

Antifungal breakpoints and susceptibility testing

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Disclosures - none

ESCMID Online Lecture Library
@ by author

Agenda

- Setting the scene
- Methodology outline
- ECOFF/breakpoint setting
- Current guidelines

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Background

- The mortality rate associated with fungal disease is dire
- Susceptibility is just one factor affecting outcome
- Limited therapeutic drug class options
- Global antifungal drug budget is significant (est. \$US 5.7 billion in 2014)

Why Susceptibility Test?

- Intrinsic resistance
- Acquired resistance (increasing in some settings)
- Some identifications challenging/slow
- Historically multiple/differing methodologies, with no/limited breakpoints to allow comparison/interpretation

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Methodology

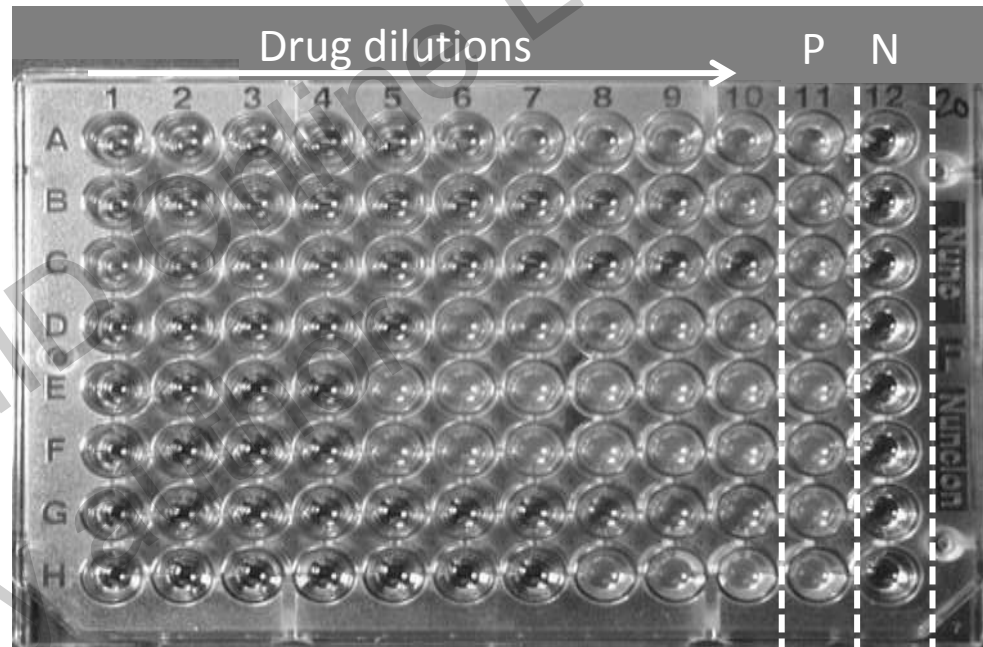
- Commercially available tests
 - Including disc diffusion, Sensititre, Vitek and Etest



- Broth dilution reference methods
 - CLSI & EUCAST

EUCAST Method

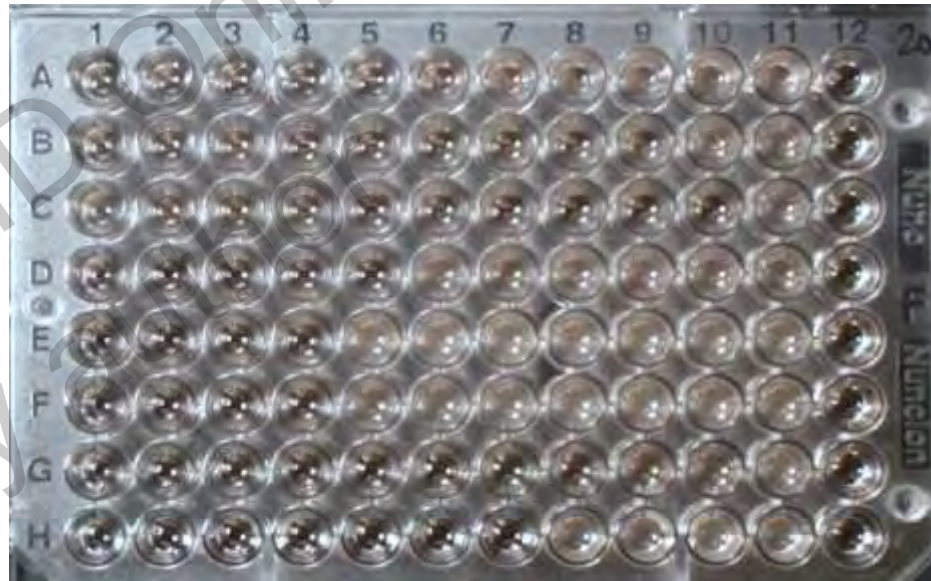
- Flat bottom plates
- RPMI with 2% glucose and MOPS
- Incubate at $35 \pm 2^\circ\text{C}$



