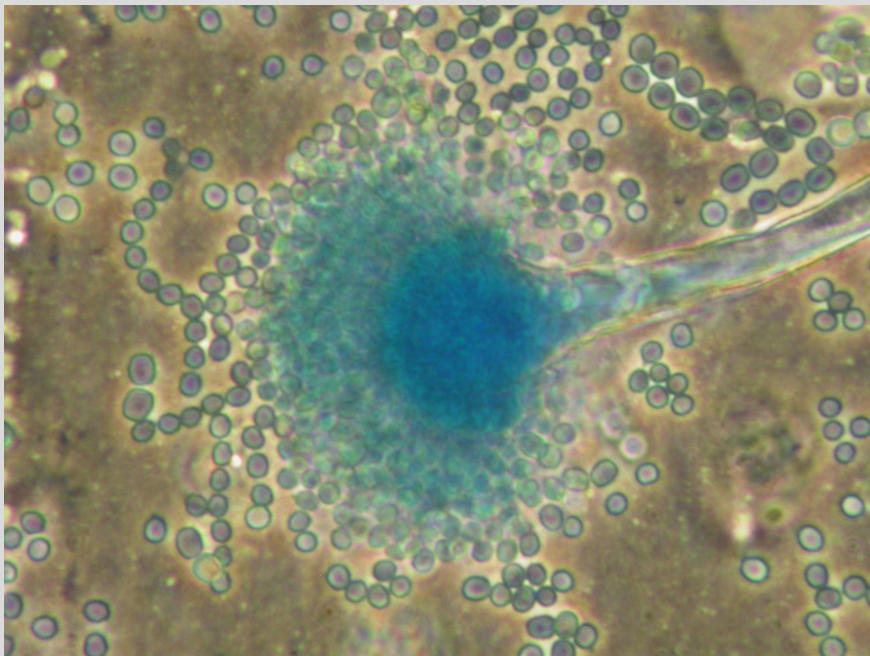


Aspergillus

Resistance mechanisms

Susceptibility testing & breakpoints

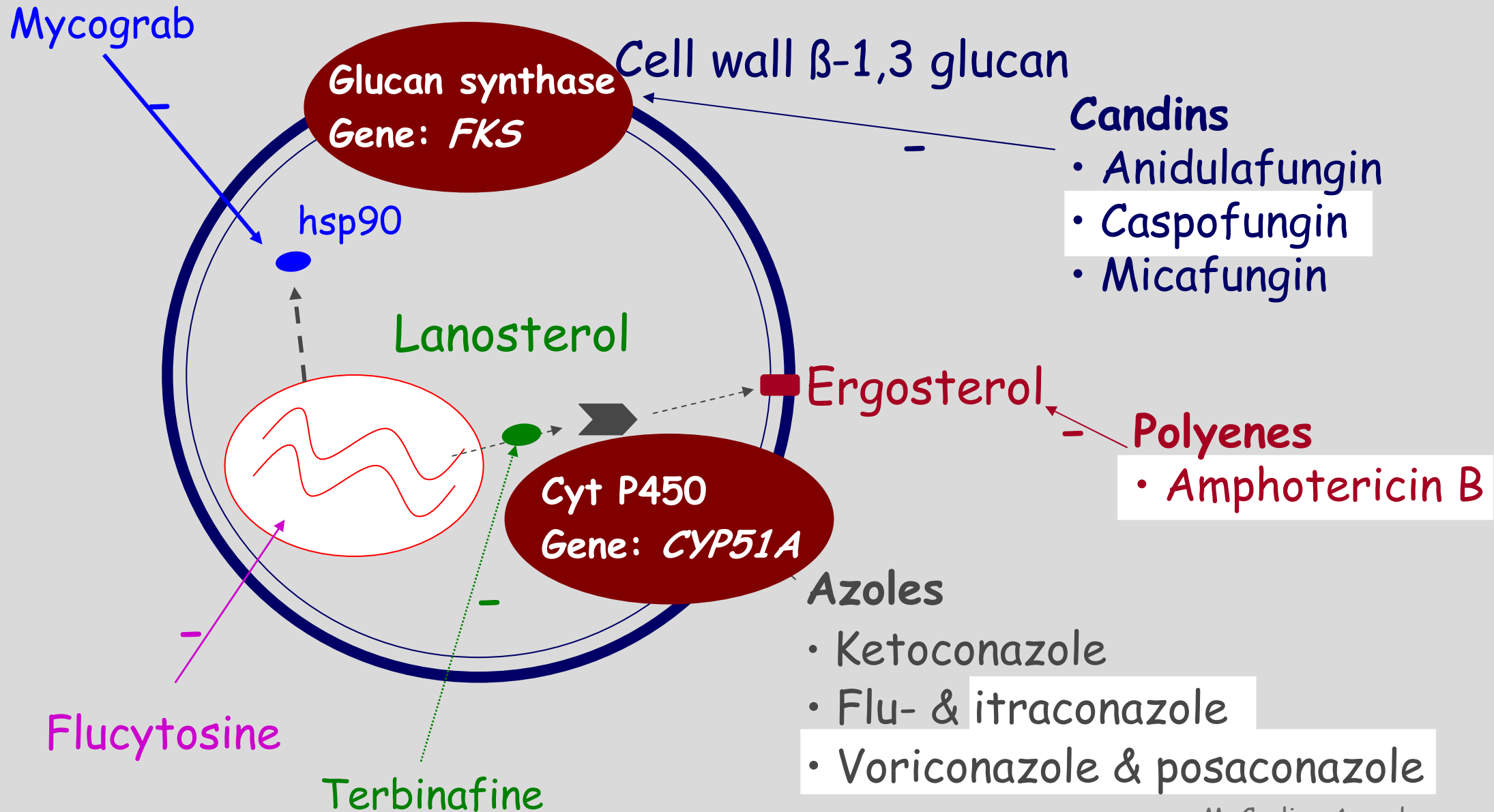


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Agenda

- Drug classes and resistance mechanisms
 - In vivo efficacy
- Non-molecular Susceptibility Testing Methods
 - Azoles, candins and amphotericin
- Interpretation of the MIC or MEC result
 - Wildtypes \gg non-wild types \gg resistance
 - Epidemiological cut off values (ECOFFs) & Breakpoints

Systemic Antifungals: Mode of Action

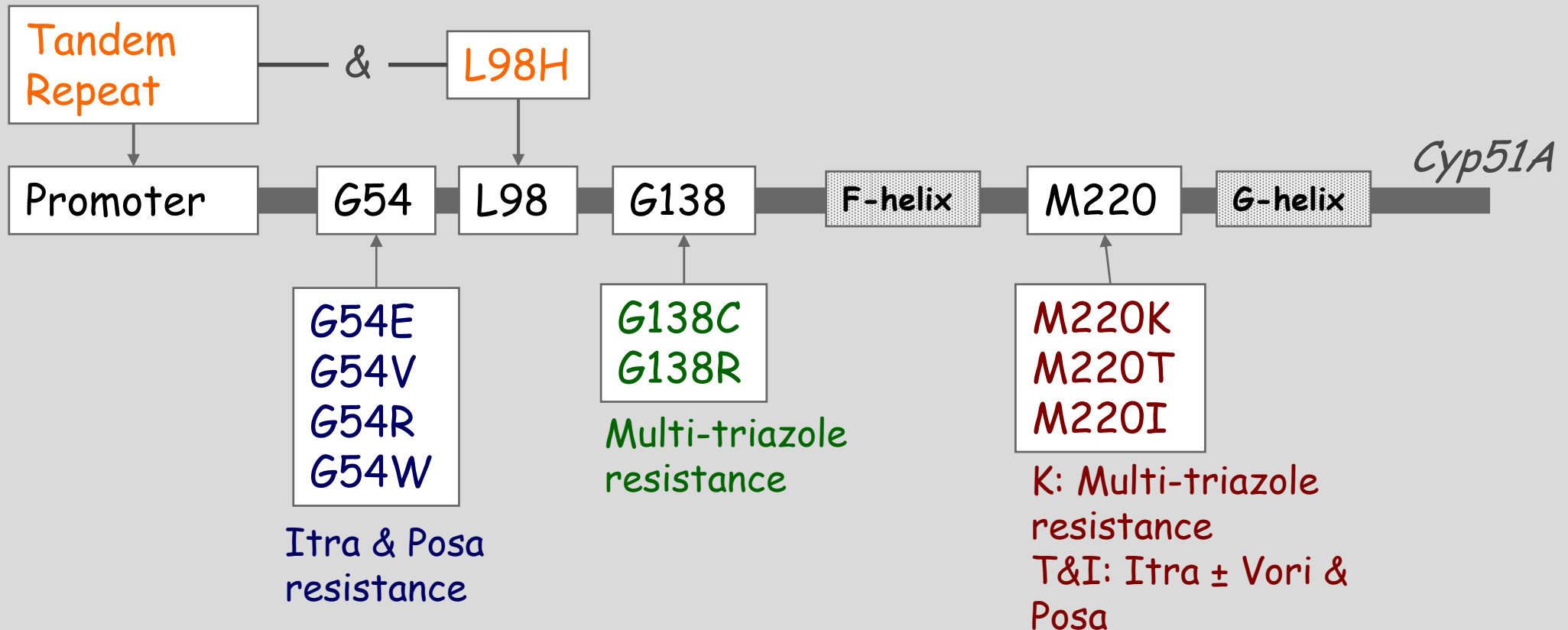


Acquired Resistance in *Aspergillus*

	Azoles	Echinocandins	Amphotericin B
Target	P450 demethylase	Glucan synthase	Ergosterol
Target gene mutation	<i>CYP51A</i>	<i>FKS1</i>	
Target up-regulation	<i>CYP51A+</i> Promotor	✓	
Efflux pumps	✓		

