CLSI Update

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Objectives

- The main focus of CLSI has been on developing Epidemiological Cut-off Value (ECVs) for drugs-bugs
- Introduce the concept of (ECVs),
- Start the process of educating the community about ECVs

Basic Assumptions

- ECVs are a feature of a single species

  –they cannot be applied or extrapolated to a genus or other larger grouping

- ECVs are "the same everywhere"

  –they do not change over time or vary geographically

Clinical Breakpoints (CBPs) VS. (ECVs)

- **ECVs are not always the same as CBPs**
- **CBPs are used to indicate those isolates that are likely to respond to treatment with a given antimicrobial**

- **ECV:**

  - Define upper limit of "wild type" MIC distribution – no acquired resistance mechanisms

  - **Establishes cutoffs to help detect emergence of reduced susceptibility, thereby may have utility in identifying strains with acquired resistance**

  - **Light-Bulb:** the organism requires further characterization

Turnidge & Paterson, Clin Micro Rev 2007;20:391-408

- The ECV distinguishes between organisms without and with phenotypically expressed resistance mechanisms for a species and a drug in a defined test system.

– Within a species, it is the highest MIC of organisms lacking phenotypically expressed resistance.

The CBP

— MIC-concentrations decided by "Committee" to distinguish treatable from non-treatable organisms.

— CBP may render the Wild Type Susceptible (S), Intermediate (I) or Resistant (R) but must not divide wild type organisms.

EUCADST

When should the ECV be considered in lieu of a formal clinical breakpoint

- When clinical data support the use of the drug for an approved indication and wild type organisms of one or several species.
- When there is a lack of convincing Pk/Pd data

ECVs in lieu of Clinical Breakpoints

- Can ECVs be used in lieu of clinical breakpoints?
- Never by default
- Yes, after consideration

When not to Use ECV

ECVs should not be used:

- when CBPs have been published
- as a strong predictor of clinical response to therapy but rather as an indicator of possible acquired resistance mechanisms which could impact response to treatment

ECV

Allows the clinician to differentiate between

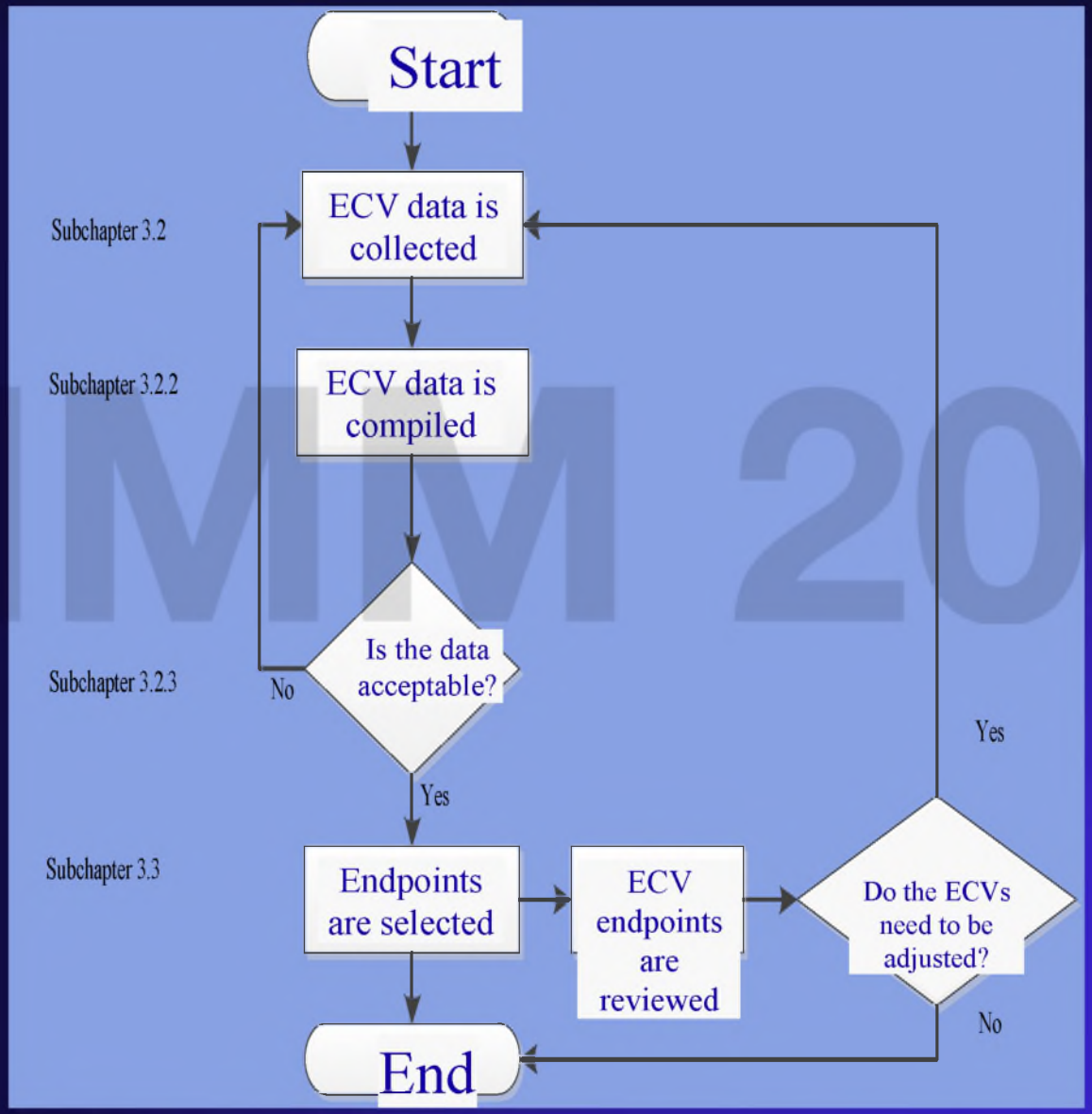
- Normal
  - –I can rely on my clinical experience for this infection
- Different
  - –I should be aware this case may respond differently

