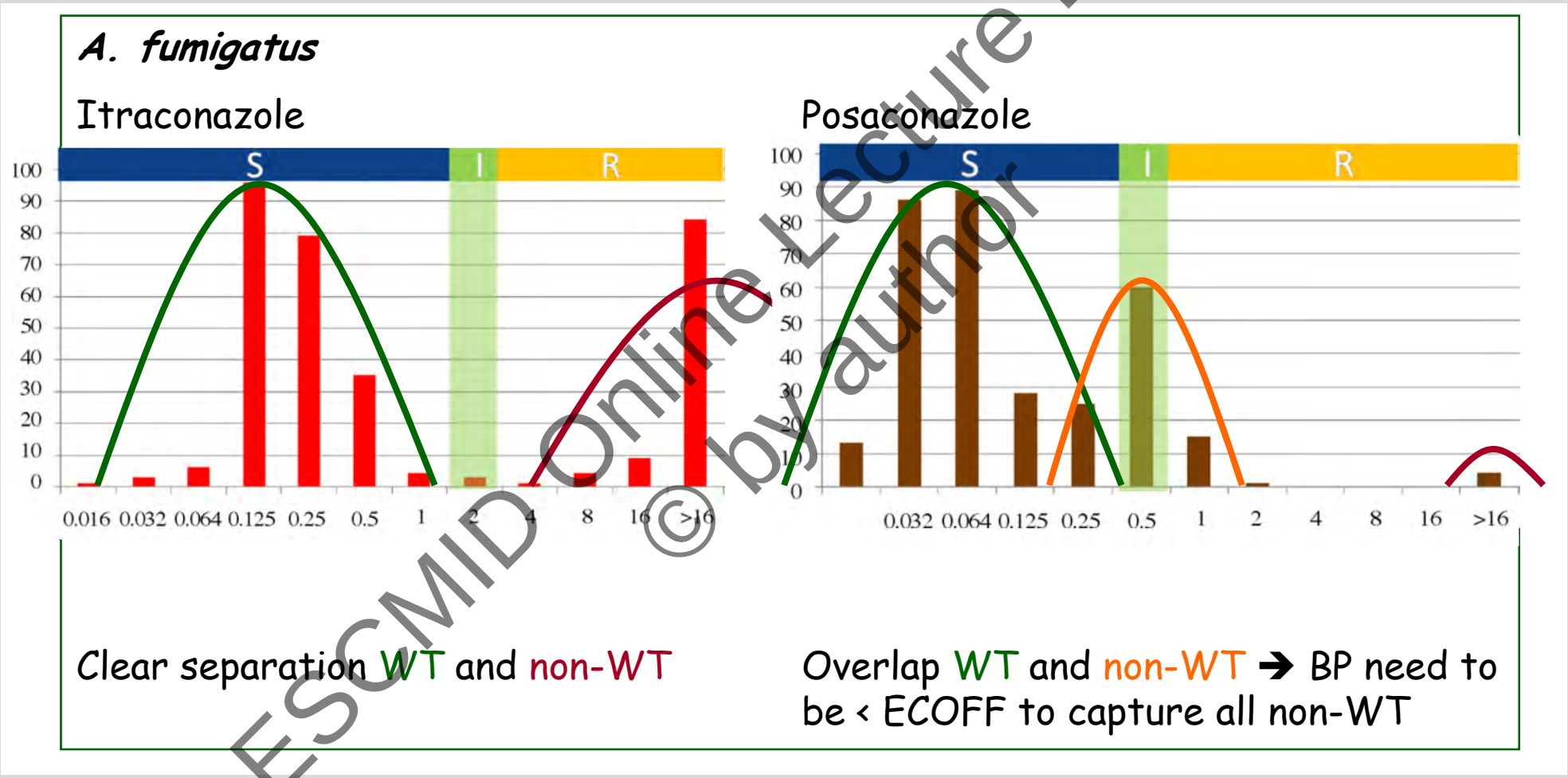
Posaconazole ECOOF: Overlap WT & non-WT

8 Individual dataset for *A. fumigatus*: MIC_{50} ~0.1 mg/L; ECOFF 0.25 mg/L

	MIC (mg/L)												
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32
EUCAST		7	70	**384**	**205**	55	12	3	3	3	4		
EUCAST			5	**28**	**50**	11	4		1		1		
EUCAST	3	1	7	5	6	8	3	1			3		
EUCAST			113	**116**	**128**	100	46	16	2		5		
EUCAST			6	49	**163**	57	7	6	2		1	14	
EUCAST		5	12	**98**	**77**	48	44	69	18	1	2	1	6
EUCAST 24h					1	3	15	1					
CLSI			21	1	73	87	1	1					