Azole resistance: *CYP51A* mutations

Up-regulation of gene expression
Multi-triazazole resistance

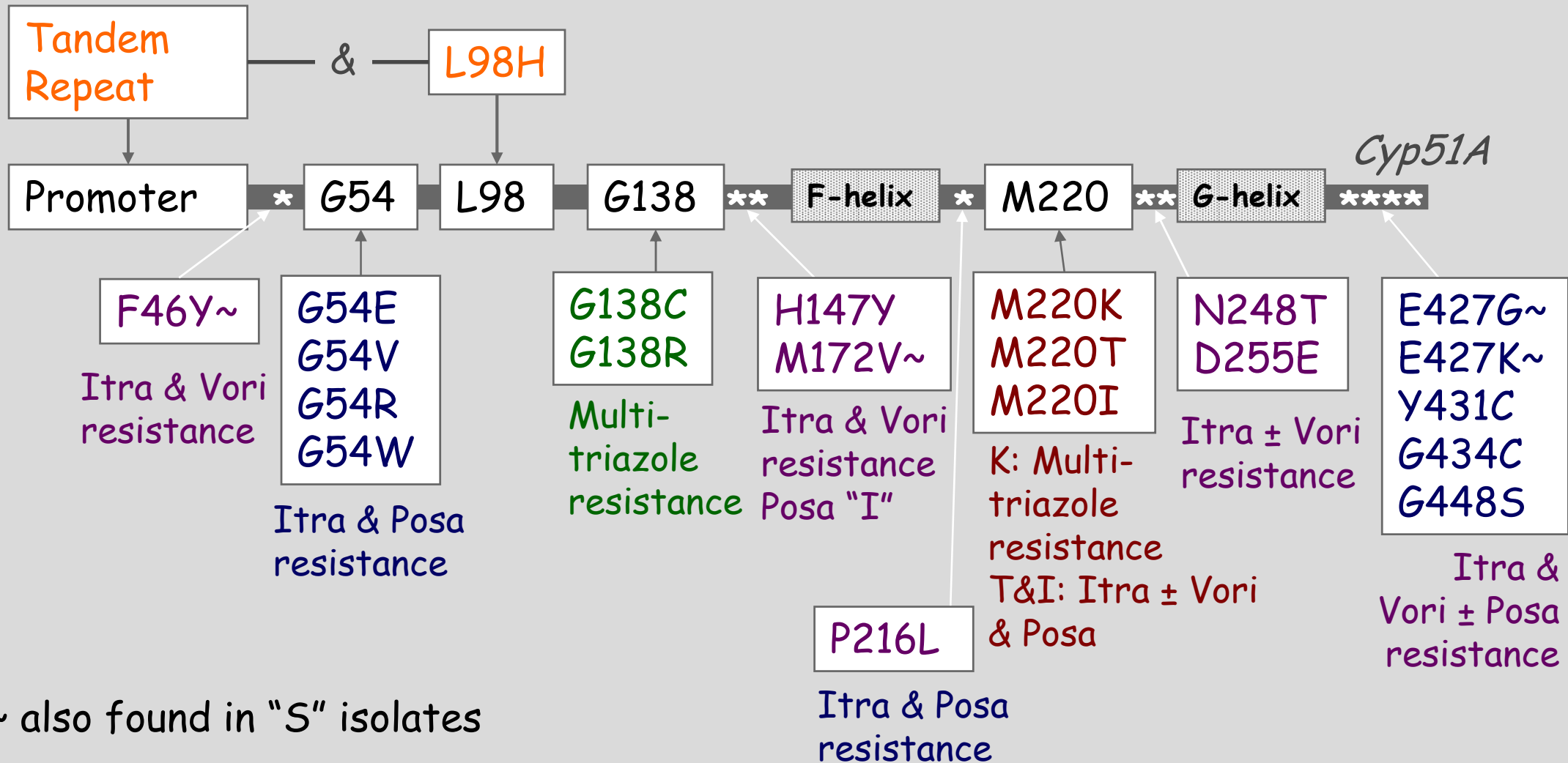


New mutations are continuously reported

Azole resistant isolates without *CYP51A* mutations have been reported

Azole resistance: *CYP51A* mutations

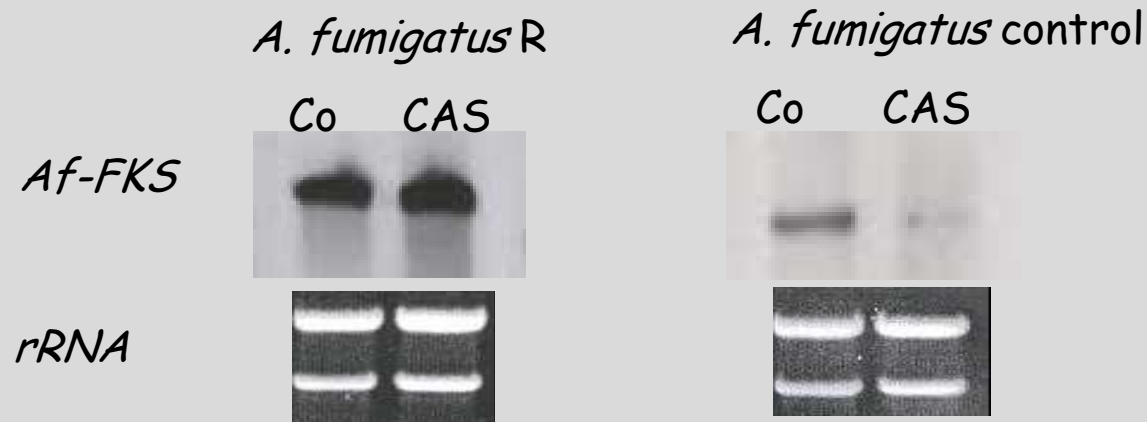
Up-regulation of gene expression
Multi-triazazole resistance



Echinocandin resistance

- Mutations in *FKS1* gene coding glucan synthase
 - S678Y laboratory generated mutant
 - S678P laboratory generated mutant

- Over-expression of the gene



Co: untreated *A. fumigatus*,

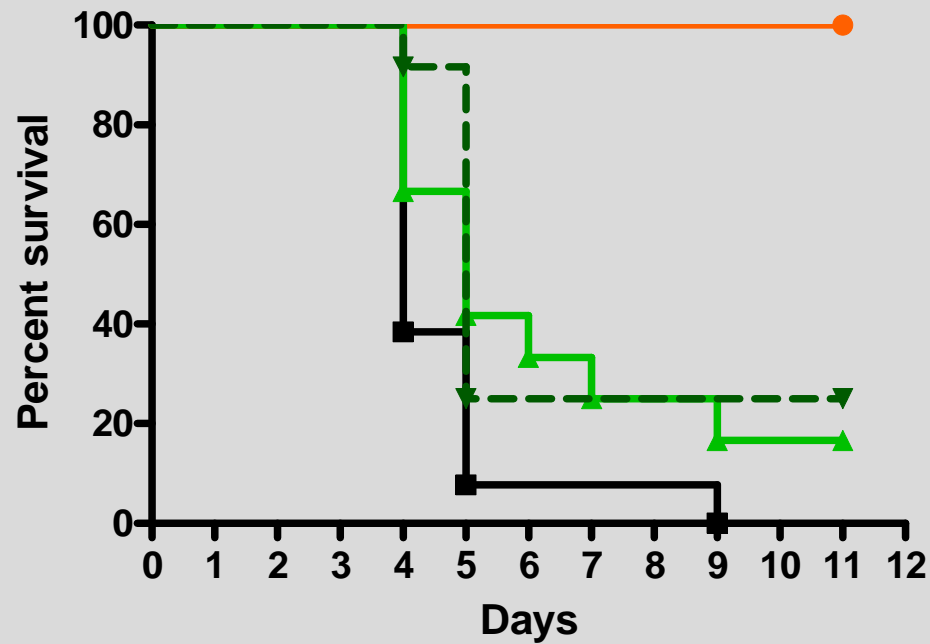
CAS: CAS treated *A. fumigatus*



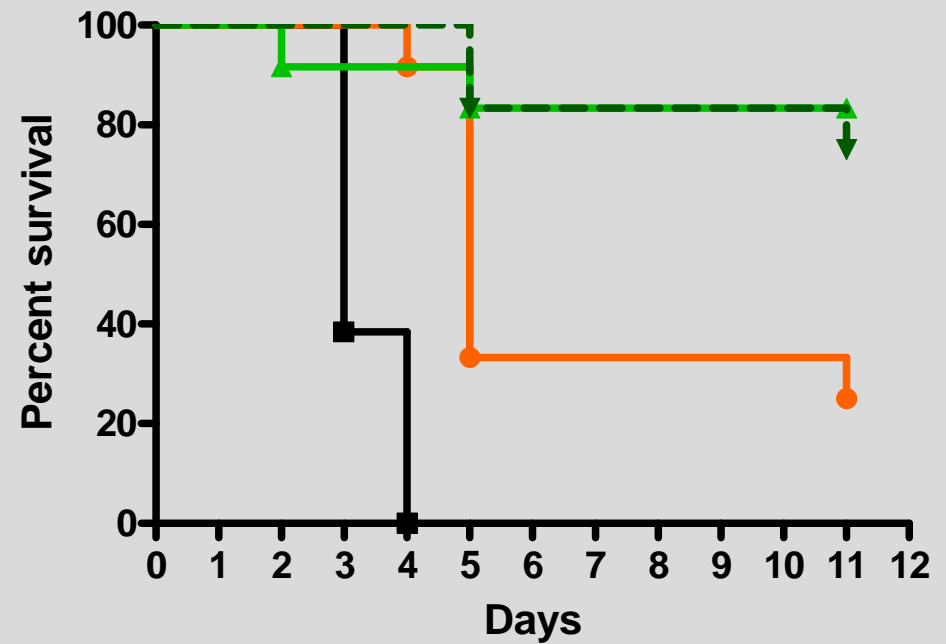
Correlation to *in vivo* efficacy

Mice inoculated with *A. fumigatus* and subsequently treated for 10 days

Pan-Azole R isolate (Pos > 4; M220K)



Caspofungin R isolate (up-regulation)

