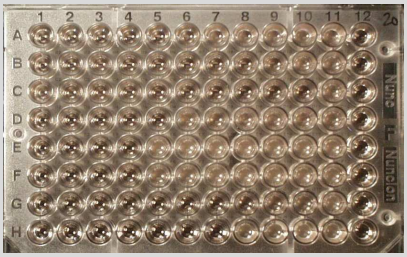
Breakpoints - why do we differ?

CLSI >< EUCAST >< NSMM



Maiken Cavling Arendrup
mad@ssi.dk
SSI

Agenda

- CLSI, EUCAST, NRMM
  - Organisation
  - Current Breakpoints
- BP setting process
  - CLSI
  - EUCAST
- Issue concerning change in CLSI methodology
  - Impact on BPs
- Conclusion

Organisation CLSI-EUCAST-NRMM

	CLSI	EUCAST	NRMM
Members	Appointed by CLSI	1/country Appointed by national body	"Appointed" by Gunnar
Composition	Academics Industry Government etc	Academics	Academics
Funding for studies/expenses	Yes	No	No
No. of labs generating MIC values	1	As many ref labs as possible	3

M. Cavling Arendrup

CLSI AFST members

- Chair CLSI: John Rex (Astra Zenica)
- Chair CLSI AFST: Mahmoud Ghannoum
- Academics
  - Mike Pfaller, Tom Walsh, Dave Andes, Barbara Alexander, Luis Ostrosky-Zeichner
- Industry
  - Merck, Pfizer, BioMerieux, Trek Diagnostic Systems, Clinical Microbiological Institutes
- Government/outsiders
  - Elizabeth Johnson, FDA

M. Cavling Arendrup

EUCAST AFST and NRMM members

- EUCAST steering com
  - JL Rodriguez Tudela (Chair)
  - P Donnelly (Secretary)
  - MC Arendrup
  - M Cuenca-Estrella
  - W Hope
  - C Lass-Floerl
- Total AFST
  - 1 member / country

- NRMM
  - MC Arendrup (Chair)
  - E Chryssanthou (Secretary)
  - V Fernandez
  - P Gaustad
  - P Koukila-Kähkölä
  - (P Sandven associated)

M. Cavling Arendrup

Antifungal "S, R" breakpoints for *Candida*

Breakpoints given as: S: ≤X; R: >Y Proposed Breakpoints- not yet approved!!

	CLSI	EUCAST (specific for)	NRMM (2009)
Ampho	≤1	≤1; >1	≤1; >1
Anidula	≤2	≤0.032; >0.032 (*C. alb*)	≤0.125 DK
		≤0.064; >0.064 (*C. g,k,t*)	≤0.5 SE
Caspo	≤2	-	≤1 DK, NO
			≤2 SE, FI
Mica	≤2	-	-
Fluco	≤8; >32	≤2; >4 (*C. a,t,p*)	≤2; >4
Itra	≤0.125; >0.5	-	≤0.125; >0.5
Posa	-	≤0.064; >0.064 (*C. a,t,p*)	≤0.125 DK, SE
Vori	≤1; >2	≤0.125; >0.125 (*C. a,t,p*)	≤0.125

EUCAST BP: Clin Microbiol Infect 2008 14: 193-5 & 2008 14: 985-987

M. Cavling Arendrup

Agenda

- CLSI, EUCAST, NRMM
  - Organisation
  - Current Breakpoints
- BP setting process
  - CLSI
  - EUCAST
- Issue concerning change in CLSI methodology
  - Impact on BPs
- Conclusion

CLSI breakpoint establishing procedure

- MIC distributions
- MIC-clinical outcome relationships
- PK/PD

Step 1: Voriconazole MIC distribution

- MIC distribution

TABLE 1. Susceptibility of *Candida* species to voriconazole by MIC: ARTEMIS Global Antifungal Surveillance Program, 2001 to 2004[a]

Species (no. of isolates tested)	Cumulative % of strains at MIC (μg/ml):										
	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8
C. albicans (4,701)	80	94	98	99	99	99	99	99	99	100	
C. glabrata (1,183)	1	1	3	14	40	70	86	92	95	99	100
C. parapsilosis (1,253)	19	61	81	90	95	98	99	99	99	99	100
C. tropicalis (963)	9	27	64	93	99	99	99	99	99	99	100
C. krusei (243)				1	13	61	95	100			
C. lusitaniae (110)	70	92	94	97	98	98	99	100			
C. guilliermondii (128)		6	15	66	93	98	99	99	100		
C. kefyr (38)	47	87	97	100							
C. pelliculosa (28)			4	4	64	93	100				
Candida spp. (55)[b]	18	40	51	80	89	98	98	100			
All *Candida* isolates (8,702)	48	64	75	82	88	94	98	99	99	99	100

Pfaller JCM 2006

M. Cavling Arendrup

Step 2: MIC – In vivo outcome

TABLE 3. *Candida* species, geometric mean MICs, and investigator-assessed response to voriconazole therapy[a]