Maiken Arendrup

Important Question

- If ECVs are to be used for interpretation of susceptibility testing results:
  - –should they be reported to the clinician?
  - –if so, how?
- **Suggestion** – report as "N" = "Non-wild-type" with a comment/footnote"

The ECV Development Process



Data Requirements

- The MICs must have been measured with a reference method
  - –ISO 20776-1 in the case of bacteria
  - –ISO 16256 in the case of yeasts
  - –Other reference methods as they are developed and agreed upon internationally

Data Requirements

- All the MICs should, as far as is feasible, be on-scale
  - –a small proportion of wild-type population values could be included as "≤"
- ideally it should be <5%, although it is possible to provide a reasonable estimate of ECVs if the mode is not also the lowest concentration tested
  - –">" and "≥" values are acceptable in the data set provided they are clearly separated from the wild-type population

Data Pooling

- Since there is known inter and intra-laboratory variation, data from several laboratories are required for estimation of ECVs to account for this variation

- The predictive power of ECVs increases as the number of laboratories increases
  - A working rule: a minimum of 3 labs, preferably with at least 35 presumptive wild-type values although a total of ≥100 overall is usually satisfactory

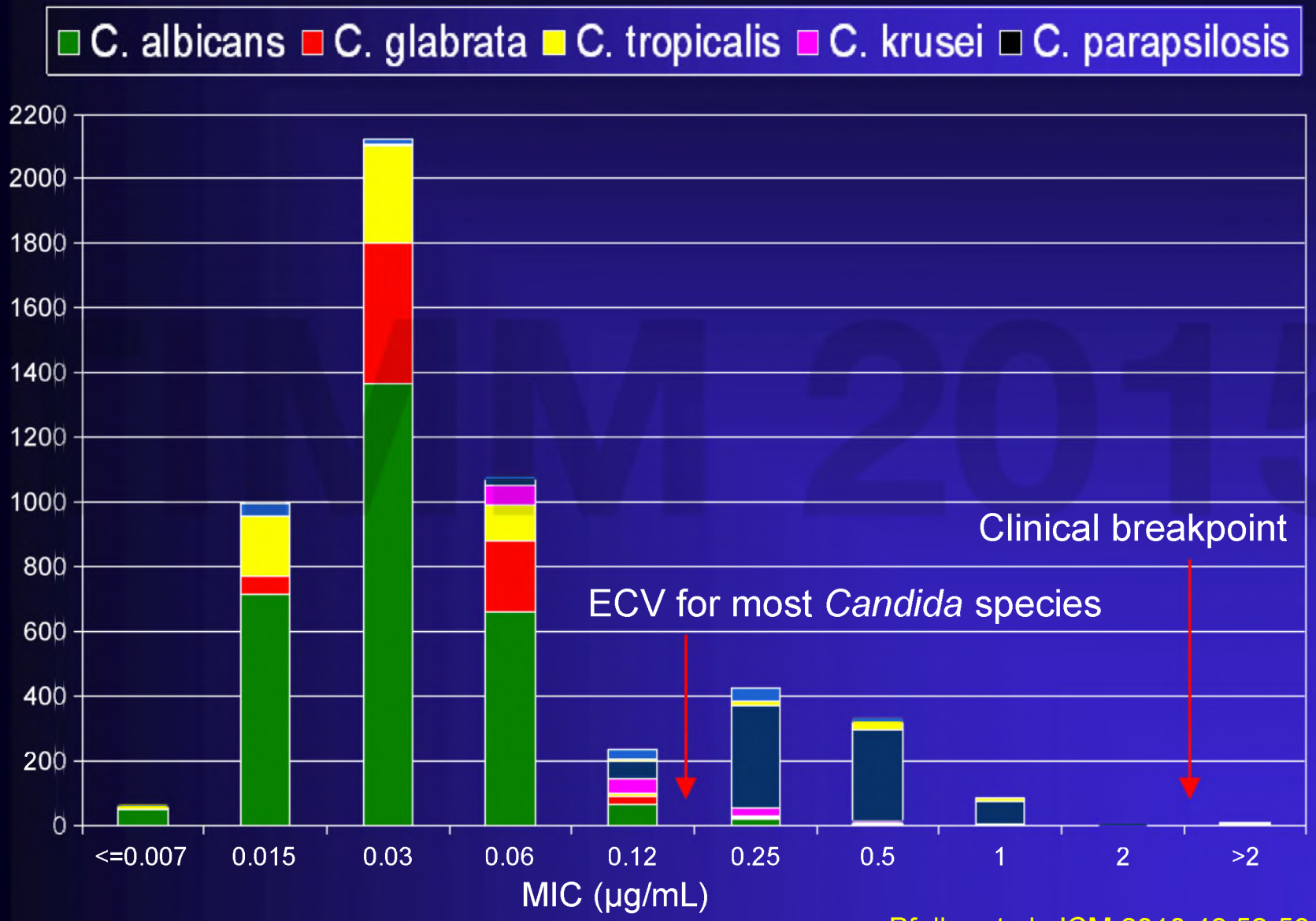
Estimation Methods

Six different methods have been proposed:

- The "Eyeball" method (Kahlmeter)
- The 95% Rule(Pfaller)
- The nortmalized resistance interpretation (Kronvall)
- The iterative statistical method (Turnidge)
- Multimodal analysis (Meletiadis)
- Cluster analysis (Canton)

Each has its own limitations

Caspofungin MIC distribution (N= 5346)



Pfaller et al. JCM 2010;48:52-56.

MIC Distributions of Three Echinocandins vs *Candida* spp. Strains Tested for the Presence of *fks1/fks2* Mutations

Species	Antifungal	No. of isolates at MIC (no. showing mutation)								
(no. tested)	agent	≤0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
C. albicans	ANF	31	7	4(1)	-	2(2)	6(6)	2(2)	-	-
(52)	CSF	15	23	3	-	2(2)	1(1)	3(3)	2(2)	3(3)
	MCF	31	7	3	2(2)	1(1)	2(2)	5(5)	1(1)	
C. glabrata	ANF	12	15	7(1)	3	-	6(3)	5(5)	5(5)	-
(53)	CSF	1	20	7	3(1)	1	8(3)	5(4)	2	6(6)
	MCF	25	9(1)	5(2)	5(3)	3(2)	1(1)	3(3)	2(2)	-
C. tropicalis	ANF	18	6	1(1)	1	1(1)	4(4)	-	-	-
(31)	CSF	8	12	3	3(1)	-	1(1)	2(2)	2(2)	-
	MCF	9	6	7	3(1)	3(2)	3(3)	-	-	-