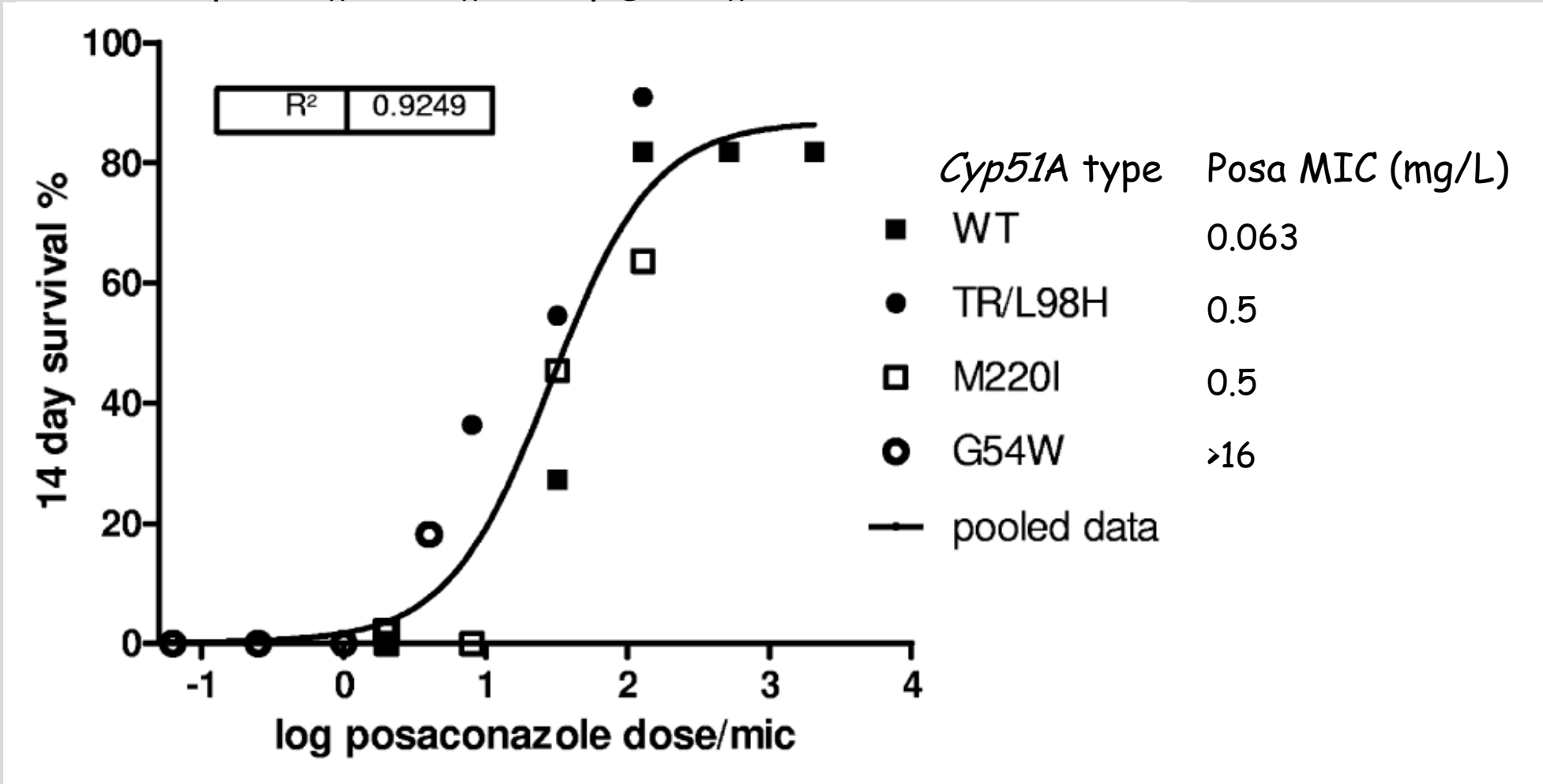


- Control
- ▼ Posaconazole High
- ▲ Posaconazole Low
- Caspofungin

- Control
- Caspofungin
- ▲ Posaconazole Low
- ▼ Posaconazole High

Posaconazole MIC-outcome correlation

Non neutropenic murine model of Disseminated IA



Susceptibility testing of moulds

■ Reference methods

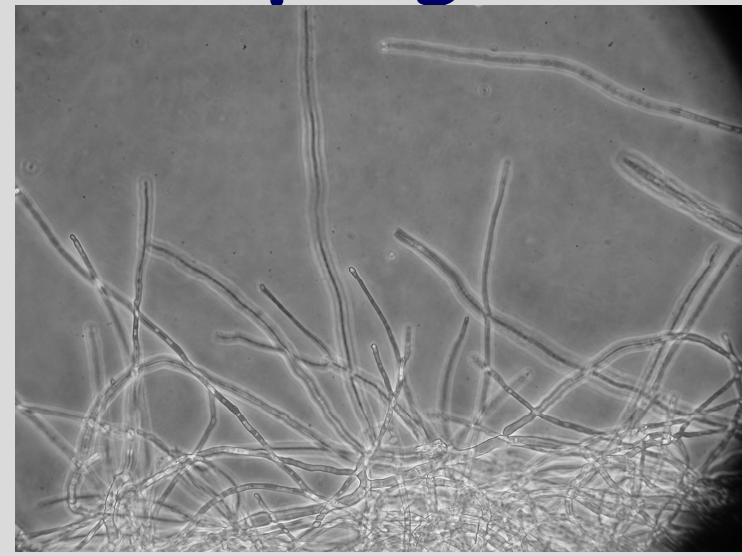
- CLSI M-38A
- EUCAST

- Visual reading of growth
- Ampho & azoles: MIC no growth
- Candins: MEC aberrant growth