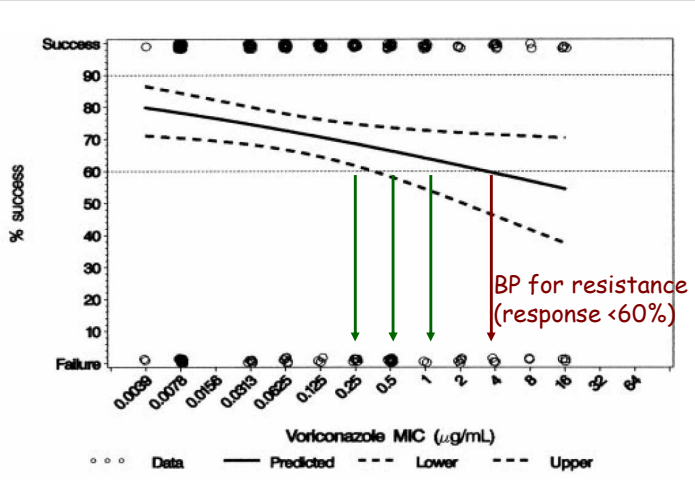
Species	No. of isolates tested	Geometric mean MIC (μg/ml)	% Success
C. albicans	96	0.0164	72
C. parapsilosis	34	0.0266	85
Candida spp.	12	0.0712	92
C. tropicalis	51	0.1283	73
C. krusei	9	0.3650	78
C. glabrata	47	0.7937	55

[a] Broth microdilution MICs were determined in accordance with CLSI M27-A2. Baseline isolates from studies 603, 608, 309/604, and 301/606 were used.

Pfaller JCM 2006

M. Cavling Arendrup

Step 2 Logistic analysis MIC-outcome



249 cases

BP suggestion

R >4 µg/ml

S ≤0.25 / 0.5 / 1 µg/ml ??

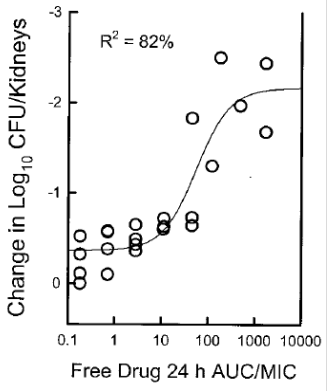
M. Cavling Arendrup

Step 3: PK/PD data

Mouse model

Isolates: 10 *C. albicans* MIC 0.007 – 0.25 µg/ml

C. albicans strain	MIC (mg/liter)		ED_{50} (mg/kg/24 h)	24-h AUC/MIC ratio	
	Voriconzole	Fluconazole		Total drug	Free drug
K1	0.007	0.25	7.93	81.4	17.9
W2	0.015	0.5	14.1	97	21.2
580	0.03	4.0	26.3	237	52
98-210	0.03	16	16.9	76	16.6
1490	0.03	0.5	19.7	60.8	13.3
5810	0.03	0.5	14.3	50.3	11.3
2-76	0.06	0.25	26	115	25.3
2183	0.06	>128	24.8	102	22.5
98-17	0.12	16	28.1	72	15.8
98-234	0.25	32	97.5	265	58
Mean ±SD					24 ± 17



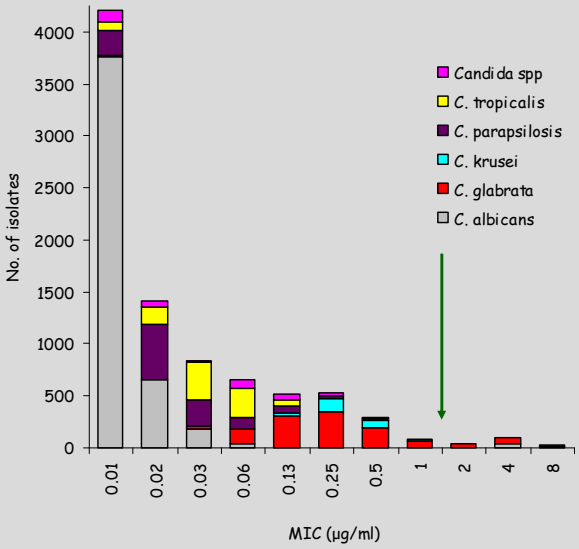
Human PK/PD

AUC ~ 20 µg*h/ml → supports a susceptibility BP of 1 µg/ml

Andes AAC 2003

M. Cavling Arendrup

Choice of susceptibility breakpoint



Arguments for S ≤1 µg/ml

MIC distributions
Not bisect *C. glabrata* population
≤1 includes 99% of isolates

Outcome
<60% response if MIC >4 µg/ml

PK/PD
Serum levels frequently ≥1 µg/ml
AUC/MIC will be ≥20.

M. Cavling Arendrup

European concerns

- Outcome ↓ *C. glabrata*
  - 55% versus 72-83% for *C. albicans, C. tropicalis* and *C. parapsilosis*
- ED_{50} achieved at AUC/MIC ~ 20 – Enough?
- No clinical experience with isolates with elevated MICs
  - *Candida* species are not all alike
  - no data to support the outcome for a *C. albicans* MIC 1 = for a *C. glabrata* MIC= 1 !