Arendrup et al AAC2010;54:426-39

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

Application of Anidulafungin ECVs to *Candida* spp Strains Tested for Presence of *fks1/fks2* Mutations

- *C. albicans* (N =52)
  - ECV, 0.12 µg/ml
  - 42 WT: 1 (2.4%) with fks mutation
  - 10 non-WT: all with fks mutations

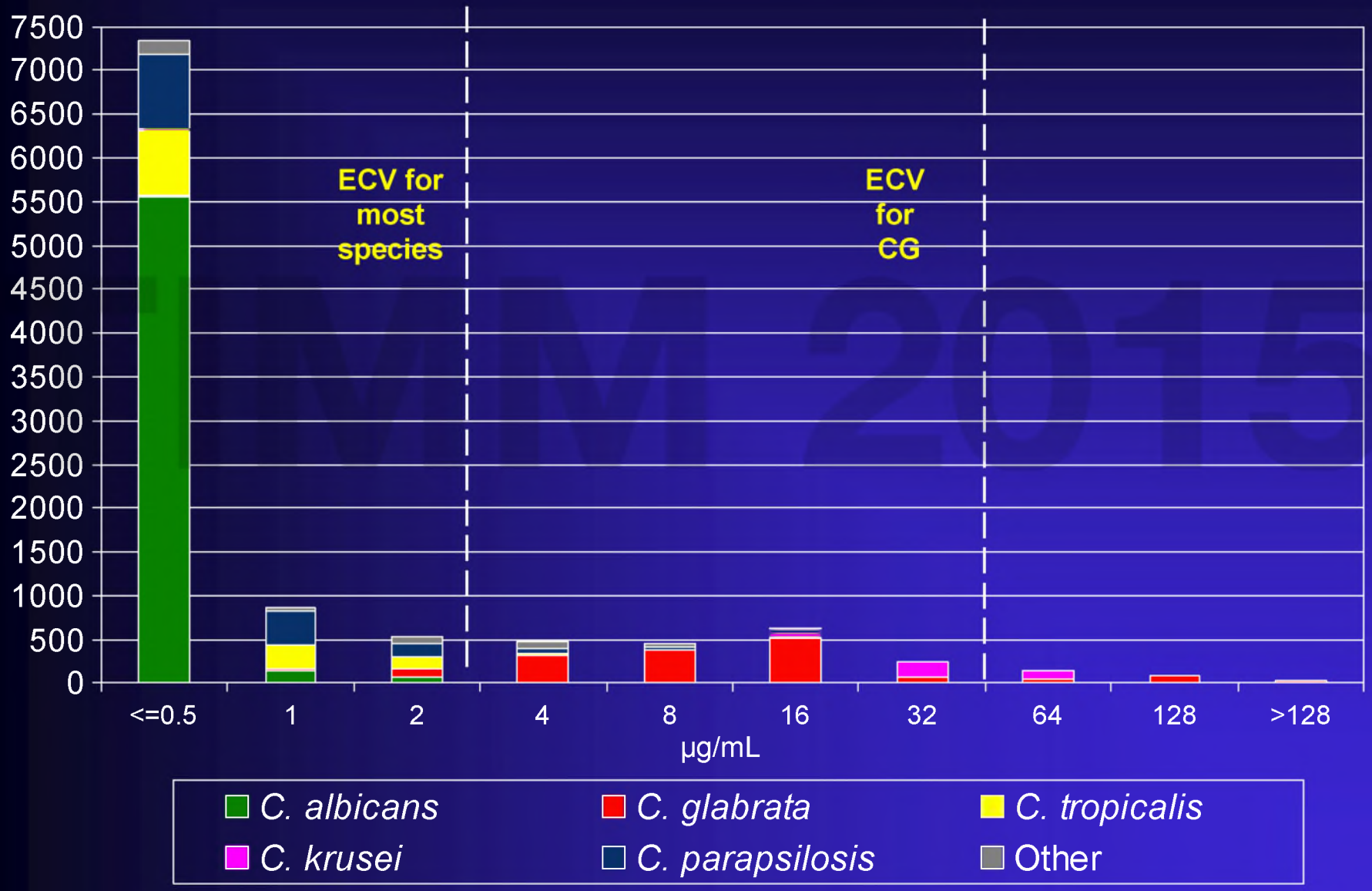
- *C. glabrata* (N=53)
  - ECV, 0.25 µg/ml
  - 37 WT: 1 (2.7%) with fks mutation
  - 16 non-WT: 13 (81%) with fks mutations