Yeasts

Fermentative yeasts E.DEF 7.2 (2012)

- $0.5-2.5 \times 10^5$ cells/mL
- 24 h and $OD \geq 0.2$
- Spectrophotometer
- 90% endpoint (AMB) / 50% endpoint (all others)



Moulds

Conidia forming moulds E.DEF 9.2 (2014)

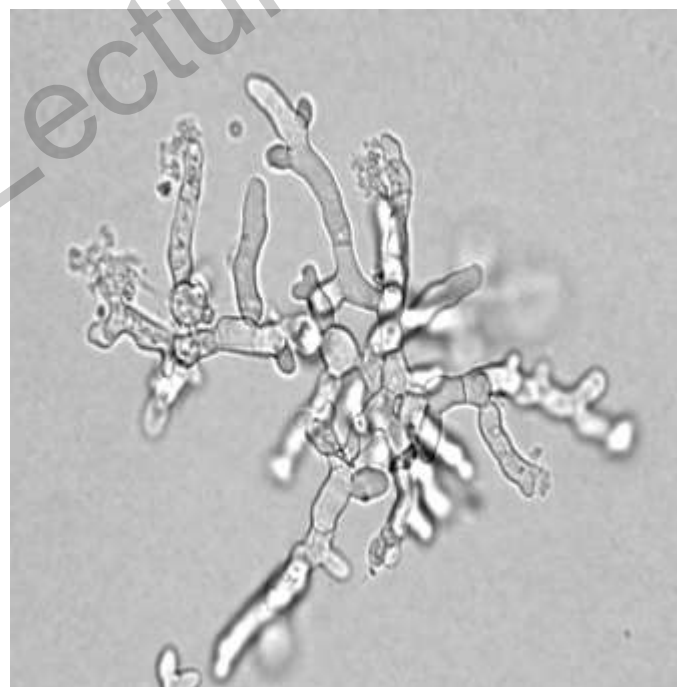
- 1-2.5 x 10⁵ cells/mL
(avoid clumps)
- 48 h → adequate growth*
(*24h may be sufficient for some zygomycetes, some moulds require 72h)
- Visual endpoint reading
 - amphotericin & azoles = no growth endpoint (MIC)
 - echinocandins = aberrant growth (MEC)



Minimum Effective Concentration (MEC)



Growth control



Aberrant growth

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ECOFFs versus Breakpoints

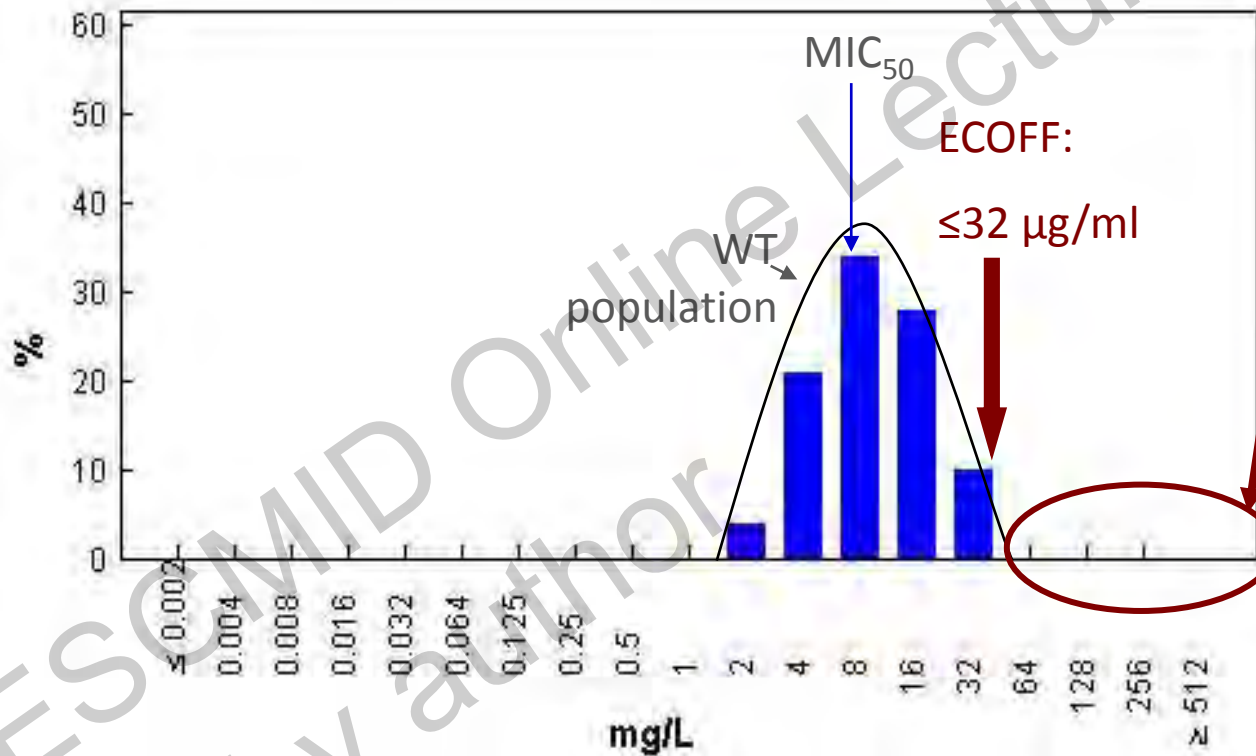
Epidemiological cut off value (ECOFFs)