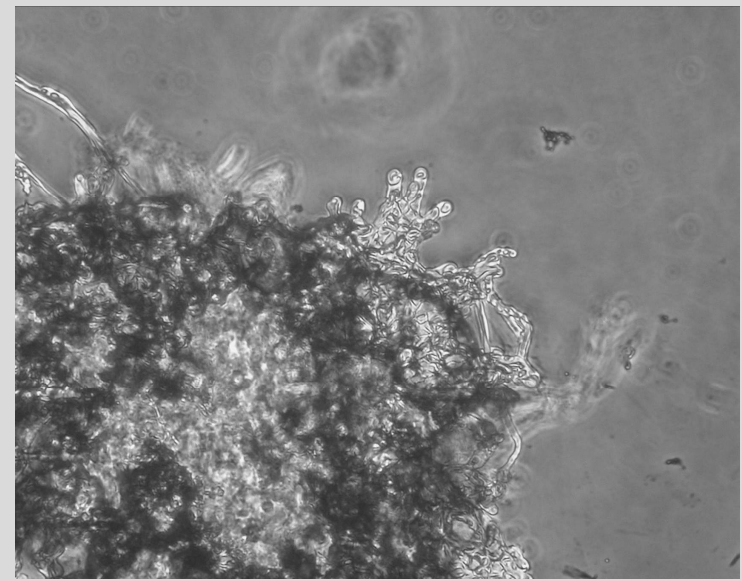
■ Alternatives

- Etest & disk diffusion
- Agar-dilution

Microdilution MIC / MEC Aspergillus



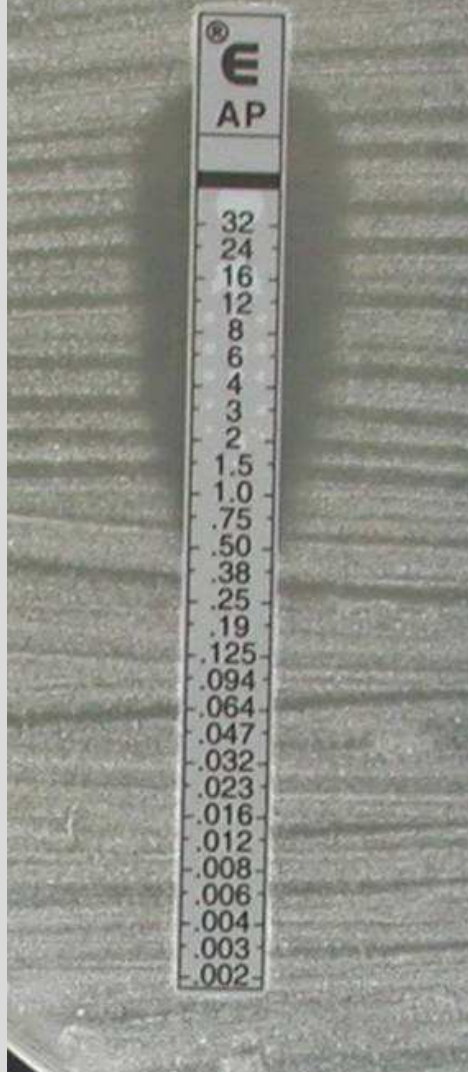
Growth control



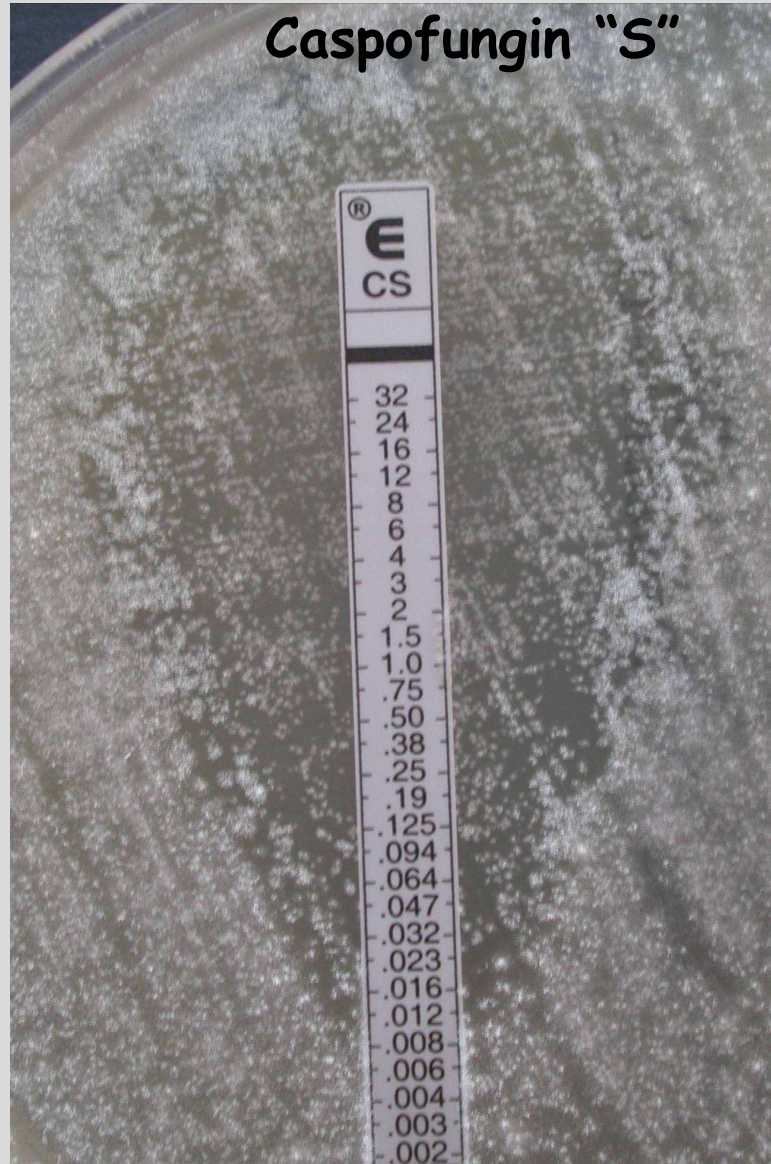
Caspofungin 0.06 µg/ml

Aspergillus susceptibility testing Etest

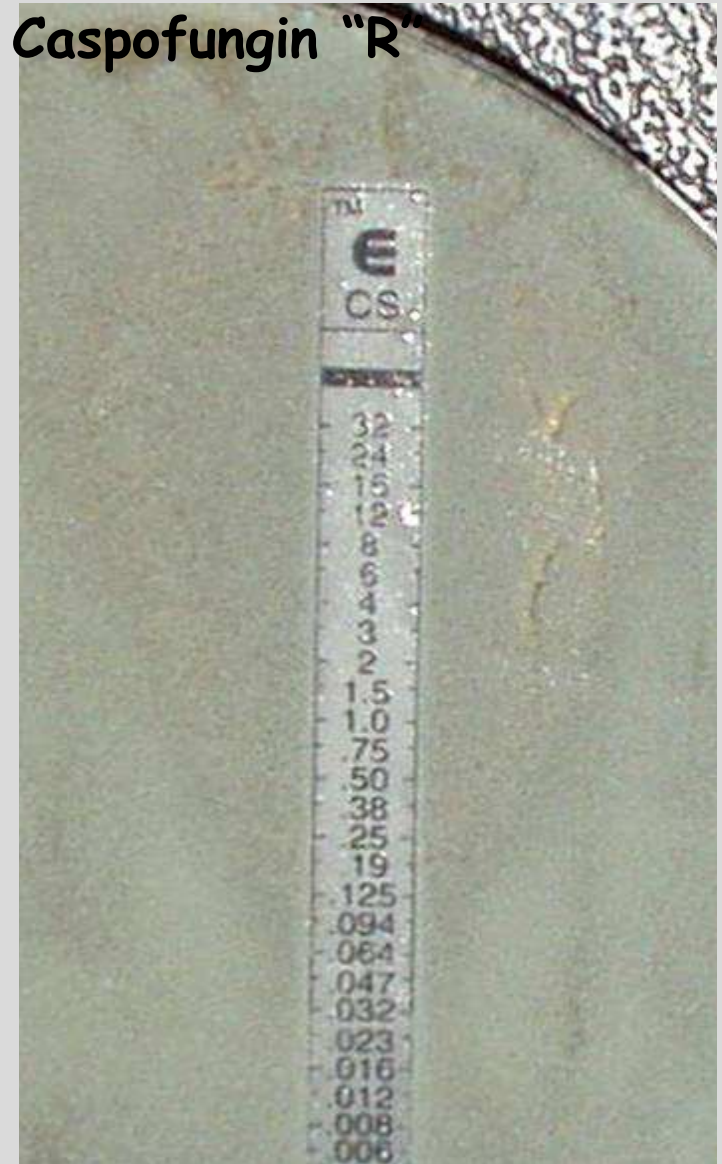
Amphotericin "S"



Caspofungin "S"



Caspofungin "R"