M. Cavling Arendrup

Virulence in a mouse model: Mortality

Species (no.)	Mortality (day)	
	10^5	10^7
C. albicans (2)	1/6 (day 7)	7/7 (day 1)
C. tropicalis (2)	0/7	1/10 (day 7)
C. glabrata (2)	0/6	0/6
C. kefyr (2)	0/6	0/8
C. lusitaniae (2)	0/6	0/11
C. parapsilosis (2)	0/7	0/8
C. krusei (3)	0/9	0/12
C. guilliermondii (2)	0/6	0/7

Virulence: Pathology

Species	Weight change	Kidney weight	Kidney (%)	Inflammation (scores)	Eye infection
C. albicans	-2.3	0.2836	1.00	+++/+++/+++/+++ /+++/+++	1/3
C. tropicalis	-2.1	0.2724	0.92	++/++/++/++/++/++	2/3
C. glabrata	0.2	0.2462	0.79	+/+/+/+/+/+	0/3
C. krusei	2.8	0.2379	0.69	0/0/0/0/(+)/(+)	0/3
Control	-0.1	0.2261	0.73	0/0/0/0/0/0	0/3

Virulence: No of Kidneys Infected

Species (no.)	No. infected / total no. kidneys	
	10^5	10^7
C. albicans (2)	10/10	-
C. tropicalis (2)	14/14	20/20
C. glabrata (2)	9/12	12/12
C. kefyr (2)	12/12	16/16
C. lusitaniae (2)	5/12	22/22
C. parapsilosis (2)	6/14	11/16
C. krusei (3)	0/18	9/24
C. guilliermondii (2)	0/12	5/14

Arendrup Infection 2002

Agenda

- CLSI, EUCAST, NRMM
  - Organisation
  - Current Breakpoints
- BP setting process
  - CLSI
  - EUCAST
- Issue concerning change in CLSI methodology
  - Impact on BPs
- Conclusion

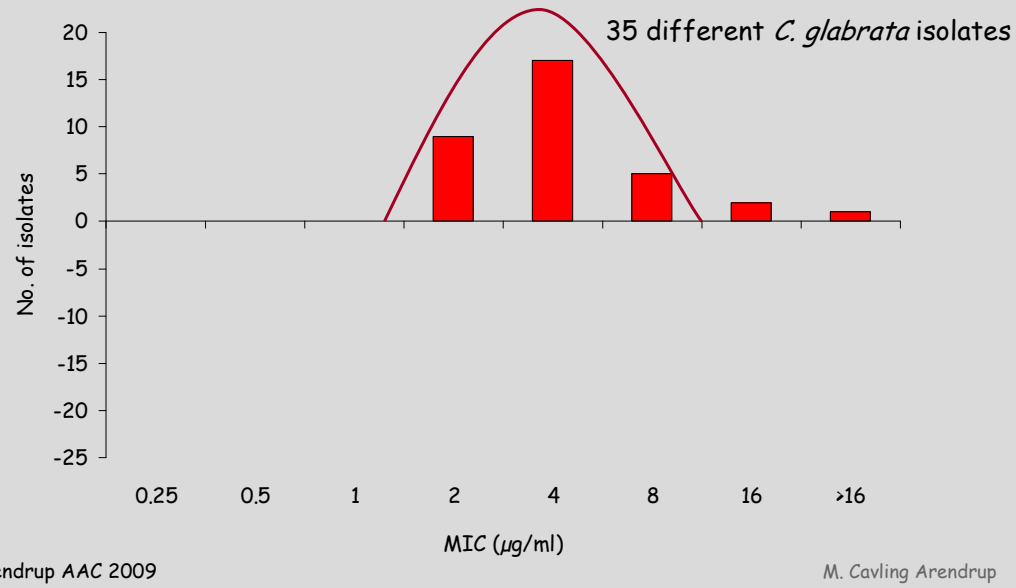
EUCAST BP establishing procedure

- MIC distributions
  - Per species
  - Several data sets
  - Epidemiological Cut Off Value (ECOFF)
- MIC-clinical outcome relationships
- PK/PD