2095 MICs

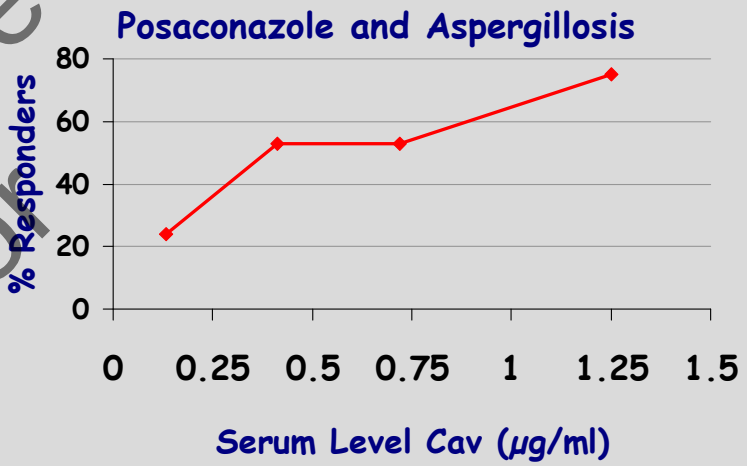
Overlap between WT and non-WT



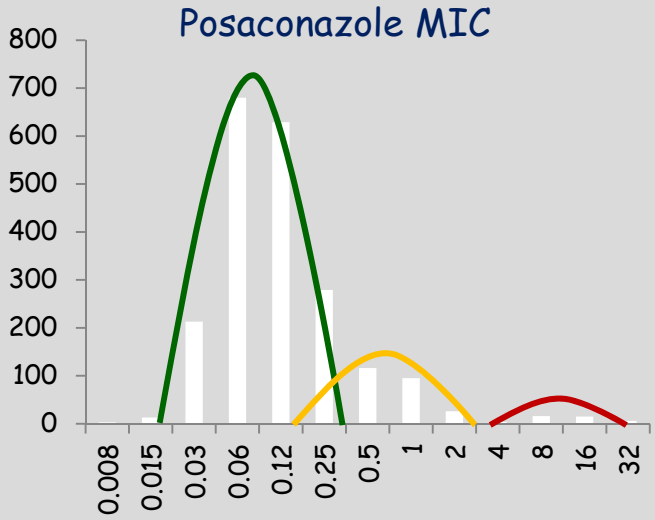
Verweij Drug Res Updates 2009

Clinical data & animal PK/PD

- Borderline exposure with the current formulation
  - C_{av} of 1.25 mg/L → better outcome
  - C_{av} of 1.25 mg/L ~ AUC of ~30 mg.h/L



- Mouse models
  - AUC:MIC of 167 → ED_{50} (include 0.125)
    - GM as endpoint, neutropenic model
  - AUC:MIC of 498 → ED_{50} (include 0.06)
    - Survival as endpoint, non-neutropenic model
  - Poorer outcome for *cyp51A* mutants
    - some of which may have an MIC of 0.25 mg/L



Walsh CID 2007; Howard JID 2011; Mavridou AAC 2010; Verweij DRU 2009

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Conservative Posaconazole BP