- Based on the wild-type MIC distributions
- Upper limit of WT isolates
- Typically describes isolates with identical susceptibility
- Typically describes isolates with no resistance mechanisms

Clinical breakpoints

- Take other factors (e.g. PK/PD and clinical data) into account
- More later...

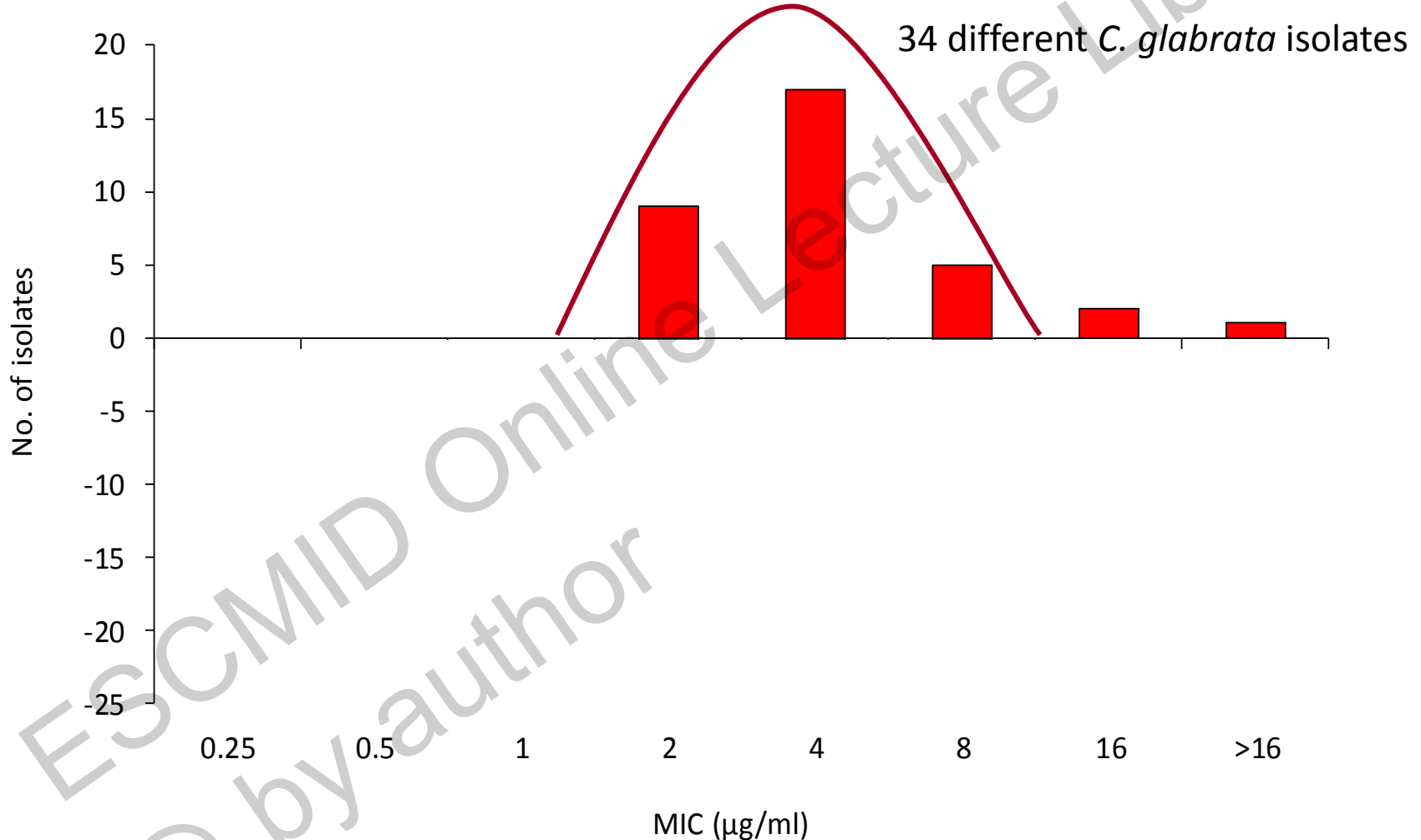
EUCAST fluconazole MIC *C. glabrata*



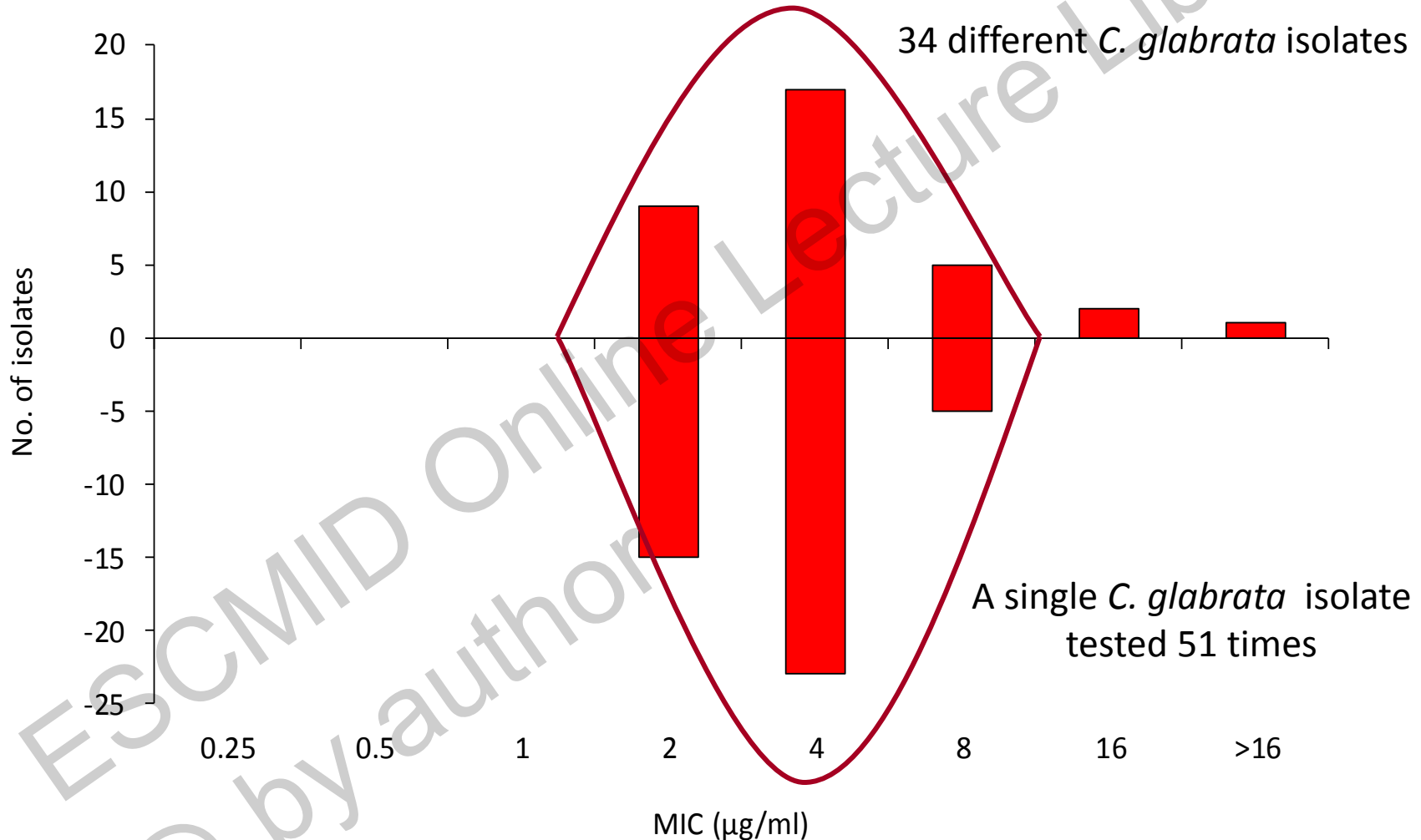
Non-WT

- May or may not respond depending on host & drug exposure
- Harbour resistance mechanisms