Agar dilution- screening agar

**Azole
S**



**Azole
R**



**Caspo
S**



**Caspo
R**

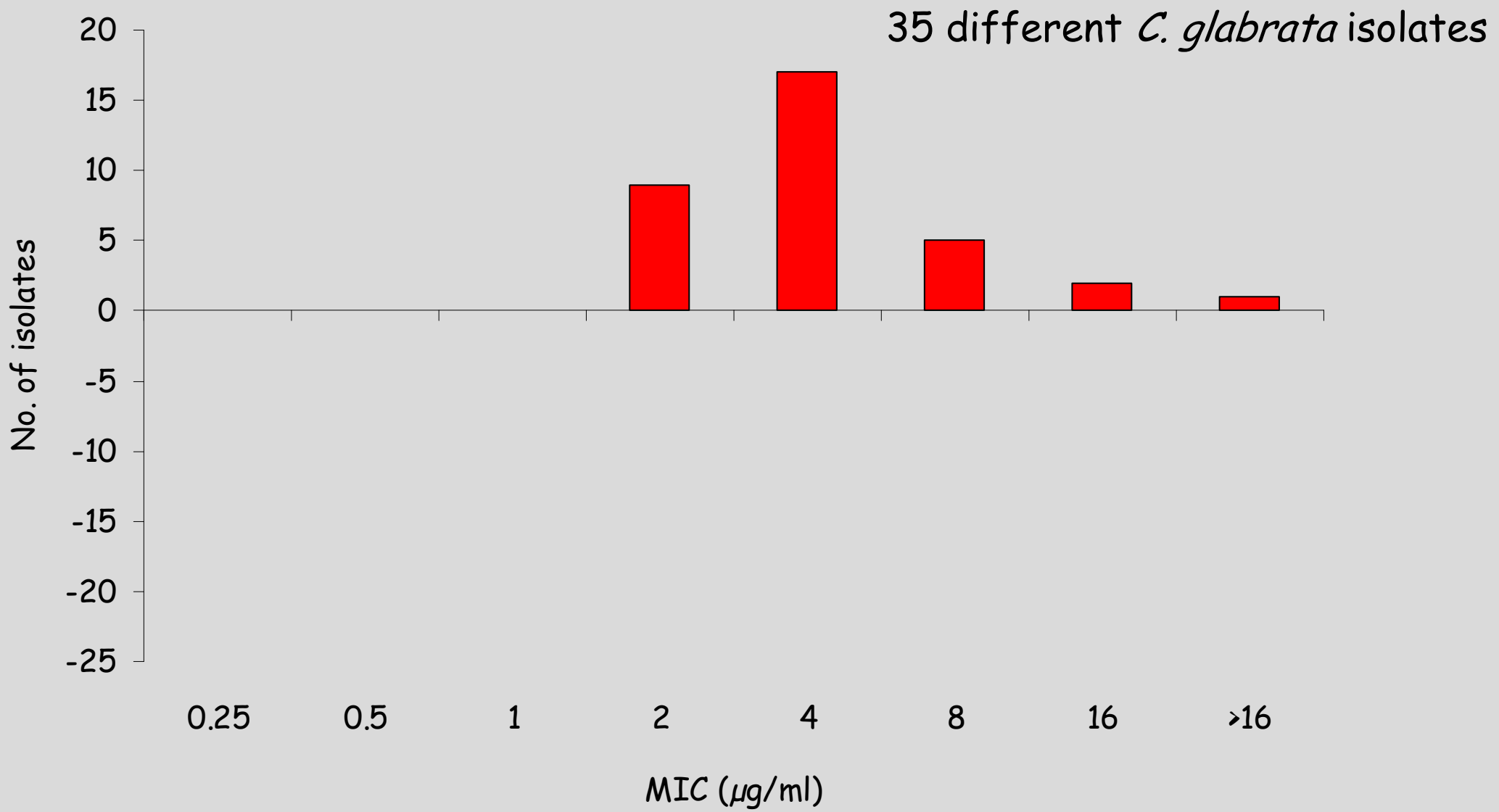
AfS678P
D. Perlin
Rocha AAC
2007



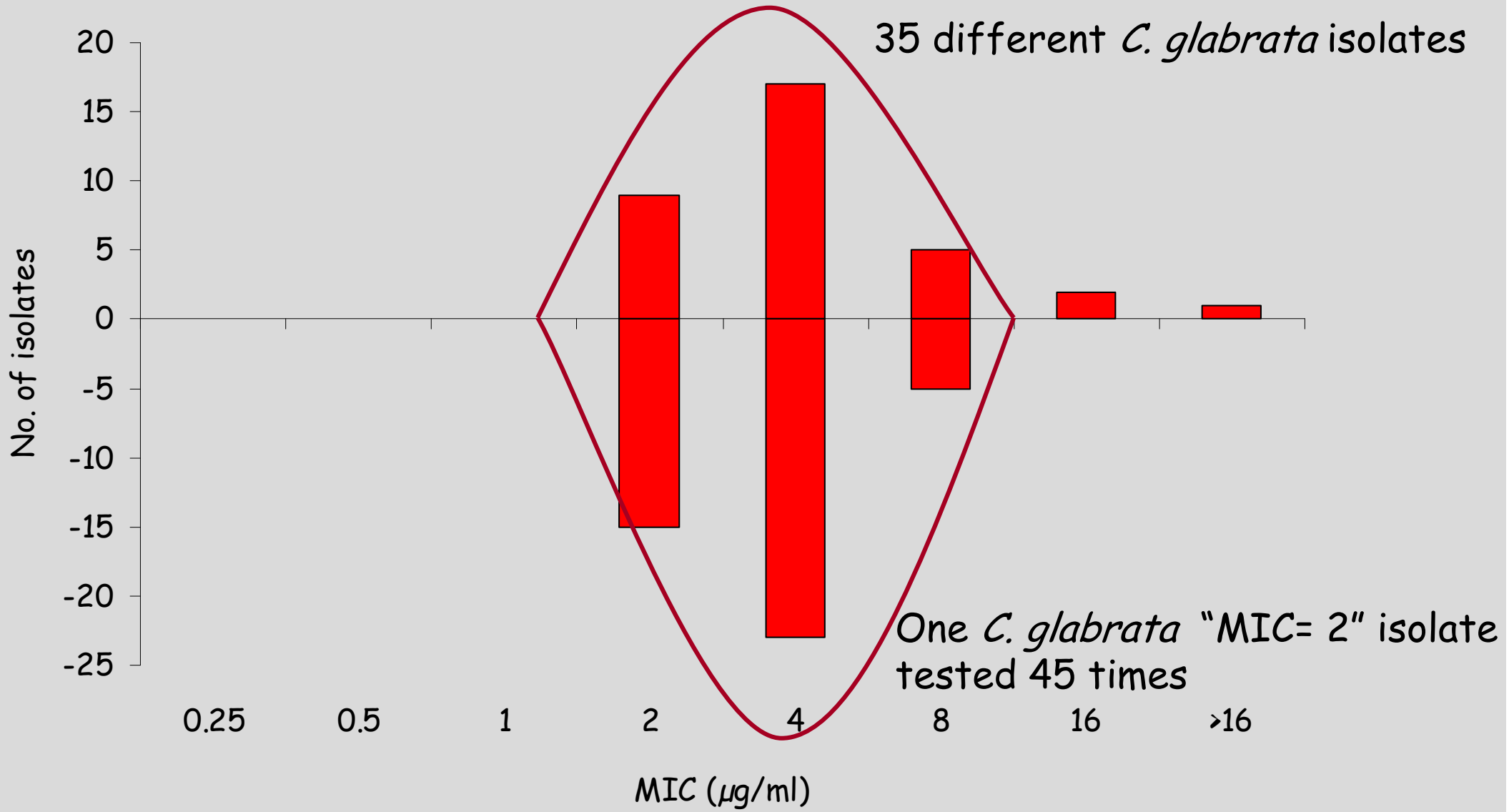
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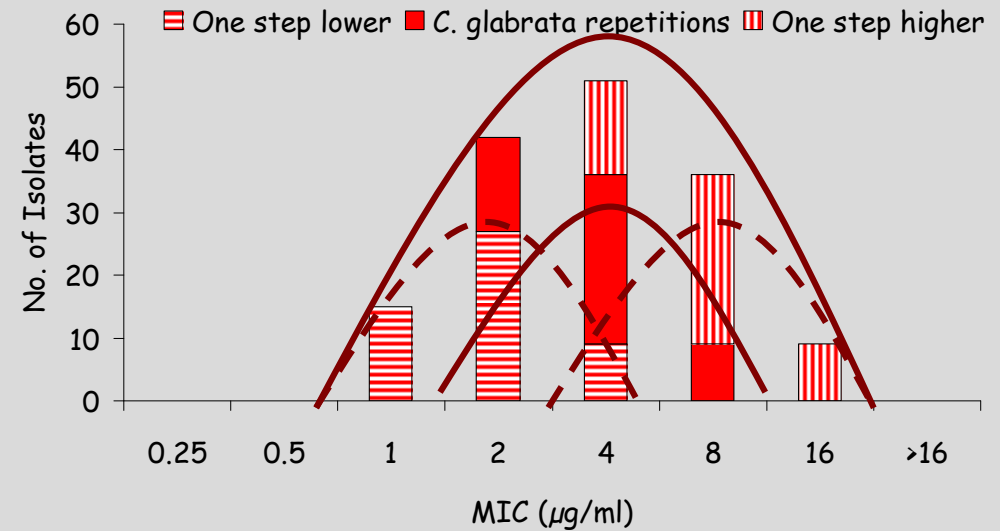
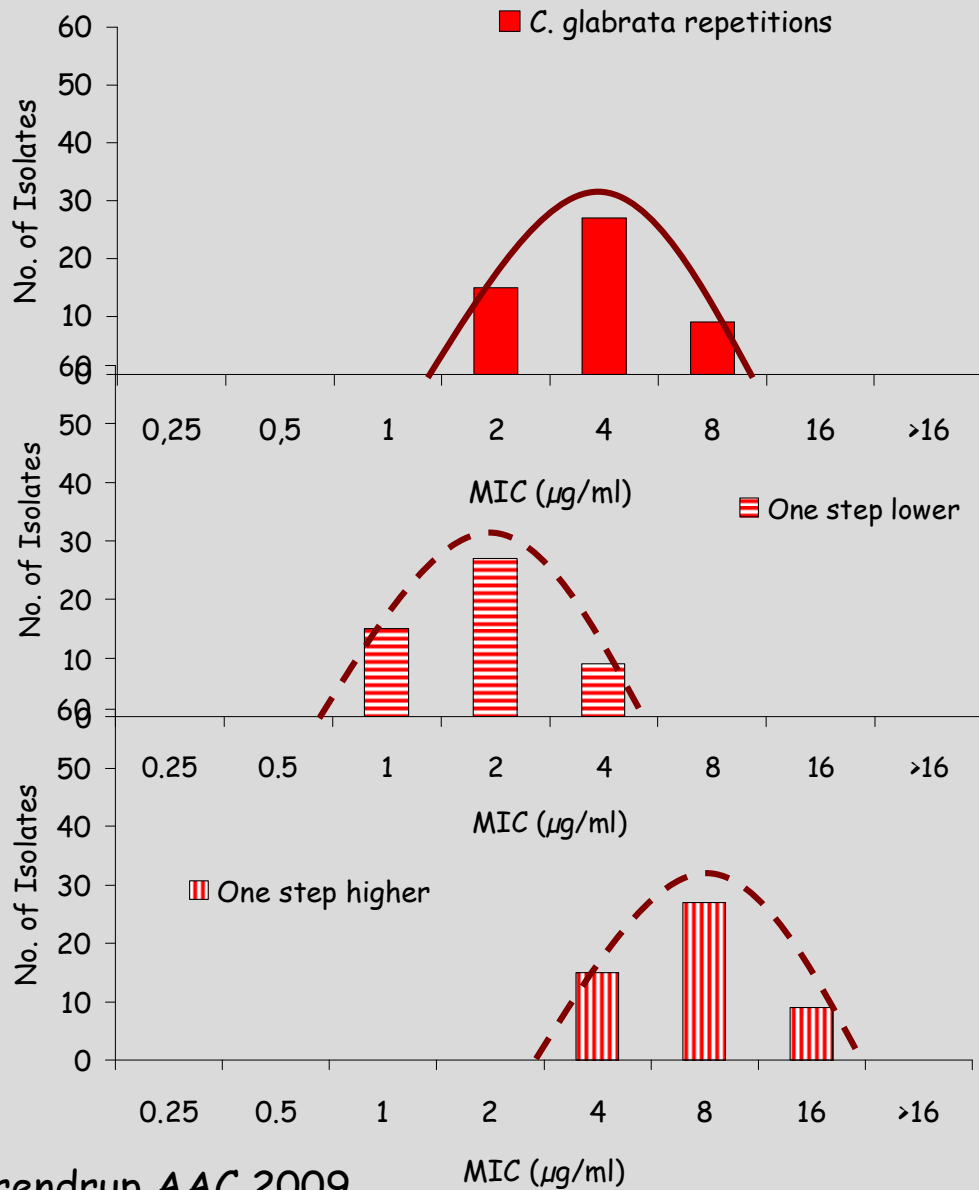
Wild-types EUCAST MICs