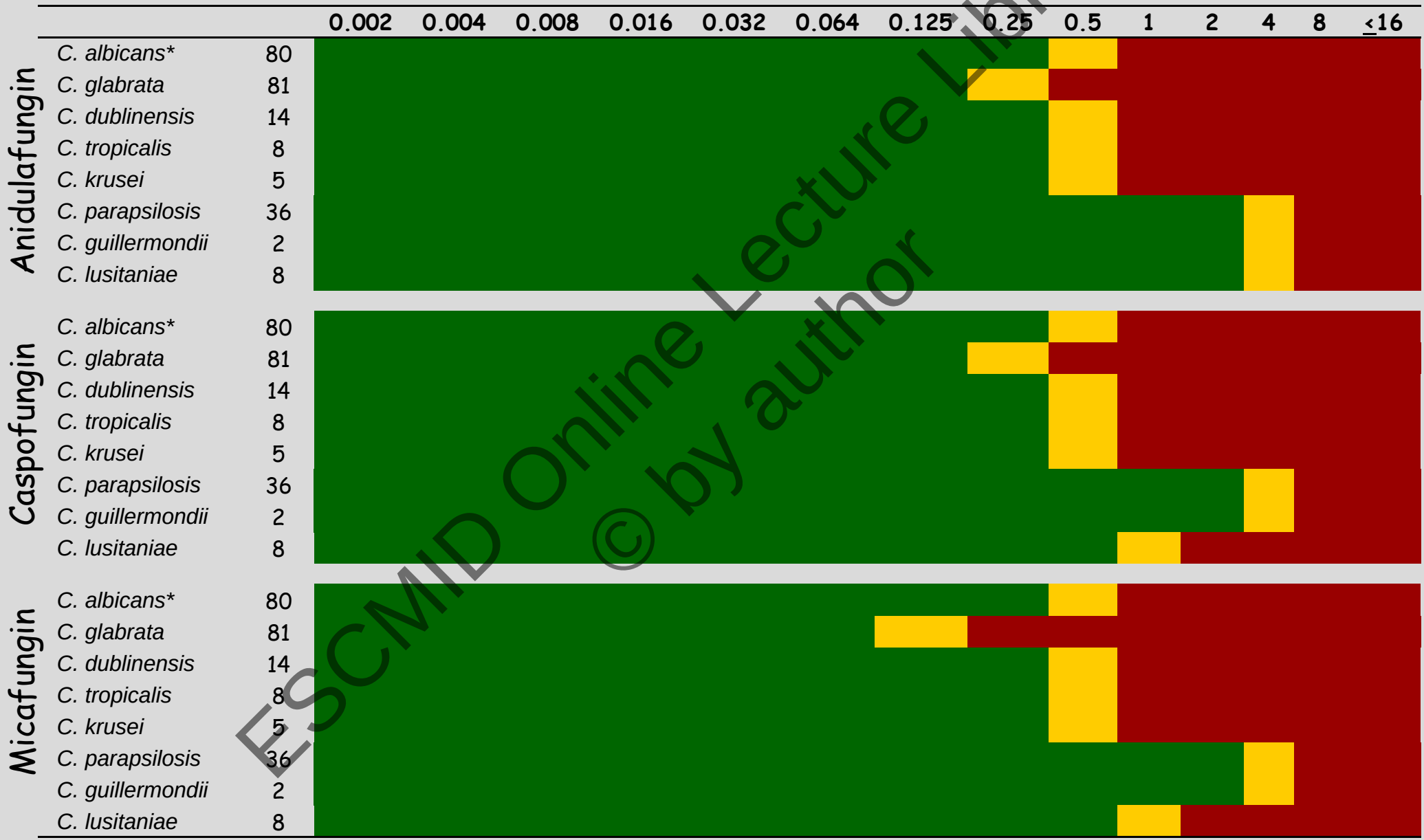
- *Aspergillus fumigatus*
  - S ≤0.125, R >0.25 mg/L
    - Provided adequate drug exposure has been confirmed using therapeutic drug monitoring
  - TDM targets:
    - >1 mg/L at steady state for salvage treatment
    - >0.7 mg/L for prophylaxis
- App. 15% WT may be classified as non-S
- Itraconazole "S" → probably also posaconazole "S"

Jang Clin Pharmacol Ther. 2010; FDA; soon at www.EUCAST.org

Commercial testing methods

- Interpretation of endpoints
  - Caveats applying CLSI or EUCAST breakpoints
  - Echinocandins
    - Etest
    - Vitek

Echinocandin Etest & CLSI BP



Axner-Elings JCM 2011

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Echinocandin Etest & CLSI BP

		0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	<16
Anidulafungin															
*C. albicans**	80	**43**	31	5	1										
C. glabrata	81		8	**67**	6										
C. dublinensis	14	2	**11**	1											
C. tropicalis	8			2	**5**						1				
C. krusei	5				**5**										
C. parapsilosis	36						1				14	**11**	6	1	3
C. guillermondii	2												1	1	
C. lusitaniae	8				**7**	1									
Caspofungin															
*C. albicans**	80				17	**29**	26	6	2						
C. glabrata	81						23	**58**							
C. dublinensis	14				2	**7**	5								
C. tropicalis	8						2	**5**					1		
C. krusei	5								**3**	2					
C. parapsilosis	36								16	**20**					
C. guillermondii	2									1	1				
C. lusitaniae	8								**6**	2					
Micafungin															
*C. albicans**	80		36	**38**	6										
C. glabrata	81		4	**74**	3										
C. dublinensis	14		6	**7**	1										
C. tropicalis	8			2	**5**					1					
C. krusei	5					1	**4**								
C. parapsilosis	36							2	8	**24**	2				
C. guillermondii	2									2					
C. lusitaniae	8				1	**6**	1								