➢ BP never higher than ECOFF unless supported by data

M. Cavling Arendrup

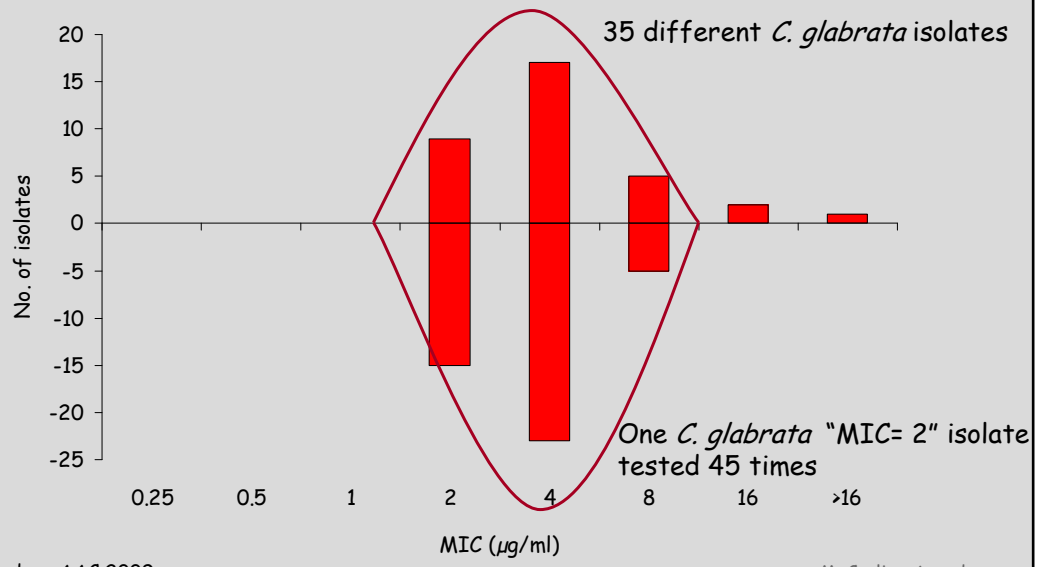
Wild-types EUCAST MICs



Arendrup AAC 2009

M. Cavling Arendrup

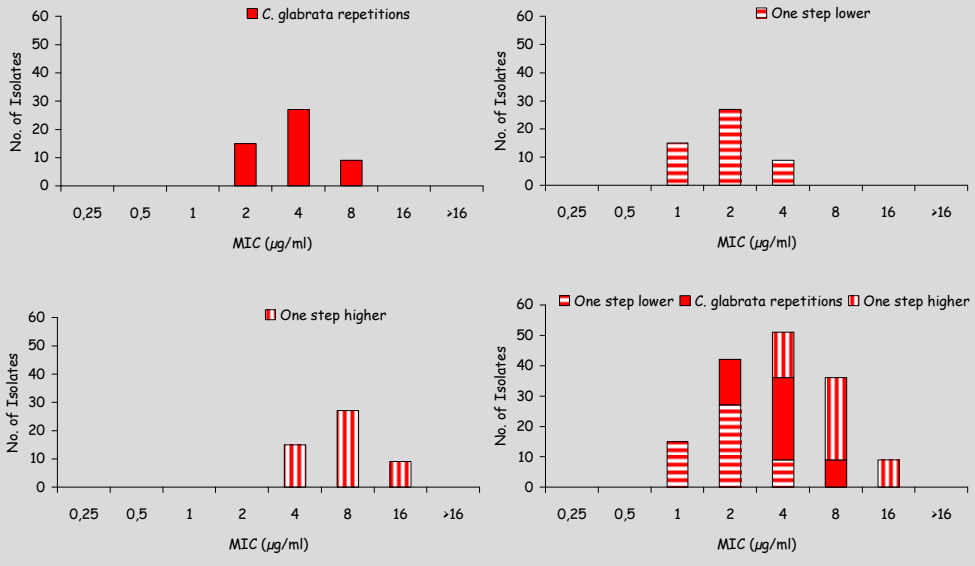
Wild-types EUCAST MICs



Arendrup AAC 2009

M. Cavling Arendrup

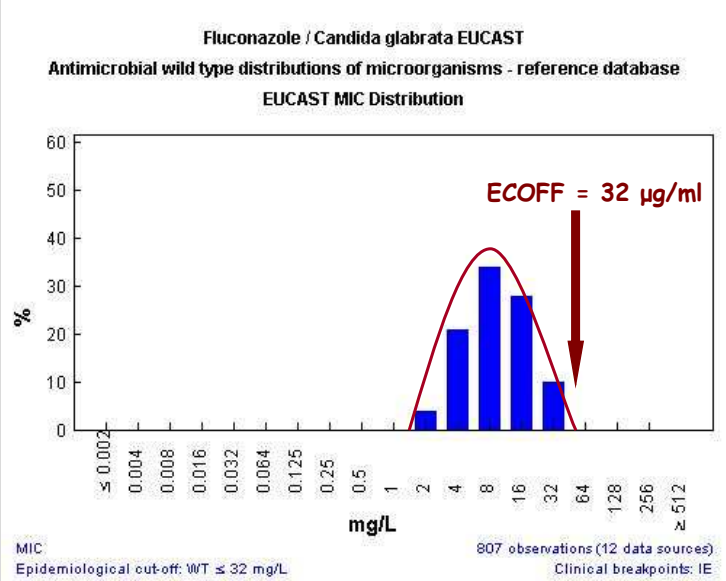
Wild-type distributions incl. variability



Arendrup AAC 2009

M. Cavling Arendrup

EUCAST fluconazole MIC *C. glabrata*



http://www.eucast.org/mic_distributions_of_wild_type_microorganisms/

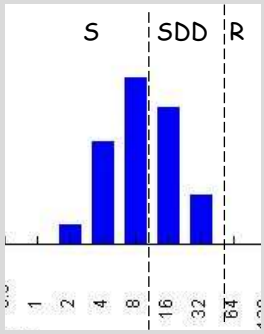
M. Cavling Arendrup

Correlation MIC and outcome

177 candidaemia cases in Taiwan 1999-2005

Table 3
In vitro susceptibilities of *Candida glabrata* isolates to five antifungal agents.

Antifungal	MIC (µg/mL)			Susceptible (%)[a]
	Range	MIC_{50}	MIC_{90}	
Fluconazole	2 to >64	8	32	63
Voriconazole	0.06–64	0.25	1	93
Caspofungin	0.25 to >64	0.5	1	96
Flucytosine	0.06 to >64	0.06	0.12	99
Amphotericin B	0.25–2	1	1	98



The different levels of susceptibility to fluconazole (susceptible, susceptible-dose dependent and resistant) were not significantly associated with 30-day mortality ($P = 0.09$).