- *C. tropicalis* (N=31)
  - ECV, 0.12 µg/ml
  - 25 WT: 1 (4%) with fks mutation
  - 6 non-WT: 5 (83%) with fks mutations

Pfaller et al JCM 2010; 48:1592-99

Epidemiological Cutoff Values (ECVs) for Azoles

Fluconazole MIC distribution tested for 10,803 invasive *Candida* spp.



Fluconazole ECVs for the Common Species of *Candida*

Species	ECV (mcg/ml)
C. albicans	0.5
C. tropicalis	2
C. parapsilosis	2
C. glabrata	32
C. krusei	64

** Species for which clinical data is available

Pfaller and Diekema J Clin Microbiol 2012;50:2846-2856.

Fluconazole ECVs for the Less Common Species of *Candida*

Species	ECV (mcg/ml)
C. dubliniensis	0.5
C. guilliermondii	8
C. lusitaniae	2
C. kefyr	1
C. pelliculosa	4

** Species for which clinical data is lacking

Pfaller and Diekema J Clin Microbiol 2012;50:2846-2856.

Epidemiological Cutoff Values (ECVs) for Fluconazole and Five Species of *Candida* Using the 24h CLSI and EUCAST BMD Methods

Species	Method	No. Isolates	MIC Mode (mcg/ml)	ECV (%)
C albicans	CLSI	8059	0.12	0.5 (98.1)
	EUCAST	15991	0.25	1(91.9)
C glabrata	CLSI	2240	4	32(91.5)
	EUCAST	5018	16	32(89.7)
C parapsilosis	CLSI	2117	0.5	2(93.2)
	EUCAST	2536	0.5	2 (92.6)
C tropicalis	CLSI	1771	0.25	2 (98.4)
	EUCAST	2229	0.5	2(93.7)
C krusei	CLSI	473	16	64 (99.8)
	EUCAST	673	32	128(98.4)

Pfaller et al, Drug Resist Updates 2010;13:180-195.
CMI 2008;14:193-5

Voriconazole ECVs for the Common Species of *Candida*

Species	ECV (mcg/ml)
C. albicans	0.03
C. tropicalis	0.06
C. parapsilosis	0.12
C. glabrata	0.5
C. krusei	0.5

** Species for which clinical data is available

Pfaller and Diekema J Clin Microbiol 2012;50:2846-2856.

Voriconazole ECVs for the Less Common Species of *Candida*

Species	ECV (mcg/ml)
C. dubliniensis	0.03
C. guilliermondii	0.25
C. lusitaniae	0.03
C. kefyr	0.015
C. pelliculosa	0.25

** Species for which clinical data is lacking

*Pfaller et al J Clin Microbiol 2012 ;50:2846-2856.

Aspergillus spp. and Azoles

- In vitro "reduced susceptibility" remains 0-5% in most large surveys, often using itraconazole
  - Most data for *A. fumigatus* complex
- Case reports and case series suggest that multiply-azole resistant *Aspergillus* could emerge
  - ? association with agricultural azole use

Rodriguez-Tudela, et al. Antimicrob Agents Chemother 2008;52:2468.
Verweij, et al. N Engl J Med 2007;356:1481-83.
Arendrup, et al. Antimicrob Agents Chemother 2008;52:3504-11.
Howard, et al. Int J Antimicrob Agents Chemother 2006;28:450-53.
Snelders et al. PLoS Medicine 2008;5:e219.