Axner-Elings JCM 2011

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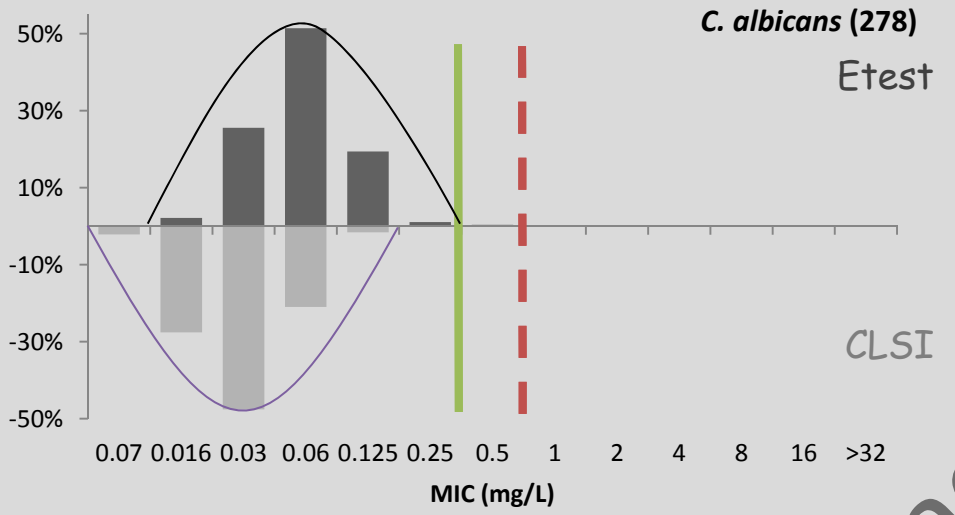
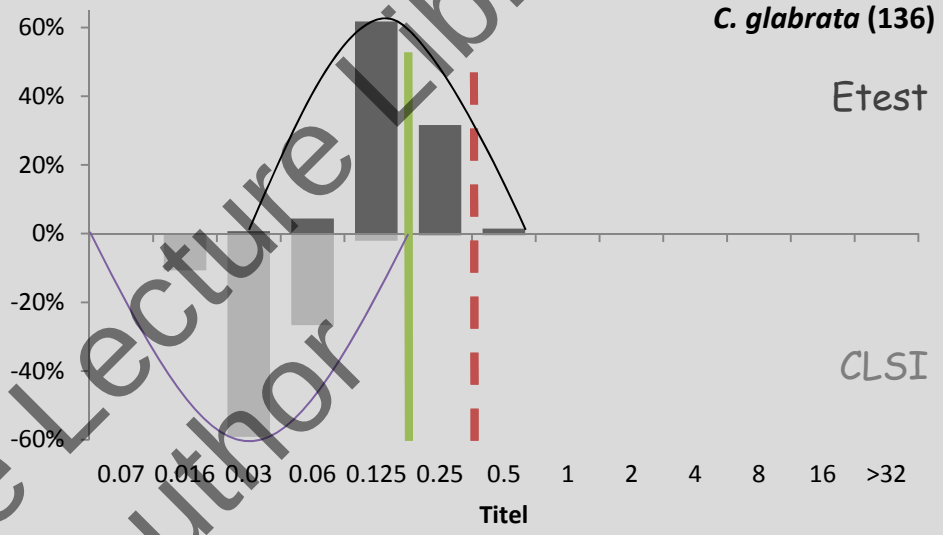
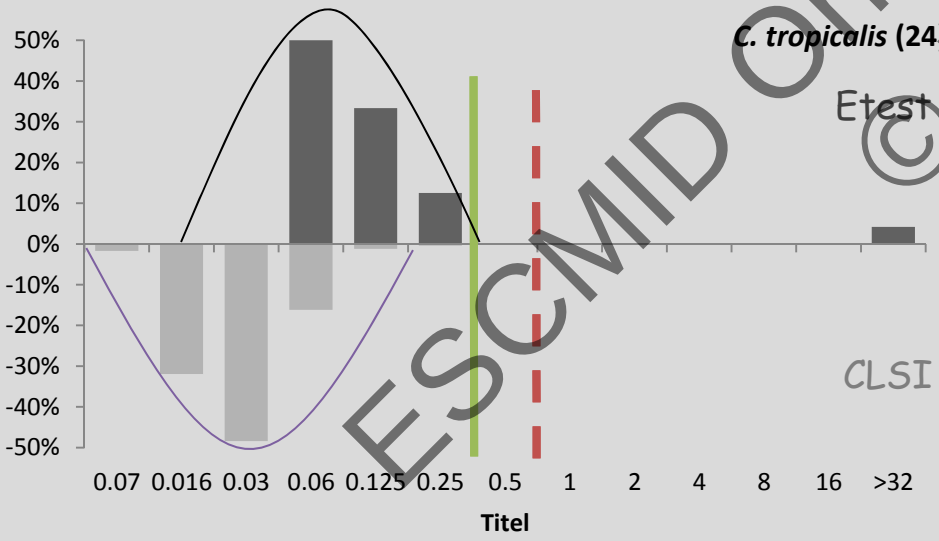
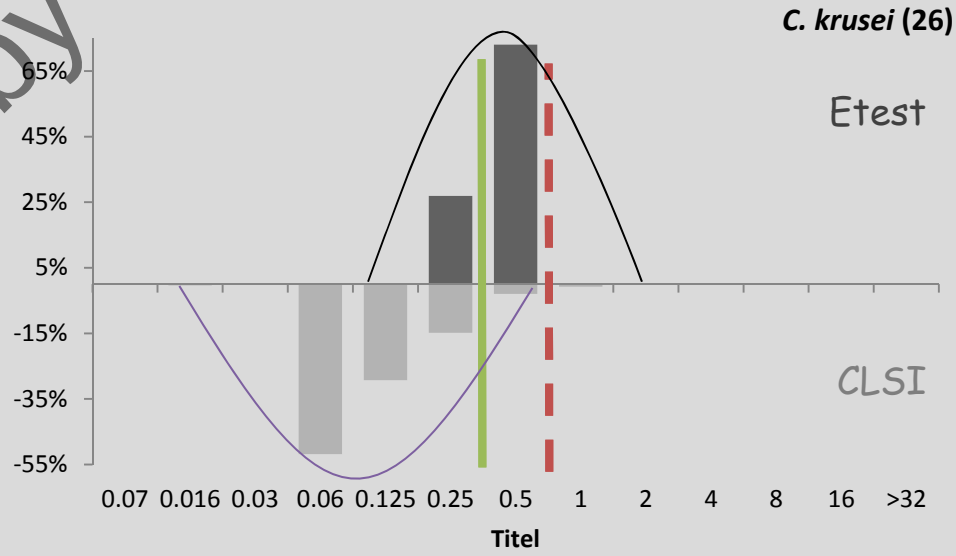
Etest: Caspofungin and CLSI BP