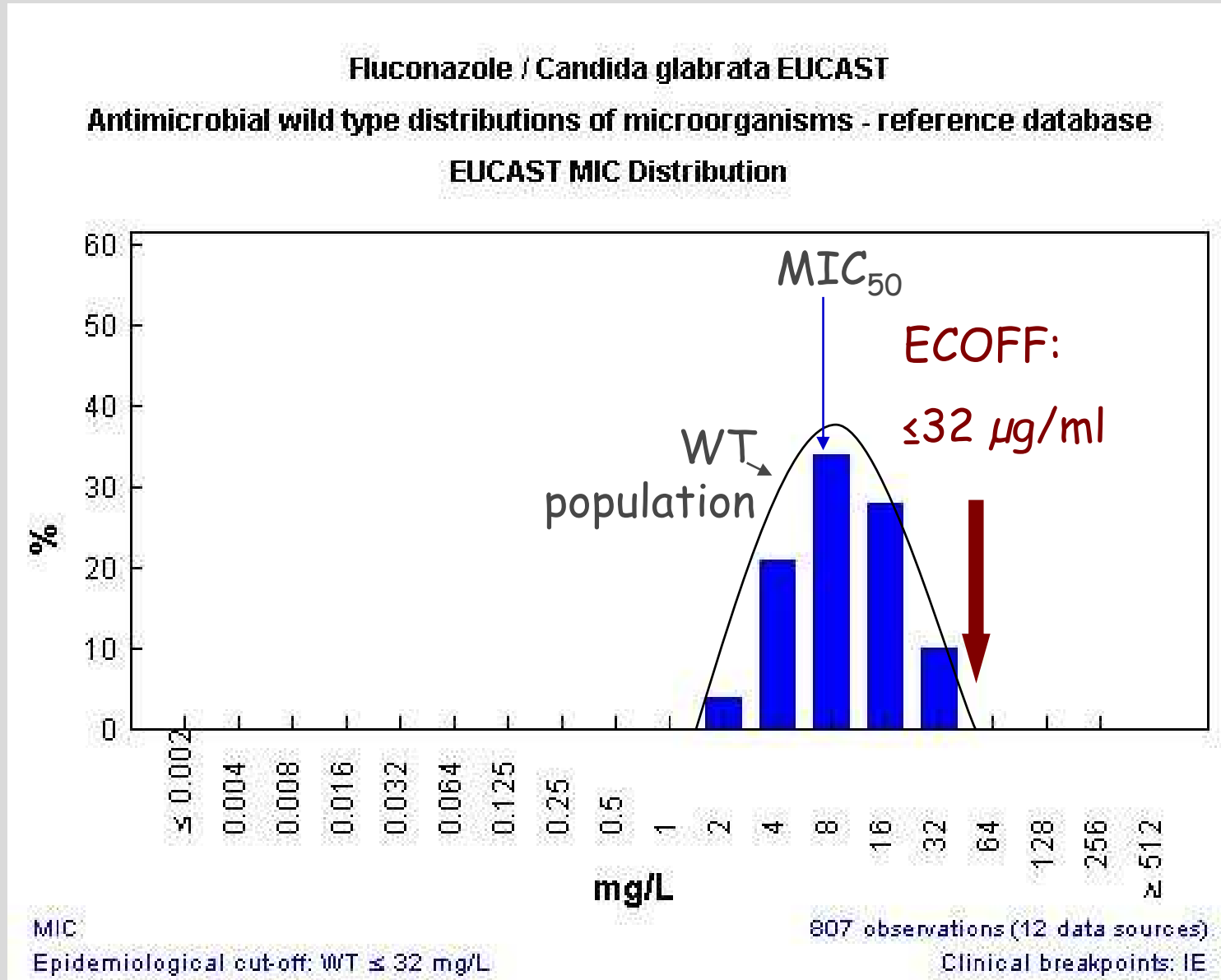
Wild-types EUCAST MICs



Wild-type distributions incl. variability

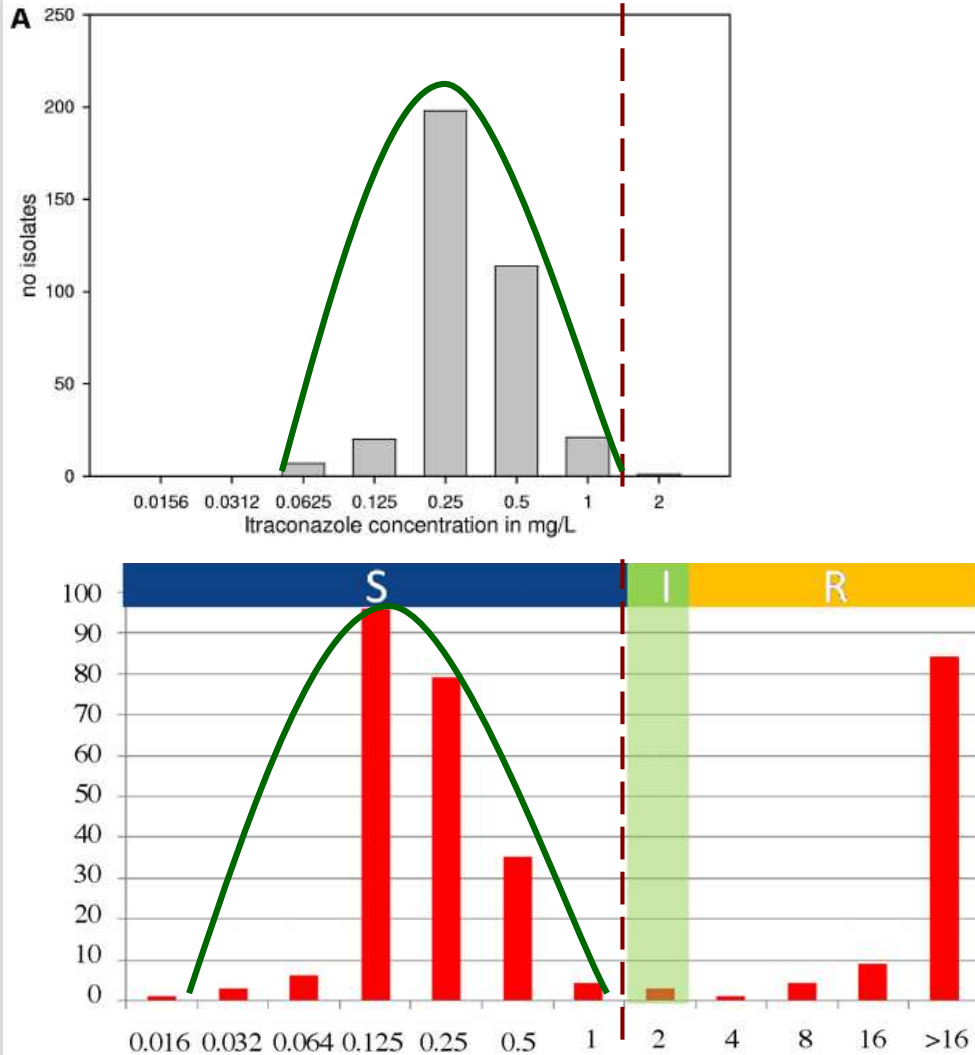


EUCAST fluconazole MIC *C. glabrata*



A. fumigatus Itraconazole MIC distribution

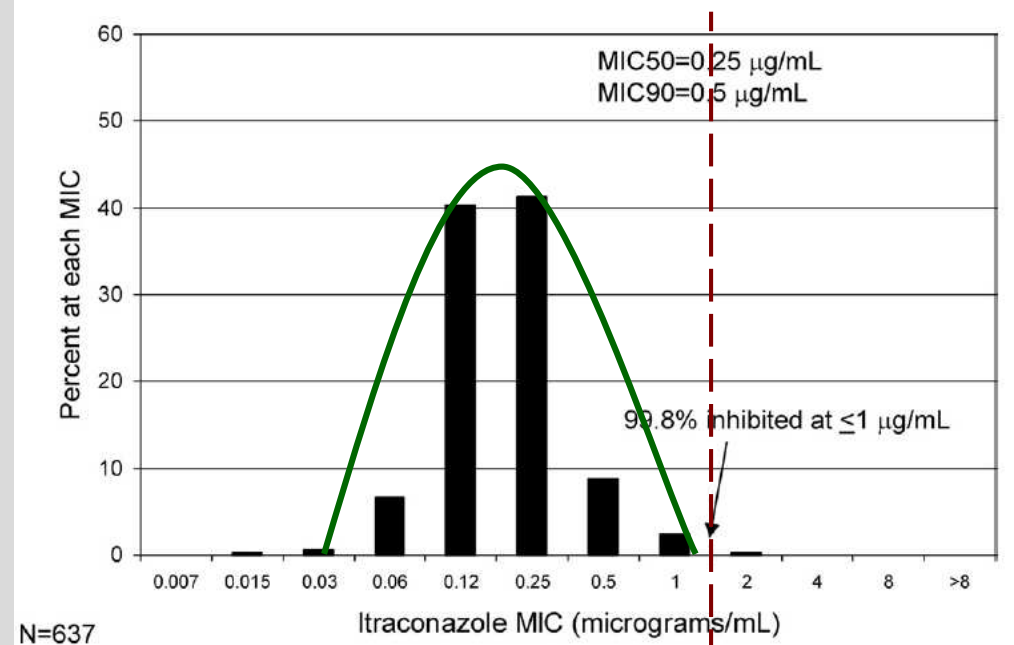
EUCAST



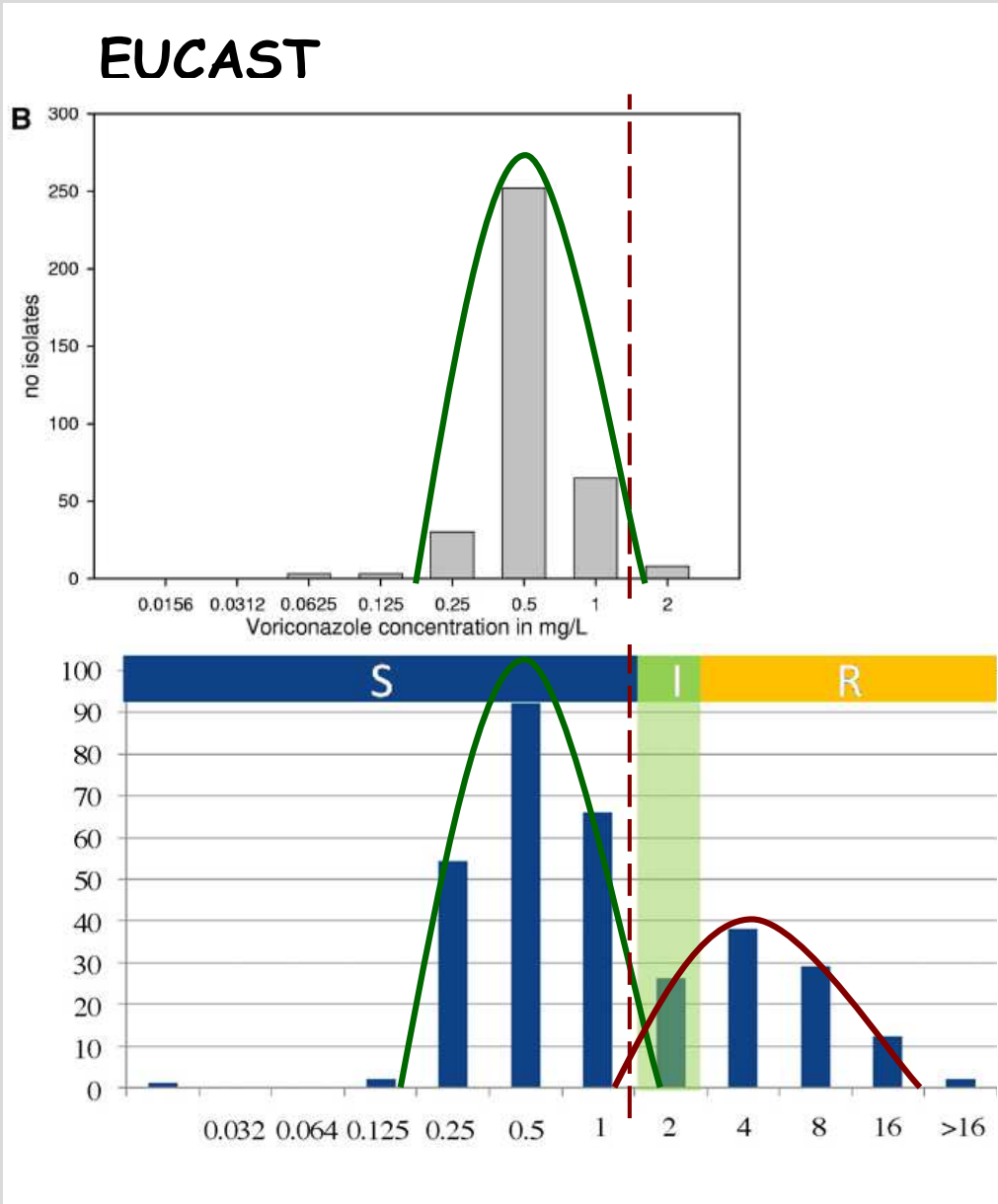
MIC_{50} at 0.25 $\mu\text{g/ml}$