Azole Resistance Mechanisms in *Aspergillus fumigatus*

- Mutations involving *cyp*51A gene (target)
  - G54 mutation

    Cross resistance between ITR and PSC

    VRC, RVC MICs < 1 microgram/mL
  - M220 mutation

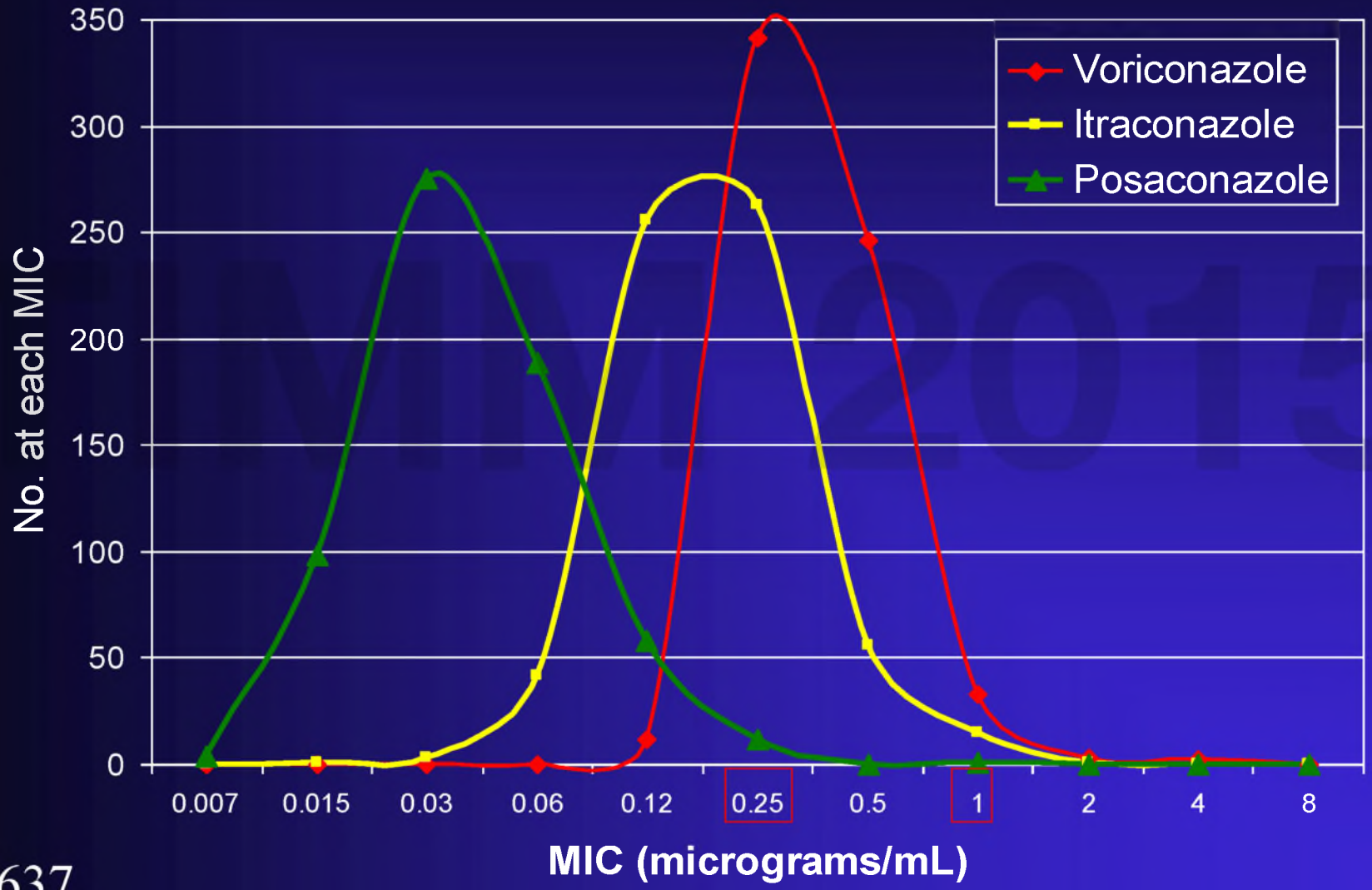
    Complete cross resistance ITR, PSC, VRC, RVC
  - Tandem repeat – L98H (TR)

    Complete cross resistance
- Decreased accumulation (slowed uptake/efflux) also described

Rodriguez-Tudela et al AAC 2008;52:2468-72.

Nascimento et al. AAC 2003;47:1719-26.