Arendrup & Pfaller submitted

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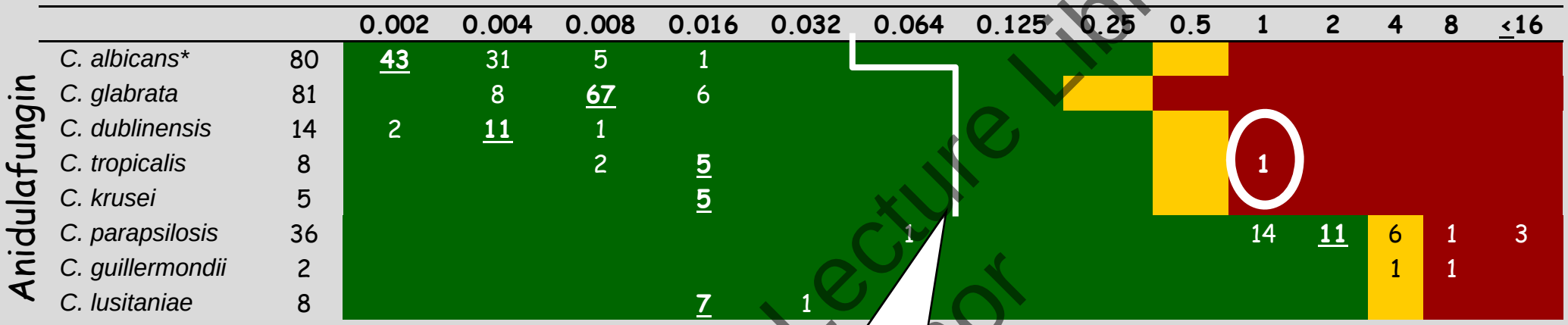
Anidulafungin Etest: EUCAST vs CLSI BP