Ryan Int J Antimicrob Agents 2009;
http://www.eucast.org/mic_distributions_of_wild_type_microorganisms/

M. Cavling Arendrup

Step 1 MIC distributions & ECOFFs

- Anidulafungin

2. MIC wild type distributions (based on MIC-values determined with EUCAST and Etest methodology*)

Species	≤0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	Tot	ECOFF≤ (mg/L)**
Candida albicans Untruncated	284	360	208	77	16	6	4	1	1							1	958	0.016-**0.032**
Truncated at 0.032					618	3	0	0	1	1	0	0	0	0	0	0	623	
Truncated at 0.016				233	5	1	0	1	0	0	0	0	0	0	0	0	240	
Candida glabrata Untruncated	55	38	60	149	62	8	6	8	2	2	0	0	2	0	0	0	392	0.032-**0.064**
Truncated at 0.032					233	1	1	0	2	0	0	0	0	0	0	0	237	
Truncated at 0.016				0	13	49	16	1	0	1	1	0	0	0	0	0	81	
Candida krusei Untruncated	2	1	12	21	7	12	2	3	0	0	0	0	0	0	0	0	60	**0.064**
Truncated at 0.032					58	7	0	0	0	0	0	0	0	0	0	0	65	
Truncated at 0.016																	0	
Candida parapsilosis	0	3	1	0	4	6	2	36	78	171	96	13	7	0	0	2	419	4
Candida tropicalis Untruncated	18	19	12	30	22	4	4	1	0	0	0	0	0	0	0	0	110	0.032-**0.064**
Truncated at 0.032					120	2	0	0	0	0	0	0	0	0	0	0	122	
Truncated at 0.016				8	22	11	0	0	0	0	0	0	0	0	0	0	41	
Candida guilliermondii	0	0	0	0	0	1	0	2	5	17	6	1	0	0	1	0	33	4

M. Cavling Arendrup

Step 2: MIC - outcome data

- No such data using EUCAST!
- Anidulafungin superior to fluconazole
  - *C. albicans* (135 patients) (81% >< 62%) P<0.05
  - *C. tropicalis* (22 patients) (93% >< 50%) P<0.05
  - *C. glabrata* (38 patients) (56% >< 50%)
  - *Candida* spp. (7 patients) (75% >< 67%

  } Good targets for anidulafungin
- Anidulafungin inferior to fluconazole
  - *C. parapsilosis* (23 patients) (64% >< 83%)