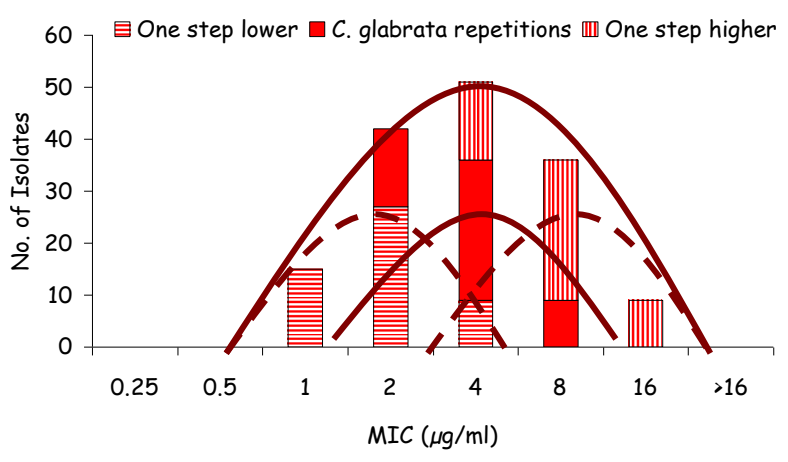
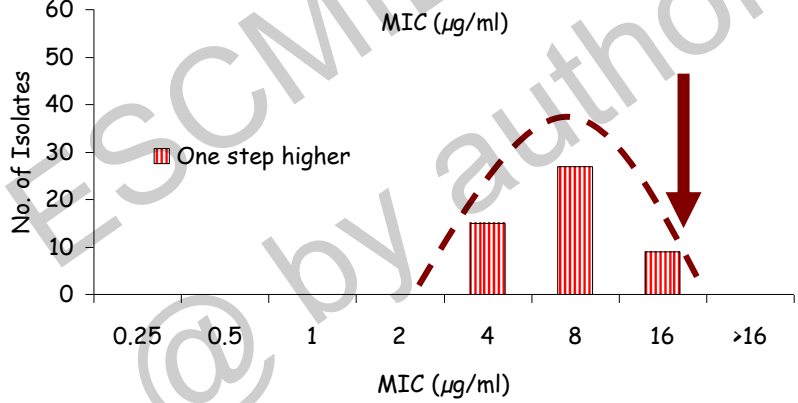
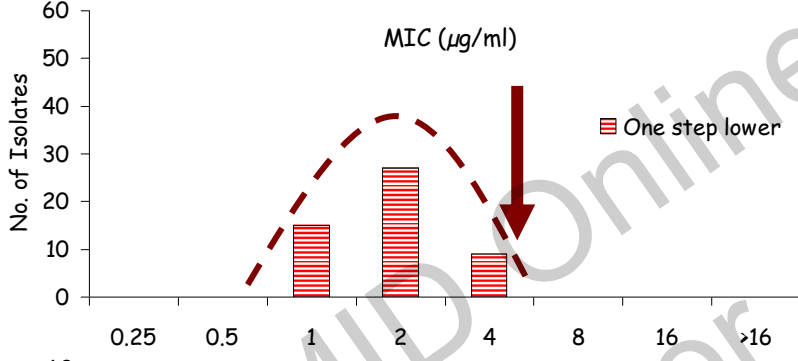
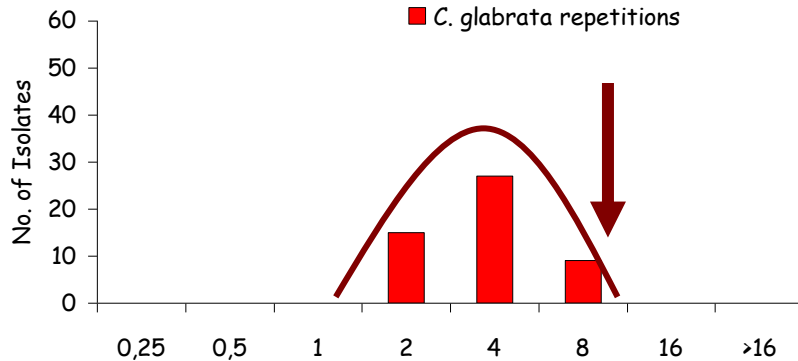
Wild-type MIC Distributions



Wild-type MIC Distributions



Variability in Wild-type Distributions



Reflects the inherent susceptibility variability of the WT population

Caspofungin and Variability (EUCAST)