EUCAST anidulafungin BP

Axner-Elings JCM 2011

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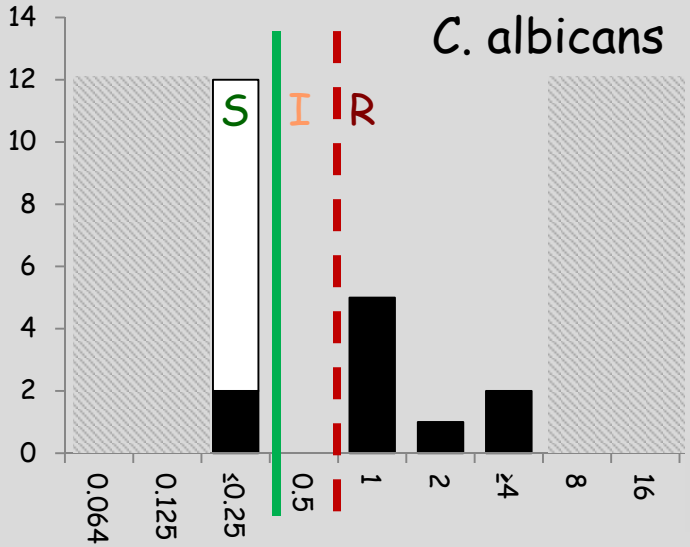
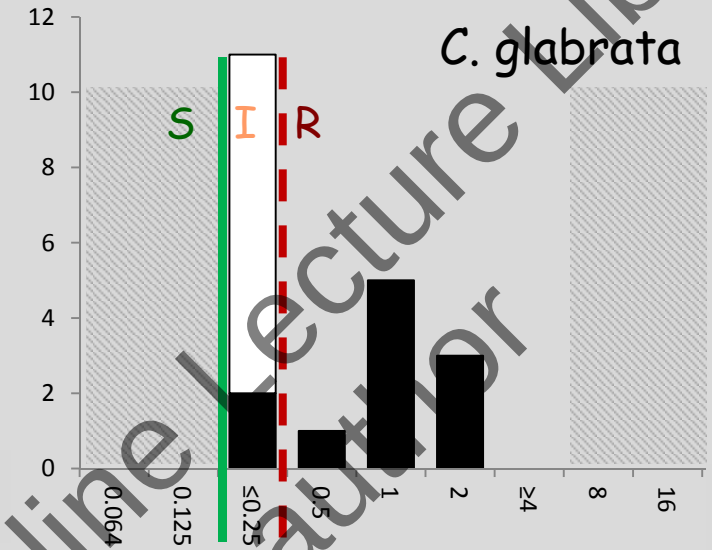
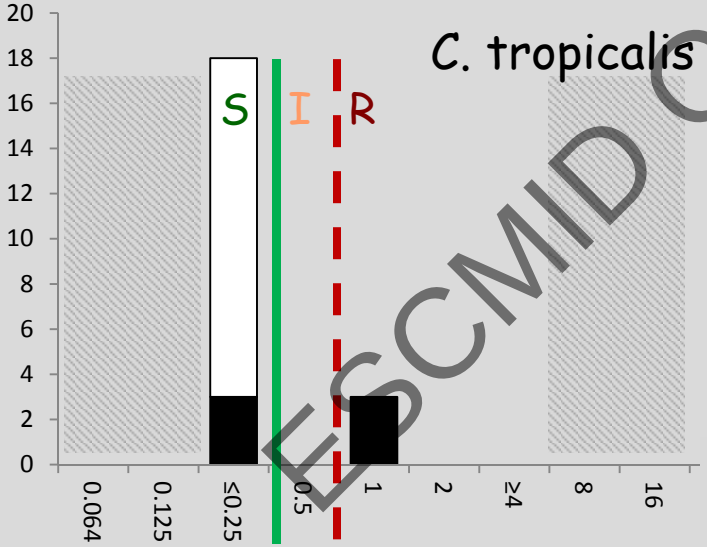
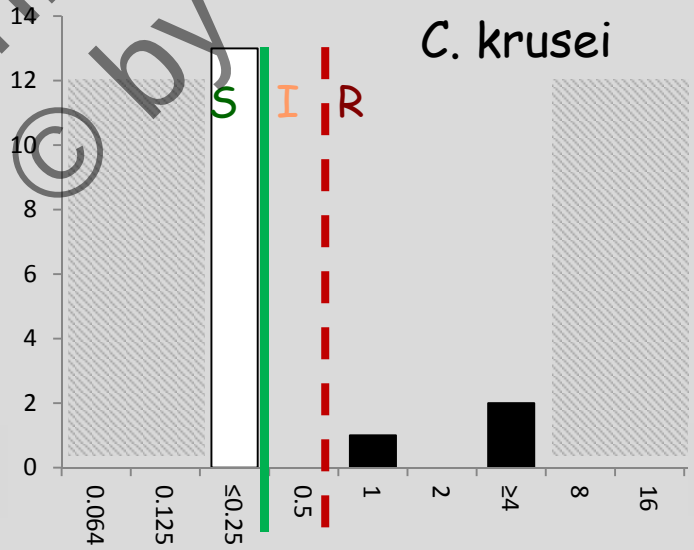
Vitek Caspofungin