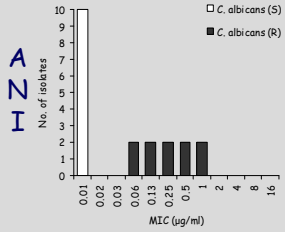
  } Poor target for anidulafungin

Reboli N Engl J Med 2007

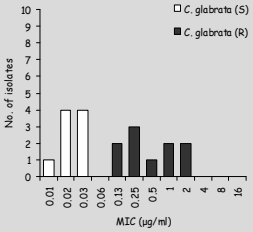
M. Cavling Arendrup

Step 2: MIC distribution *fks* mutants

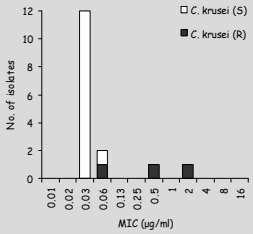
C. albicans



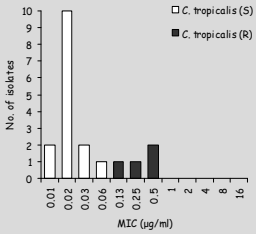
C. glabrata



C. krusei



C. tropicalis



MICs for *fks* mutants

C. albicans	≥0.06
C. glabrata	≥0.125
C. krusei	≥0.06/ ≥0.5
C. tropicalis	≥ 0.125

Arendrup AAC 2010

M. Cavling Arendrup

Step 2: MIC distribution *fks* mutants

C. albicans

C. glabrata

C. krusei

C. tropicalis

MICs for *fks* mutants

C. albicans	≥0.06
C. glabrata	≥0.125
C. krusei	≥0.06/ ≥0.5
C. tropicalis	≥ 0.125

Arendrup AAC 2010

M. Cavling Arendrup

Step 3: No PK/PD → ECOFFs as BP

Clinical breakpoints

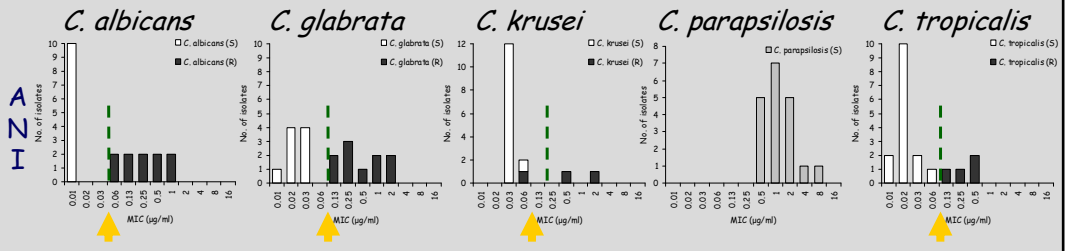
C. albicans: S ≤0.03, R >0.03 mg/L

C. glabrata: S ≤0.06, R >0.06 mg/L

C. tropicalis: S ≤0.06, R >0.06 mg/L

C. krusei: S ≤0.06, R >0.06 mg/L

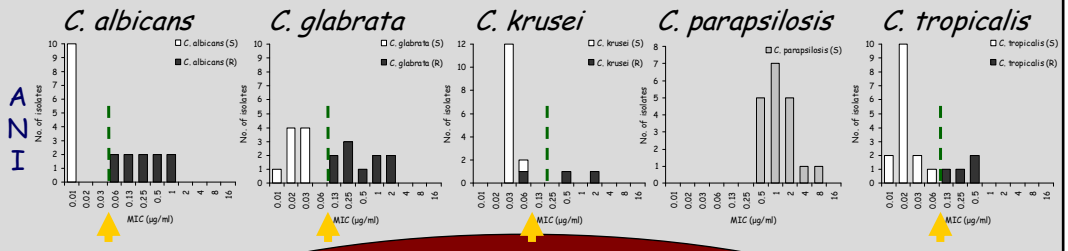
C. parapsilosis & *C. guillermondii*: no Breakpoints (not good targets)



Arendrup et al AAC 2010 and EUCAST-AFST

M. Cavling Arendrup

EUCAST



ECOFFs as BP

1/29 Very Major Error (*C. krusei* with fks mutation as S)

0 Major Errors (susceptible isolates mis-classified as R)

CLSI BP (S ≤2) with EUCAST testing

29/29 VMEs

Arendrup et al AAC 2010 and EUCAST-AFST

M. Cavling Arendrup

Mis-classifications using CLSI BP

- CLSI BP: Susceptible ≤2 µg/ml

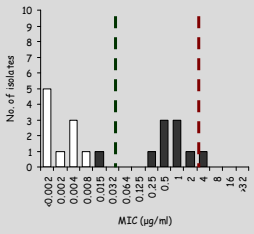
	fks hot spot mutants classified as "S"		
	Anidulafungin	Caspofungin	Micafungin
VMEs CLSI	89.2%	60.7%	92.9%

Arendrup et al AAC 2010

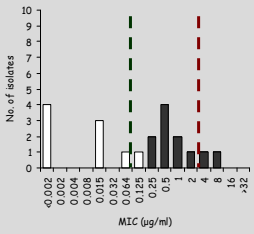
Etest and Anidulafungin

ANI

C. albicans



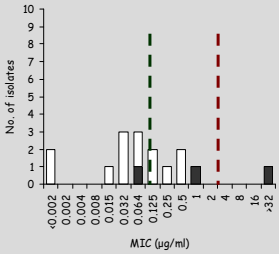
C. glabrata



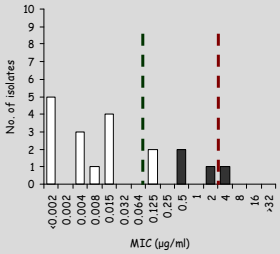
EUCAST draft BP

CLSI BP

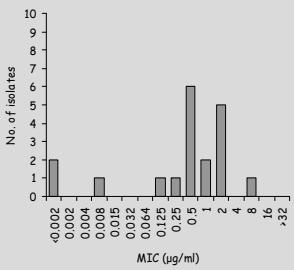
C. krusei



C. tropicalis



C. parapsilosis



Arendrup et al AAC 2010 and EUCAST-AFST

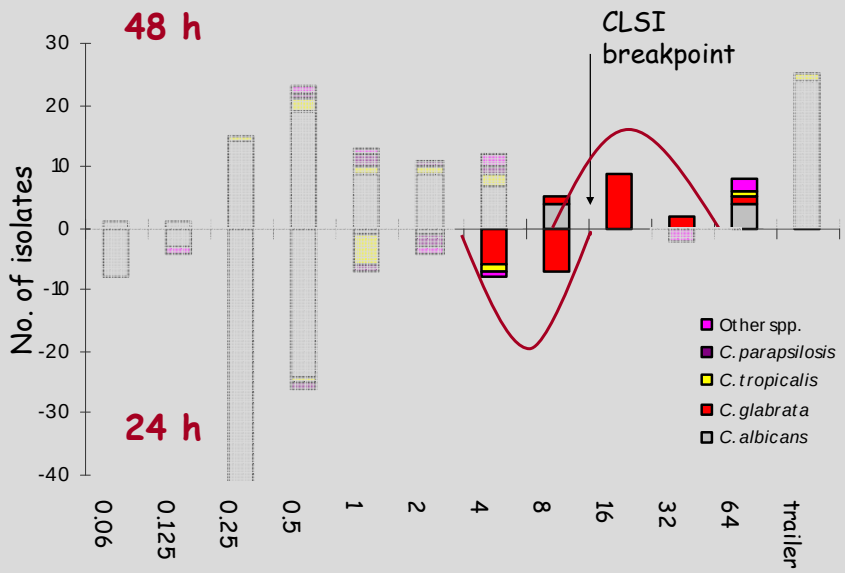
Agenda

- CLSI, EUCAST, NRMM
  - Organisation
  - Current Breakpoints
- BP setting process
  - CLSI
  - EUCAST
- Issue concerning change in CLSI methodology
  - Impact on BPs
- Conclusion