EUCAST MIC₅₀ is highlighted

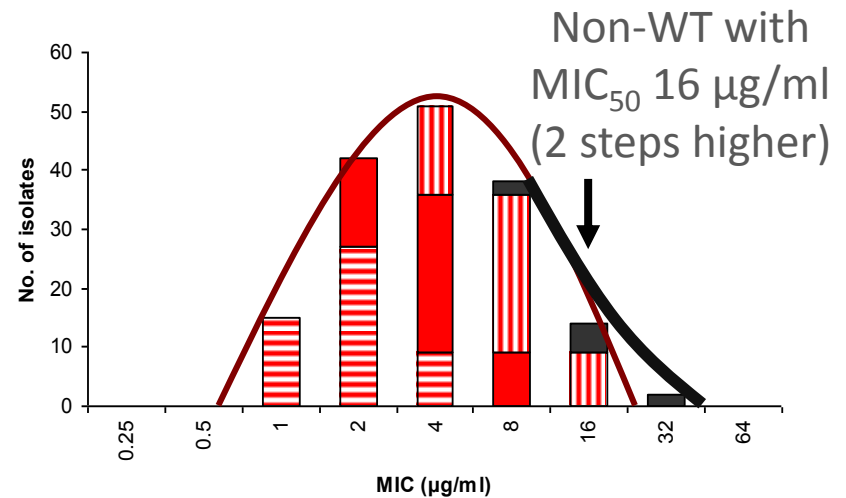
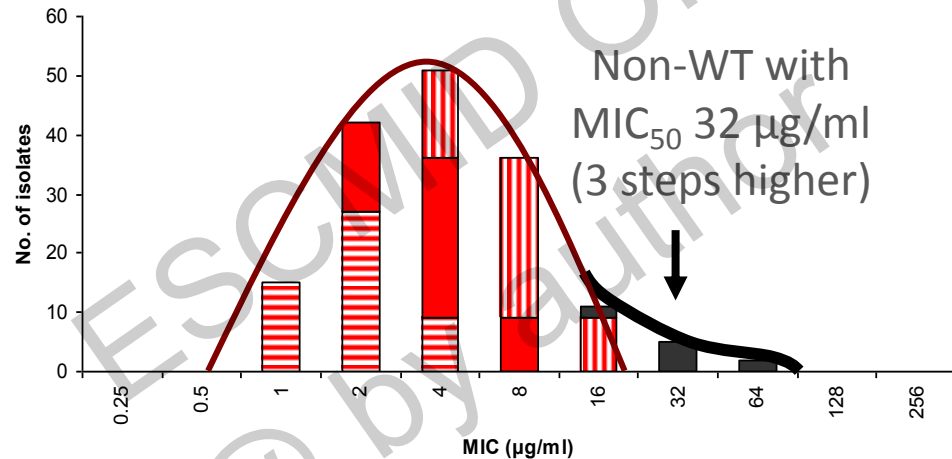
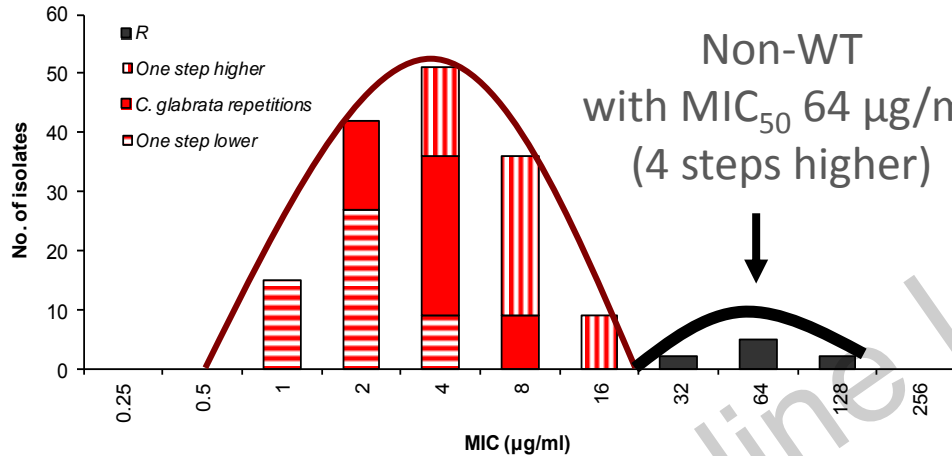
	No. of <i>C. albicans</i> isolates at the individual EUCAST MICs (mg/L)											No. Tested	
	0.002	0.004	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2		≥ 4
CFG													
Lab 1	ND	ND	ND	20	30	<u>35</u>	45						130
Lab 2	ND	ND	ND										606
Lab 3	ND	ND	ND	ND	ND		10	161	<u>219</u>	8	5		404
Lab 4	ND	ND	ND	2	105	<u>264</u>	32	54	11	3	1	4	626
Lab 5	ND	ND	6	12	<u>12</u>	5	3						38
Lab 6	ND	ND	ND										31
Lab 7	ND	ND	ND	ND	1	2	14	330	<u>373</u>		1		721
MFG													
Lab 1	ND	ND	ND	ND	107								107
Lab 2	ND	<u>121</u>	2										123
Lab 3	ND	34	<u>19</u>	12	<u>30</u>	4	1						100
Lab 4	ND	<u>78</u>	19	2		1							100
Lab 5	ND	ND	ND	<u>520</u>	35	2	1			2			560
Lab 6	4	87	<u>252</u>	239	4	4							590
Lab 7	ND	ND	<u>87</u>	2									89