FKS mutants → "S"
Vitek: 23% (7/30)*

FKS mutants → "I"
Vitek: 0% (0/30)

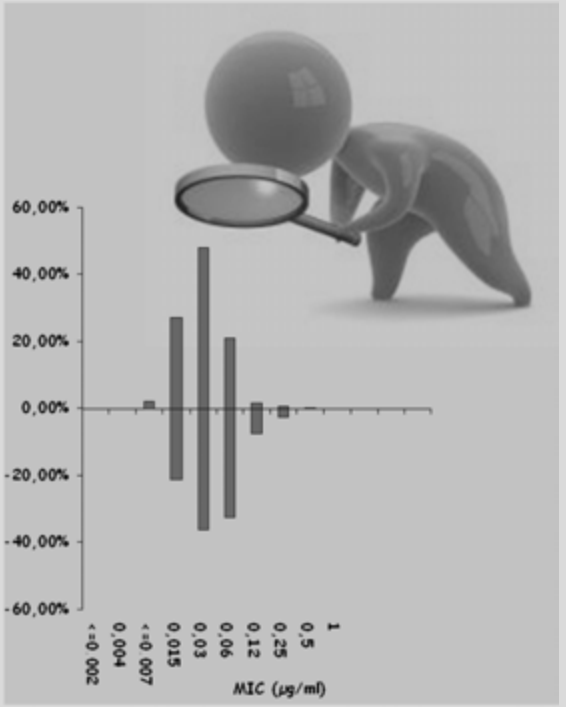
WT as I/R
Vitek: *C. glabrata* !!

* 2 *C. glabrata FKS* mutants with MIC ≤0.25 mg/L counted as S

Astvad, Johansen & Arendrup, in preparation

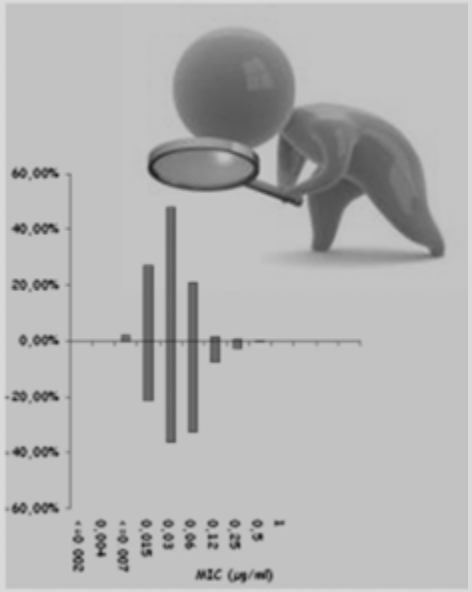
Take home messages Commercial Tests

- You have to check for each drug-bug combination
- BPs can only be adopted if MICs matches the reference method!
- Recommendations for echinocandins
  - Echinocandins:
    - Anidulafungin Etest with EUCAST BPs
    - VITEK2 problematic: MIC range doesn't cover the BP for C. glabrata



Conclusion: Susceptibility testing

- Antifungal Susceptibility testing
  - Requires training
  - Precise within ± 2 dilutions
  - The MIC_{50} reflects the susceptibility of the species
  - Correlates with the likelihood of success
  - BPs should be carefully selected
    - CLSI M27-S3 >< revised CLSI >< **EUCAST**
    - check your "commercial" MIC mirrors those of the ref method
    - incorrect breakpoints → random/incorrect categorization S, I, R
  - Available BPs
    - *Candida*: amphotericin, anidulafungin, flu-, vori- and posaconazole
    - *Aspergillus*: amphotericin, itraconazole, posaconazole and voriconazole



Available documents

- CLSI
  - M27-A3 Yeast broth dilution 120 $
  - M27-S3 QC and BPs 35 $
  - M38-A2 Mould broth dilution 120 $
  - M44-A2 Yeast disk diffusion 200 $
  - M44-S3 QC and BPs 35 $
  - M51-A Mould disk diffusion 170 $
  - M51-S1 QC and ECVs 200 $