CLSI M27-A><A2><A3 & EUCAST

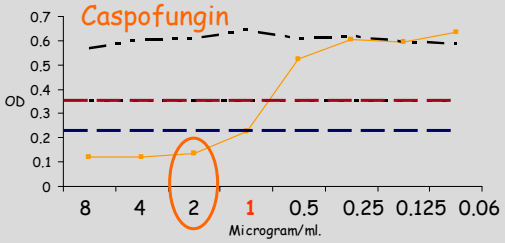
	M27-A	M27-A2	M27-A3	EUCAST
Glucose Inoculum size		0.2% Low		2% High
Incubation time	48 h	24-48 h	24 h	24 h
End point	80% inhib.	50% inhib.	50% inhib.	50% inhib

Fluconazole CLSI 48-h >< 24-h

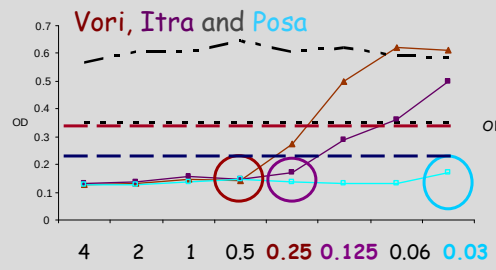


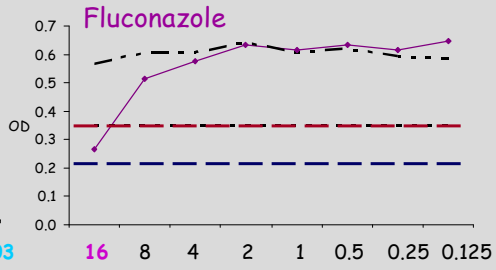
M. Cavling Arendrup

Influence of endpoint: % inhibition



	80%	50%
Caspofungin	2	1
Voriconazole	0.5	0.25
Itraconazole	0.25	0.125
Posaconazole	≤0.03	≤0.03
Fluconazole	>16	16





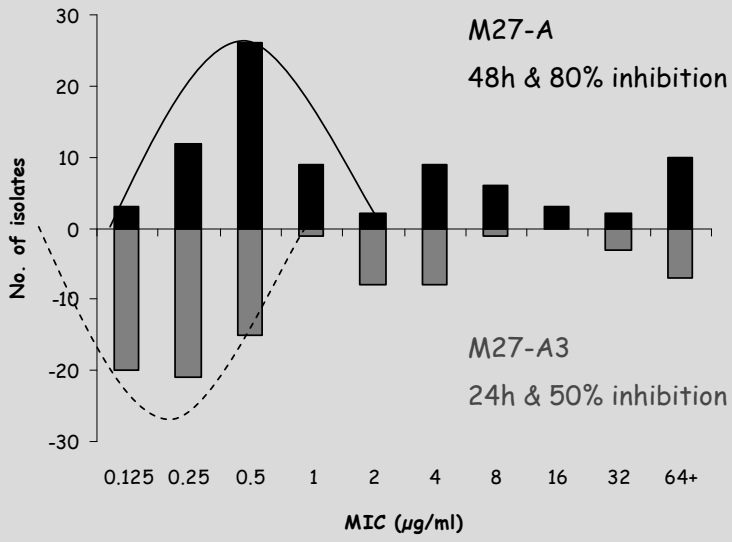
M. Cavling Arendrup

CLSI M27-A><A2><A3 & EUCAST

	M27-A	M27-A2	M27-A3	EUCAST
Glucose Inoculum size		0.2% Low		2% High
Incubation time	48 h	24-48 h	24 h	24 h
End point	80% inhib.	50% inhib.	50% inhib.	50% inhib
Fluconazole QC MIC limits				
C. parapsilosis ATCC 22019	2-8 µg/ml	0.5-4 / 1-4 µg/ml	0.5-4 µg/ml	0.5-2 µg/ml
C. krusei ATCC 6258	16-64 µg/ml	8-64 / 16-128 µg/ml	8-64 µg/ml	16-64 µg/ml