Considerable variation for caspofungin

→

EUCAST abstained from setting cut offs

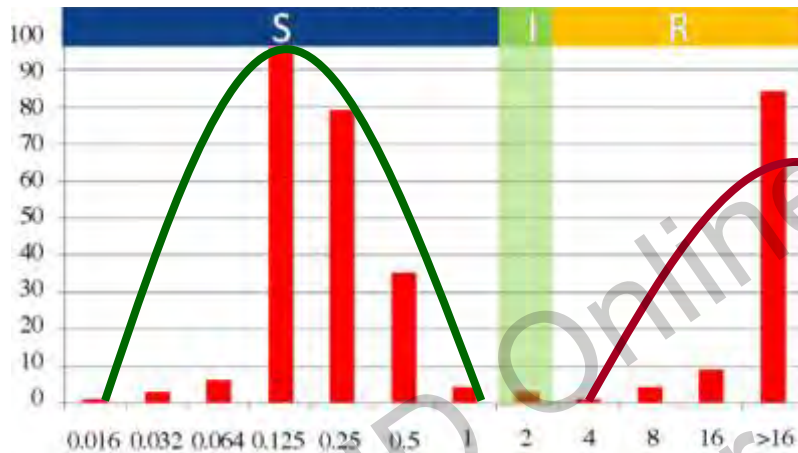
Population with WT and non-WT



Real Life Breakpoint Example

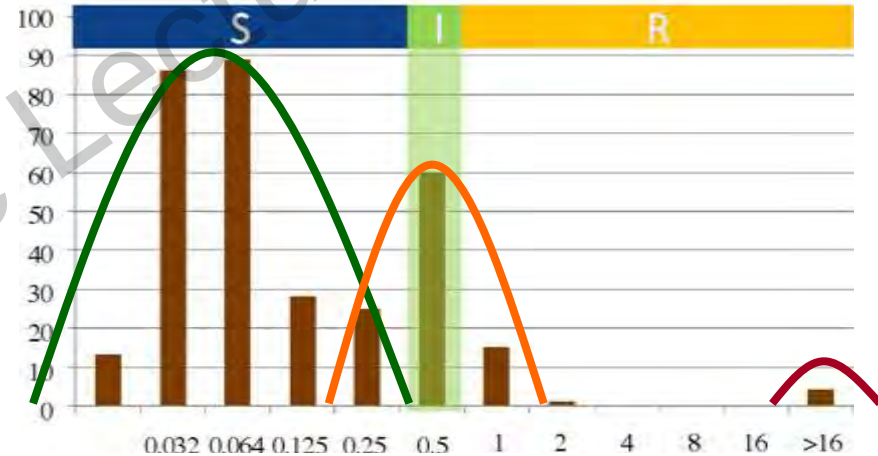
A. fumigatus

Itraconazole



Clear separation WT and non-WT

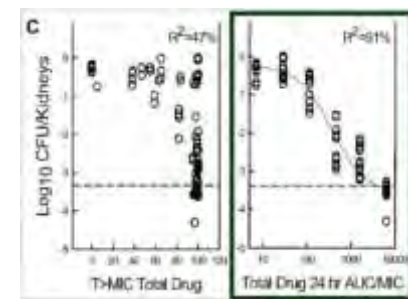
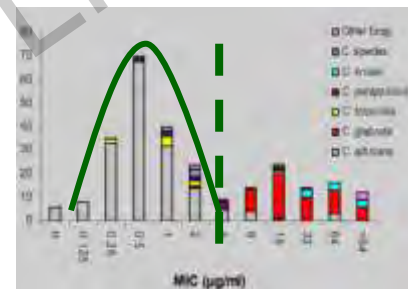
Posaconazole