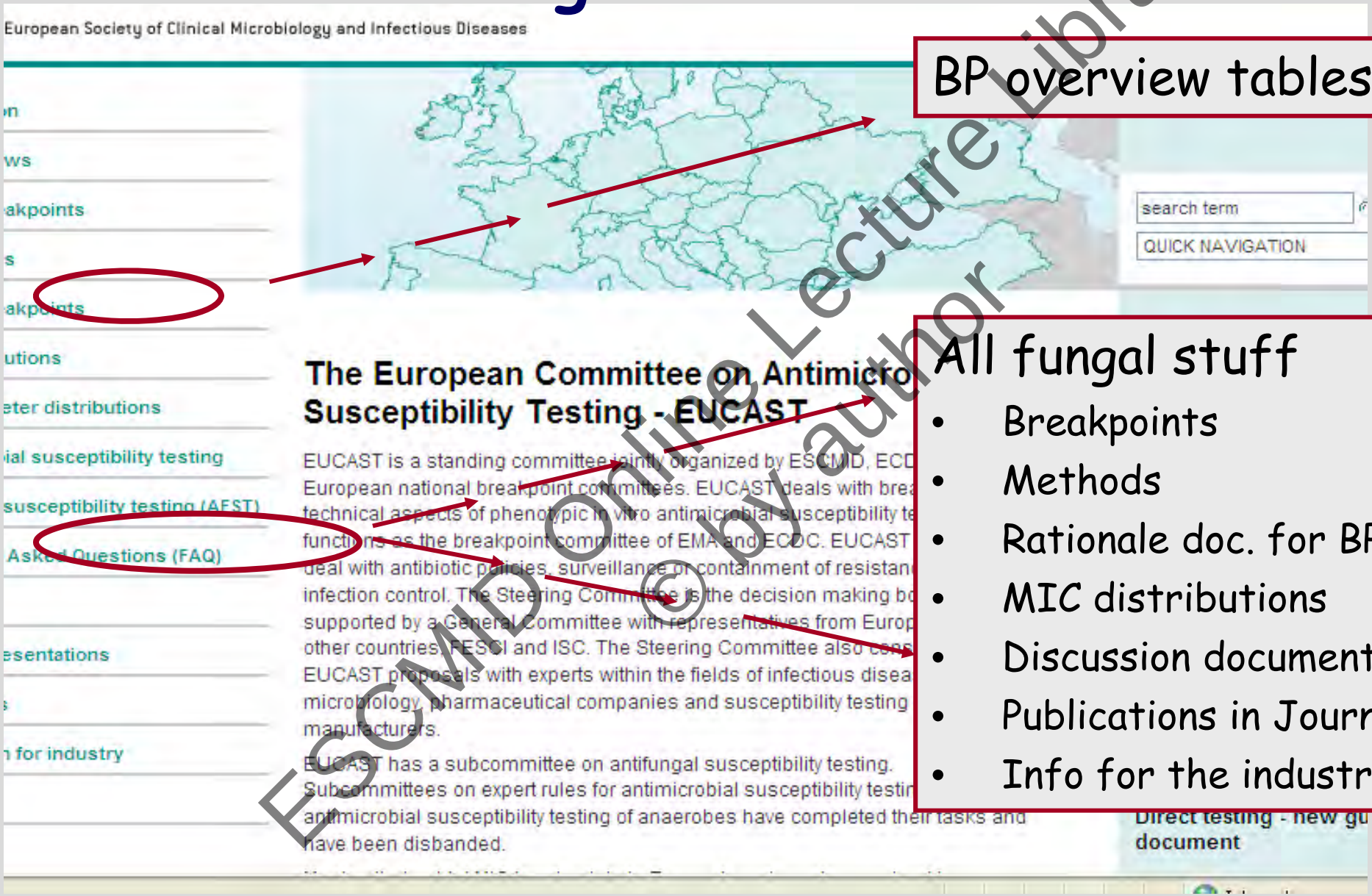
  } 880 $

- EUCAST
  - Methods EDef 7.2 (yeast) and EDef 9.1 (mould)
  - Rationale documents (breakpoints)
    - *Candida*: amphotericin, anidulafungin, flu-, vori- and posaconazole
    - *Aspergillus*: amphotericin, itraconazole, posaconazole
  - Technical notes in Clin Microbiol Infect

  } Free

www.clsi.org, www.eucast.org

www.eucast.org



BP overview tables

All fungal stuff

- Breakpoints
- Methods
- Rationale doc. for BPs
- MIC distributions
- Discussion documents
- Publications in Journals
- Info for the industry

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(in alphabetic order):

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Thank you for your attention