M. Cavling Arendrup

CLSI M27-A >< A3 Fluconazole

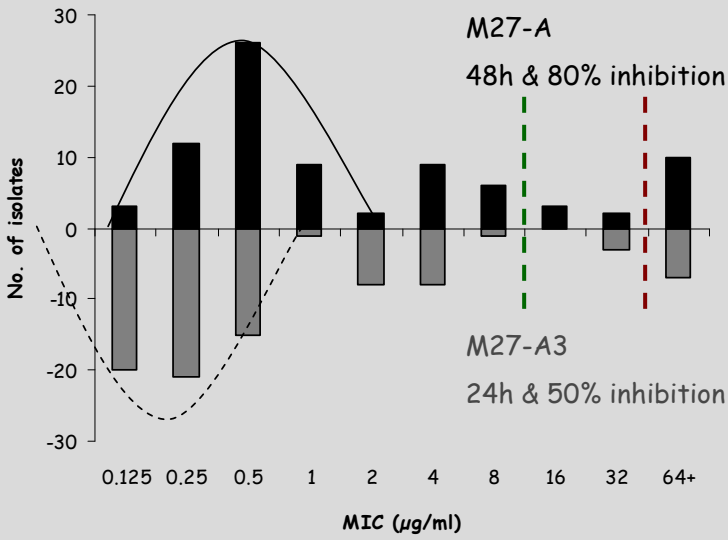


3.6% isolates are R according to M27-A but not M27-A3

Baddley AAC 2008, Arendrup AAC 2009

M. Cavling Arendrup

CLSI M27-A >< A3 Fluconazole



10 R & 5 SDD

vs

7 R & 3 SDD

30% SDD/R isolates become more susceptible

Baddley AAC 2008 , Arendrup AAC 2009

M. Cavling Arendrup

CLSI M27-A >< A3 Fluconazole

505 Candida isolates

Fluconazole 24h MIC	48h MIC 0.0625	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Total
0.0625	9	1	51	15	8							84
0.125		4	16	12	5	1						38
0.25			49	51	31	3					3	137
0.5				24	26	14	1					65
1					5	11	1				4	21
2						13	9	6	4	1		33
4							17	9	6	3	1	36
8								12	9	5	1	27
16									9	10	4	23
32										1	17	18
≥64											23	23
N	9	5	116	102	75	42	28	27	28	20	53	505

24 h: 64 not S

48 h: 101 not S

37% SDD/R isolates are re-classified as more susceptible (37/101)

Ostrosky-Zeichner AAC 2008

M. Cavling Arendrup

Caspofungin MICs (CLSI)

Organism	Caspofungin MIC_{90} (µg/ml)							
	Ch 2002 (87)	La 2002 (178)	Pf 2001 (726)	Pf 2003 (3,959)	Al 2007 (212)	O-Z 2003 (1,972)	Pf 2006 (2,656)	Pf 2008 (5,346)
C. albicans	0.5	0.25	0.25	0.25	0.5	0.5	0.06	0.06
C. glabrata	1	0.5	0.25	0.25	1	1	0.06	0.06
C. krusei	1	2	1	1	2	2	0.25	0.25
C. tropicalis	0.5	0.25	0.5	0.5	0.5	1	0.06	0.06
C. kefyr	-	-	-		-	1	0.015	0.015
C. lusitaniae	2	-	2	1	1	2	0.25	0.5
C. parapsilosis	2	2	2	4	2	2	1	1
C. guilliermondii	>16	-	>8	>8	1	1-2	1	1
C. famata	-	-	-	>8	.	-		1

Chryssanthou JCM 2002, Lavadiere IntJAA 2002, Ostrosky-Zeichner AAC 2003, Alexander JCM 2007, Pfaller JCM 2001, 03, 06 & 08,

M. Cavling Arendrup

Caspofungin MICs (CLSI)

Organism	48-h & 95% inhibition endpoint				48-h & 80%	48-h & 50%	24-h & 50% inhibition endpoint	
	Ch 2002 (87)	La 2002 (178)	Pf 2001 (726)	Pf 2003 (3,959)	Al 2007 (212)	O-Z 2003 (1,972)	Pf 2006 (2,656)	Pf 2008 (5,346)
C. albicans	0.5	0.25	0.25	0.25	0.5	0.5	0.06	0.06
C. glabrata	1	0.5	0.25	0.25	1	1	0.06	0.06
C. krusei	1	2	1	1	2	2	0.25	0.25
C. tropicalis	0.5	0.25	0.5	0.5	0.5	1	0.06	0.06
C. kefyr	-	-	-		-	1	0.015	0.015
C. lusitaniae	2	-	2	1	1	2	0.25	0.5
C. parapsilosis	2	2	2	4	2	2	1	1
C. guilliermondii	>16	-	>8	>8	1	1-2	1	1
C. famata	-	-	-	>8	.	-		1

Chryssanthou JCM 2002, Lavadiere IntJAA 2002, Ostrosky-Zeichner AAC 2003, Alexander JCM 2007, Pfaller JCM 2001, 03, 06 & 08,

M. Cavling Arendrup

Conclusion – Why do we differ??

- CLSI BPs
  - species with highest MIC drives the BP
  - BPs have not been adjusted to change in methodology
  - Risk of misclassifying R isolates as S
- EUCAST BPs
  - conservative approach
  - if data arise showing isolates with resistance mechanisms are good targets BPs have to be raised
- My recommendation: **Use EUCAST BPs**

M. Cavling Arendrup

Antifungal "S, R" breakpoints for *Candida*

Breakpoints given as: S: ≤X; R: >Y Proposed Breakpoints- not yet approved!!

	CLSI	EUCAST (specific for)	NRMM (2009)
Ampho	≤1	≤1; >1	≤1; >1
Anidula	≤2	≤0.032; >0.032 (*C. alb*) ≤0.064; >0.064 (*C. g,k,t*)	≤0.125 DK ≤0.5 SE
Caspo	≤2	-	≤1 DK, NO ≤2 SE, FI
Mica	≤2	-	-
Fluco	≤8; >32	≤2; >4 (*C. a,t,p*)	≤2; >4
Itra	≤0.125; >0.5	-	≤0.125; >0.5
Posa	-	≤0.064; >0.064 (*C. a,t,p*)	≤0.125 DK, SE
Vori	≤1; >2	≤0.125; >0.125 (*C. a,t,p*)	≤0.125

EUCAST BP: Clin Microbiol Infect 2008 14: 193-5 & 2008 14: 985-987

M. Cavling Arendrup


STATENS
SERUM
INSTITUT
Thank you for your attention