Overlap WT and non-WT → further analysis needed to identify all non-WT

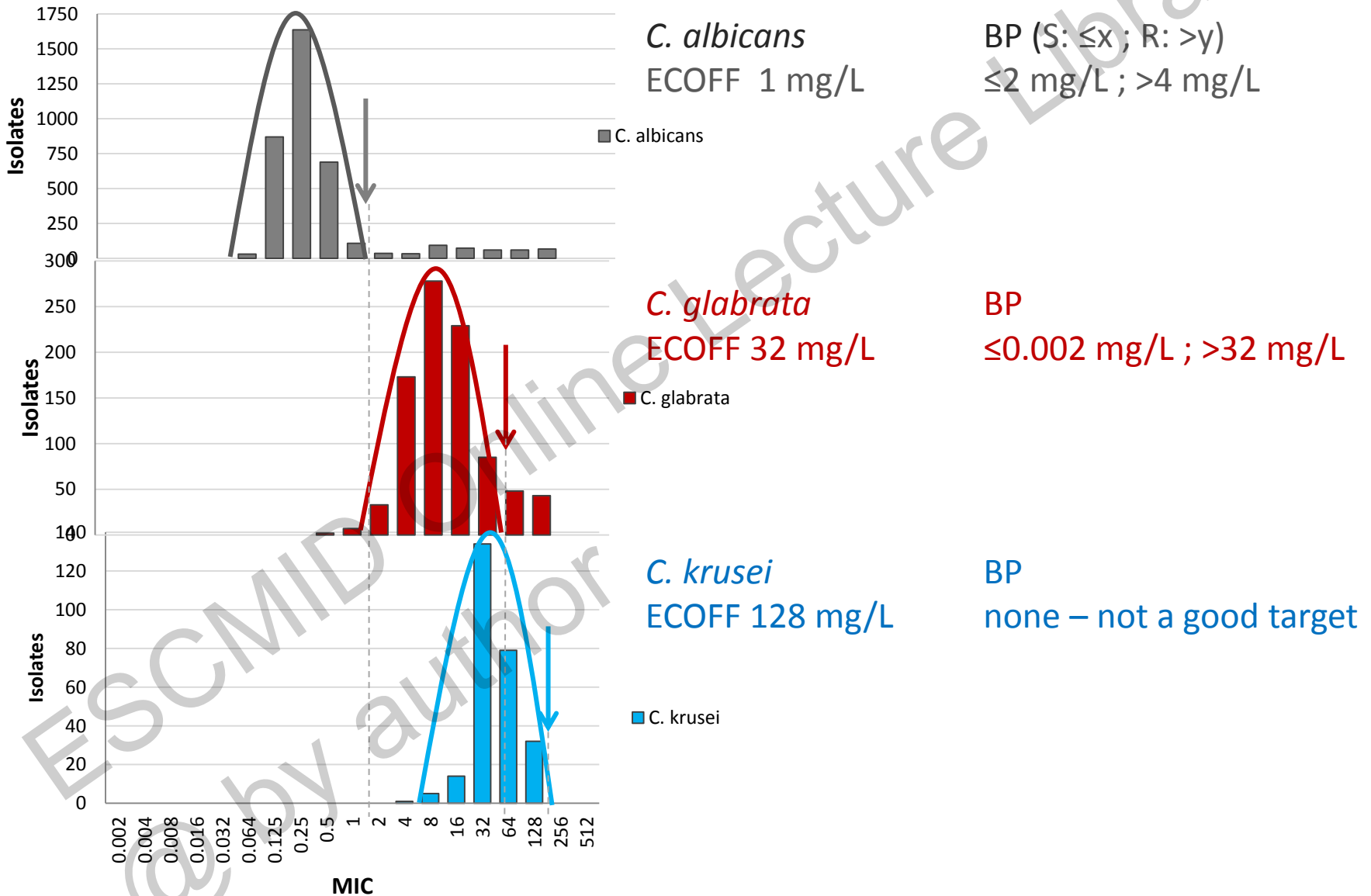
EUCAST BP Setting Process

- MIC distributions
 - Per species
 - Several data sets
 - Epidemiological Cut Off Value (ECOFF)
- Dosing regimens used
- PK/PD
- MIC-clinical outcome relationships
 - Per species
 - For wild type and non-wild type isolates



MIC (µg/L)	Candidemia		OPC \geq 100 mg/d		All	
	No. cure/Total	% response	No. cure/Total	% response	No. cure/Total	% response
0.5	98/107	92	26/26	100	124/133	93
1	6/6	100	4/4	100	10/10	100
2	1/1	100	1/1	100	2/2	100
4	3/3	100	5/9	56	8/12	67
8	2/5	40	7/32	22	9/37	24
16	3/4	75	0/60	0	3/64	5

ECOFF versus Breakpoint (flucon)



C. albicans
 ECOFF 1 mg/L

BP (S: $\leq x$; R: $> y$)
 ≤ 2 mg/L ; > 4 mg/L

C. glabrata
 ECOFF 32 mg/L

BP
 ≤ 0.002 mg/L ; > 32 mg/L

C. krusei
 ECOFF 128 mg/L

BP
 none – not a good target

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Website



Home Contact Sitemap

Antifungal susceptibility testing (AFST)

- Organization
- EUCAST News
- Clinical breakpoints
- Expert rules
- Resistance mechanisms
- MIC distributions ECOFFs
- Zone distributions ECOFFs
- AST of bacteria
- AST of fungi
 - Methods of antifungal susceptibility
 - Rationale documents for antifungals
 - Documents for discussion in AFST
 - Publications in journals
 - Meetings and Minutes
- AST of veterinary pathogens
- Frequently Asked Questions (FAQ)

The European Committee on Antimicrobial Susceptibility Testing – EUCAST

search term Search

Antifungal susceptibility testing (AFST) ▾

Antifungal susceptibility testing (AFST)

Methods for susceptibility testing of *Candida* and *Aspergillus* are developed and validated by the EUCAST subcommittee on AFST.

New and revised documents open for consultation will until accepted be published in the → EUCAST News section together with all other consultations from EUCAST.

Information on subcommittee organisation and members are available on the webpage describing the → Organisation of EUCAST.

- Clinical breakpoints
- Methods of antifungal susceptibility testing
- MIC distributions for antifungal agents
- Rationale documents
- Documents for discussion
- Publications in journals

www.eucast.org

ECOFF/Breakpoint Updates

- Recent updates (since ECCMID 2014):
 - Mould E.DEF 9.2 definitive document
 - Itraconazole *Candida* rationale document
 - BP table (itra *Candida* BP added, caspo BP comment revised)
- Forthcoming documents to look out for in 2015:
 - Amphotericin B *Candida* rationale document
 - Posaconazole *Candida* rationale document
 - Voriconazole *Candida* rationale document
 - Isavuconazole *Aspergillus* rationale document

Acknowledgements

The EUCAST Steering Committee

Maiken Arendrup
Joseph Meletiadis
Johan Mouton
Manuel Cuenca-Estrella
Cornelia Lass-Flörl



The EUCAST General (Sub) Committee

Thank you for your attention