Voriconazole, Posaconazole, Itraconazole
Wild Type Distributions
Aspergillus fumigatus, 2005-2007



N=637

Pfaller et al JCM 2009;47:3142-6

Resistance Mechanisms and Azole Cross Resistance in *A. fumigatus*

Strain type (N)	GM MIC (µg/mL)			
	ITR	VRC	RVC	PSC
WT (361)	0.32	0.53	0.60	0.08
G54 (9)	16	0.42	0.32	1.6
M220 (6)	16	1.0	1.85	0.65
TR (17)	16	3.7	6.8	0.68

Rodriguez-Tudela et al AAC 2008;52:2468-72

MIC Distributions of Three Azoles vs *A. fumigatus* Strains Tested for the Presence of *cyp*51A Mutations

Antifungal agent	No. tested	No. isolates at MIC (no. showing mutation)										
		0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
ITR	28							3	13	1	1	10(8)
PSC	28							16	3(2)	8(6)	1	
VRC	28						2	13	4(1)	7(6)	1(1)	1

Lockhart et al AAC 2011; 55:4465-4468.

Note: All mutant strains were from China and contained the TR/L98H mutation in *cyp51A* (as well as S297T and F495I): all had unique microsatellite genotypes.

In vitro resistance confirmed by the sterol quantification method.

Cross-Resistance Between Itraconazole, Posaconazole, and Voriconazole Among 43 isolates of *A. fumigatus* with Decreased Susceptibility to Itraconazole

Antifungal agent	MIC (mcg/ml) Range	Mode	ECV	% ≤ ECV
ITR	2->8	2	1	0
PSC	0.007-2	0.5	0.25	46.5
VRC	0.12-4	0.25	1	93.0

Pfaller et al, J Clin Microbiol 2009;47:3142-6

MIC Distribution and CLSI ECV for Azoles and *A. fumigatus*

Antifungal agent (N)	MIC (mcg/ml) Range	Mode	ECV	% ≤ ECV
ITR (2,591)	0.03-≥4	0.5	1	98.8
PSC (1,684)	≤0.015-≥4	0.06	0.5	97.8
VRC (2,851)	0.03-≥4	0.25	1	98.2

Espinel-Ingroff et al, JCM 2010;48:3251-7

ECV for In Vitro Susceptibility Testing of Fungi With No/Lacking Interpretive Breakpoints

Antifungal Agent	Species	ECV (µg/mL)[c]
		97.5% Statistical
Amphotericin B	*C. albicans*	2
	C. glabrata	2
	C. krusei	2
	C. parapsilosis	2
	C. tropicalis	2
Flucytosine	*C. albicans*	0.12
	C. glabrata	0.12
	C. krusei	32
	C. parapsilosis	0.25
	C. tropicalis	0.25

[c] ECVs that capture at least 97.5% of the statistically modeled population

ECV for In Vitro Susceptibility Testing of Fungi With No/Lacking Interpretive Breakpoints - Echinocandins

Antifungal Agent	Species	ECV (µg/mL)[c]
		97.5% Statistical
Anidulafungin	*C. dubliniensis*	0.12
	C. lusitaniae	1
Caspofungin	*A. flavus*	0.5
	A. fumigatus	0.5
	A. niger	0.25
	A. terreus	0.12
Micafungin	*C. dubliniensis*	0.12

[c]ECVs that capture at least 97.5% of the statistically modeled population

ECV for In Vitro Susceptibility Testing of Fungi With No/Lacking Interpretive Breakpoints - Azoles

Antifungal Agent	Species	ECV (µg/ml)[c]
		97.5% Statistical
Isavuconazole	*A. flavus*	Skewed
	A. fumigatus	Skewed
	A. niger	Skewed
	A. terreus	Skewed
	A. nidulans	Skewed
Itraconazole	*A. flavus*	1
	A. fumigatus	1
	A. terreus	2
	A. niger	4
	A. nidulans	2
	C. albicans	0.12
	C. glabrata	4
	C. krusei	1
	C. lusitaniae	1
	C. parapsilosis	0.5
	C. tropicalis	0.5
Posaconazole	*A. flavus*	0.5
	A. fumigatus	0.5
	A. nidulans	2
	A. niger	2
	A. terreus	1
Voriconazole	*A. flavus*	2
	A. fumigatus	1
	A. nidulans	0.5
	A. niger	2
	A. terreus	2

[c]ECVs that capture at least 97.5% of the statistically modeled population

CLSI Working Group on ECVs

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