ECOFF: $\leq 1 \mu\text{g/ml}$

CLSI



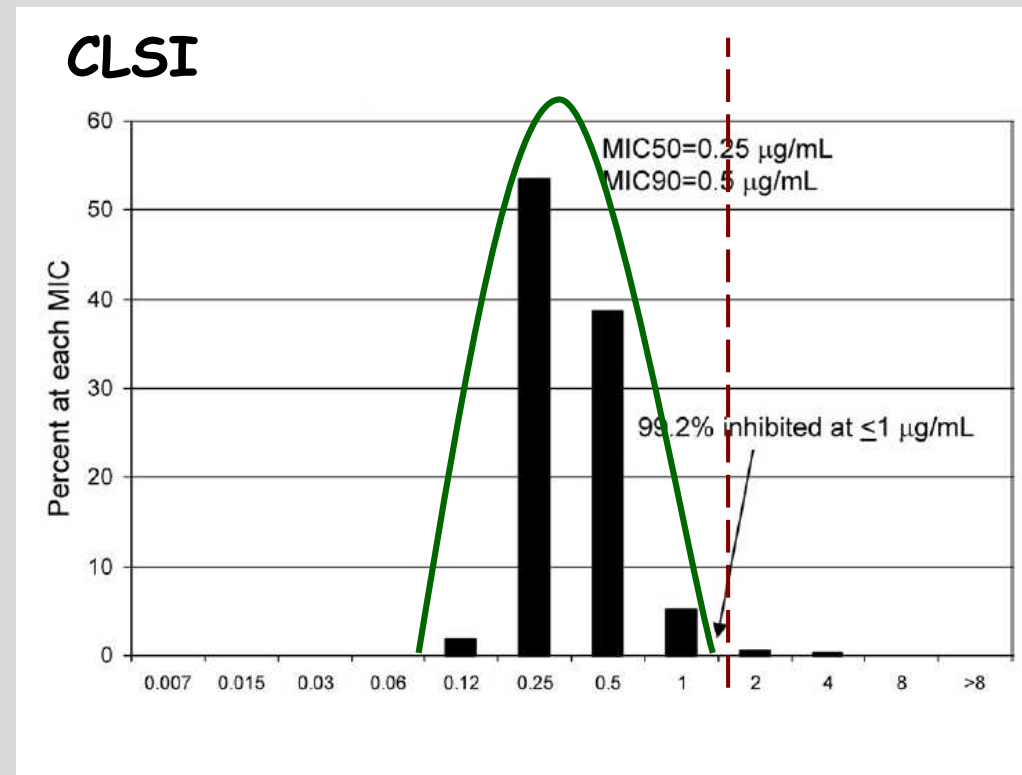
A. fumigatus Voriconazole MIC distribution



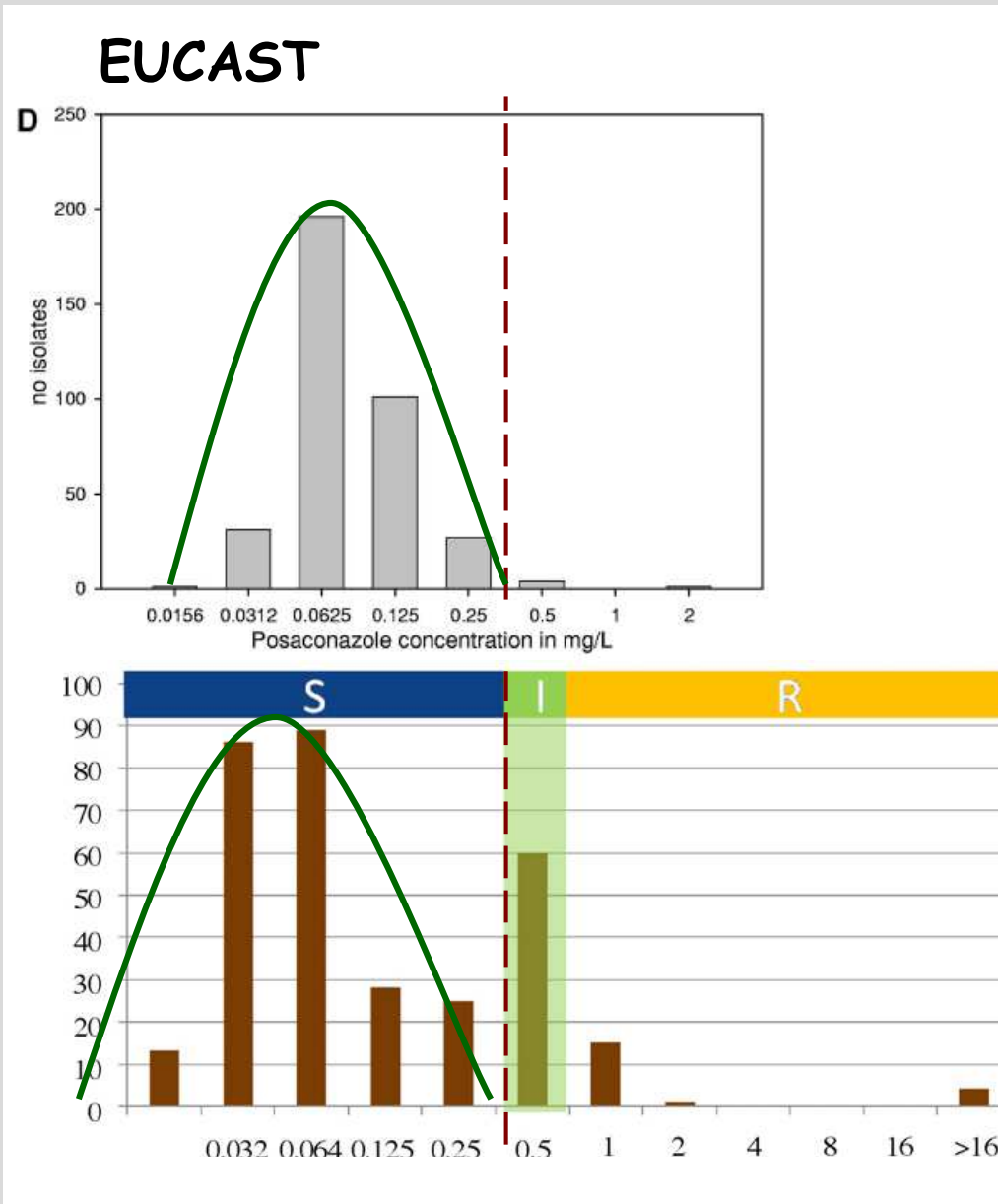
MIC_{50} EUCAST at 0.5 $\mu\text{g/ml}$

MIC_{50} CLSI at 0.25 $\mu\text{g/ml}$

ECOFF: $\leq 1 \mu\text{g/ml}$



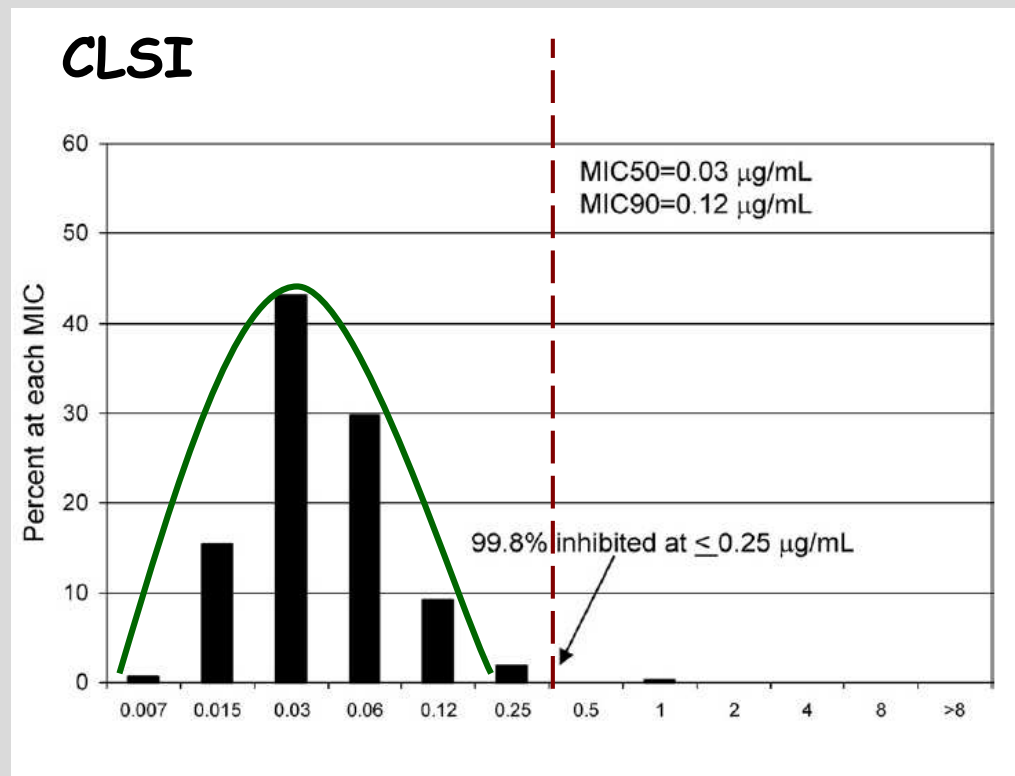
A. fumigatus Posaconazole MIC distribution



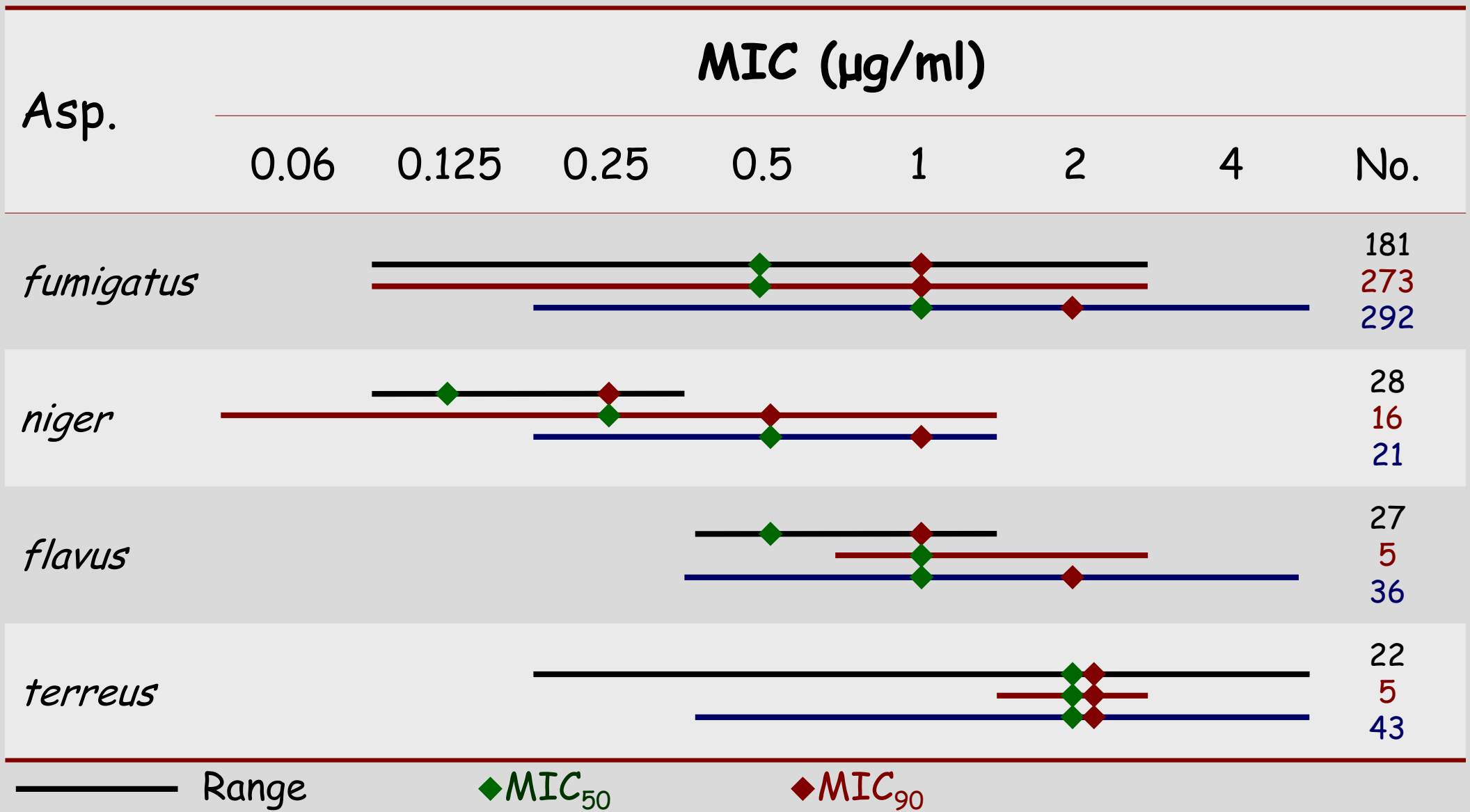
MIC_{50} EUCAST at 0.064 $\mu\text{g/ml}$

MIC_{50} CLSI at 0.032 $\mu\text{g/ml}$

ECOFF: $\leq 0.25 \mu\text{g/ml}$



Aspergillus and Amphotericin B





ECOFFs and Breakpoint suggestions

A. fumigatus

	ECOFF / Susceptible	Intermediate	Resistant
Amphotericin B	$\leq 1 \mu\text{g/ml}$	-	-
Itraconazole	$\leq 1 \mu\text{g/ml}$	2	>2
Voriconazole	$\leq 1 \mu\text{g/ml}$	2	>2
Posaconazole	$\leq 0.25 \mu\text{g/ml}$	0.5	>0.5

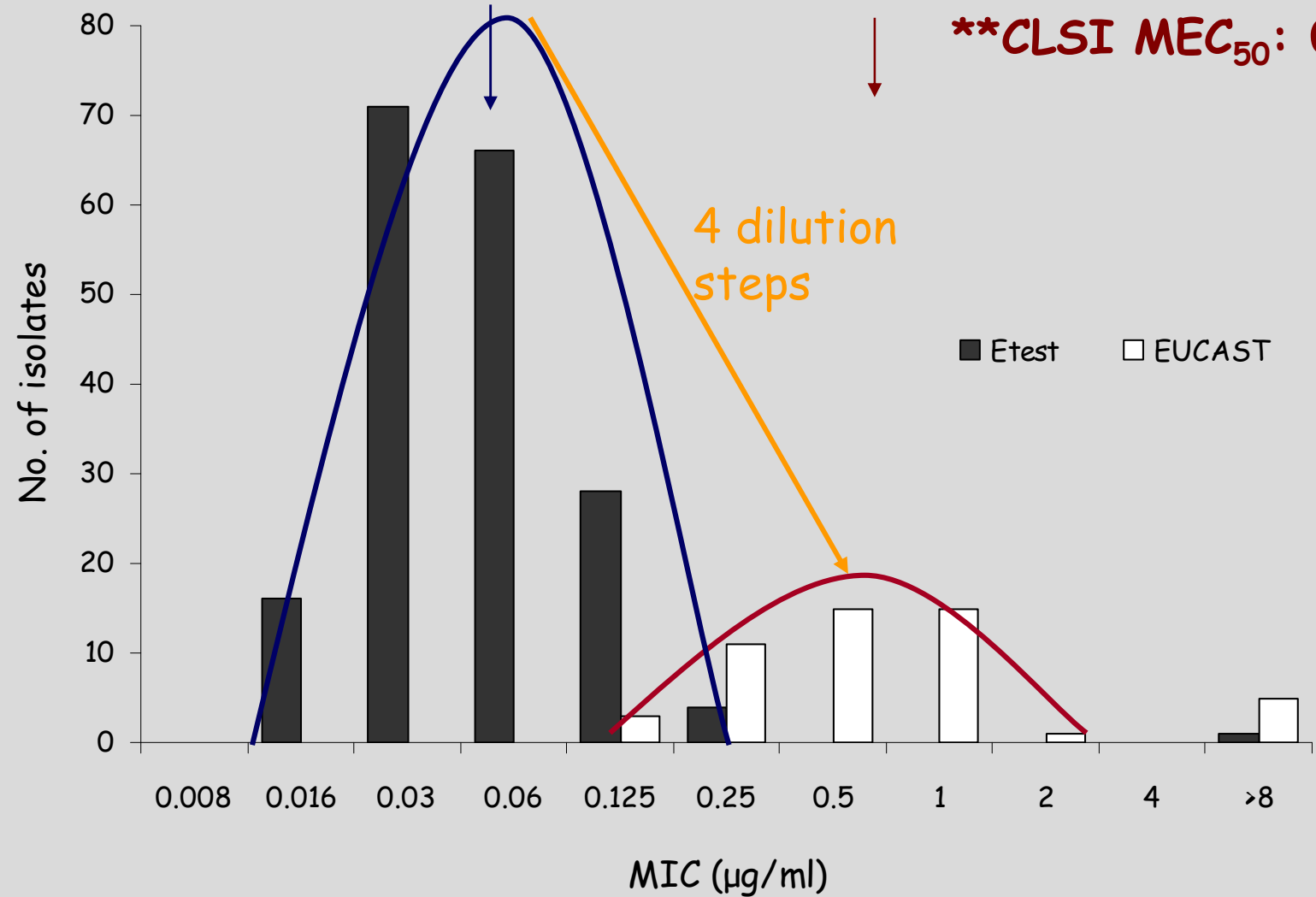
Caspofungin MEC - Etest and Microdil.

Etest MEC_{50} : 0.03 $\mu\text{g/ml}$
 Etest MEC_{50} : 0.06 $\mu\text{g/ml}$

*CLSI- MEC_{50} : 0.25 $\mu\text{g/ml}$ 
 EUCAST- MEC_{50} : 0.5 $\mu\text{g/ml}$ 

**CLSI MEC_{50} : 0.015 $\mu\text{g/ml}$ 

4 dilution steps



Conclusion

■ Azole resistance

- *CYP51A* mutations
- Over-expression of the target enzyme
- Efflux pumps
- Mono → pan azole
- Itraconazole Screening agar

	<i>A fumigatus</i> ECOFF
Itra	≤1 µg/ml
Vori	≤1 µg/ml
Posa	≤0.25 µg/ml

■ Echinocandin resistance

- *FKS1* mutations
- Over-expression of the target enzyme
- Susceptibility testing difficult even for “experts”!



STATENS
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Thank you for your attention