Instituto de Salud Carlos III

Antifungal susceptibility and setting breakpoints

ESCMID CONFERENCE
INFECTIONS IN IMMUNOCOMPROMISED HOST
17-18 NOVEMBER 2011, ISTANBUL

Conflict of interest disclosure

- In the past 5 years, M.C.E. has received grant support from **Astellas Pharma, bioMerieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering Plough, Soria Melguizo SA**, **Ferrer International**
- He has been an advisor/consultant to the **Panamerican Health Organization, Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer, and Schering Plough**.
- He has been paid for talks on behalf of **Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough**.

Problems for AST of Fungi

Different morphologies even for yeasts

Budding Yeast

C. albicans

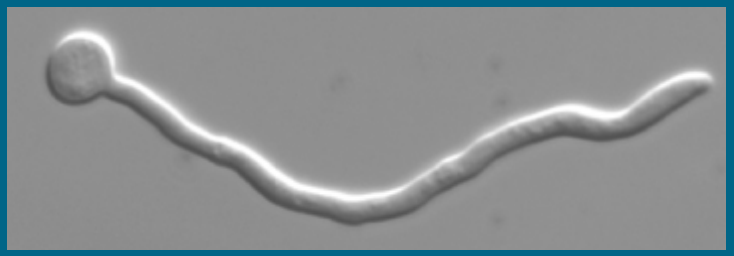
C. glabrata

Pseudohypha

C. albicans

C. parapsilosis

Hypha



C. albicans

Problems for AST of Fungi

Different rate of growth

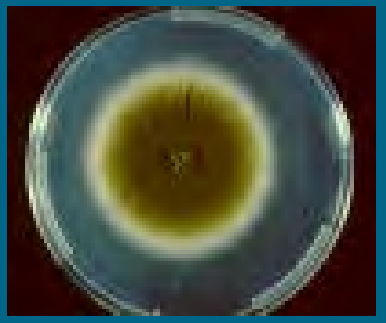
Mucorales

18-24 h

Yeasts

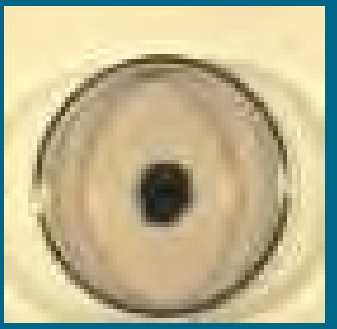
24-48 h

Aspergillus



48-72 h

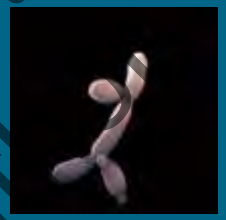
Black Yeasts



Several days

Problems for AST of Fungi

Different respiratory behaviour

Fermentative yeasts as →  *Candida*

 *Cryptococcus*

Non-Fermentative yeasts as {  *Trichosporon*

 *Rhodotorula*

From 1992

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Reference methods for Yeasts

- M27 A3 by CLSI, dilution
- Document 7.1 by EUCAST (7.2 soon), dilution
- M44 A2 by CLSI, diffusion****

AST

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Reference methods for MOLDS

- M38 A2 by CLSI, dilution
- Document by EUCAST, Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds

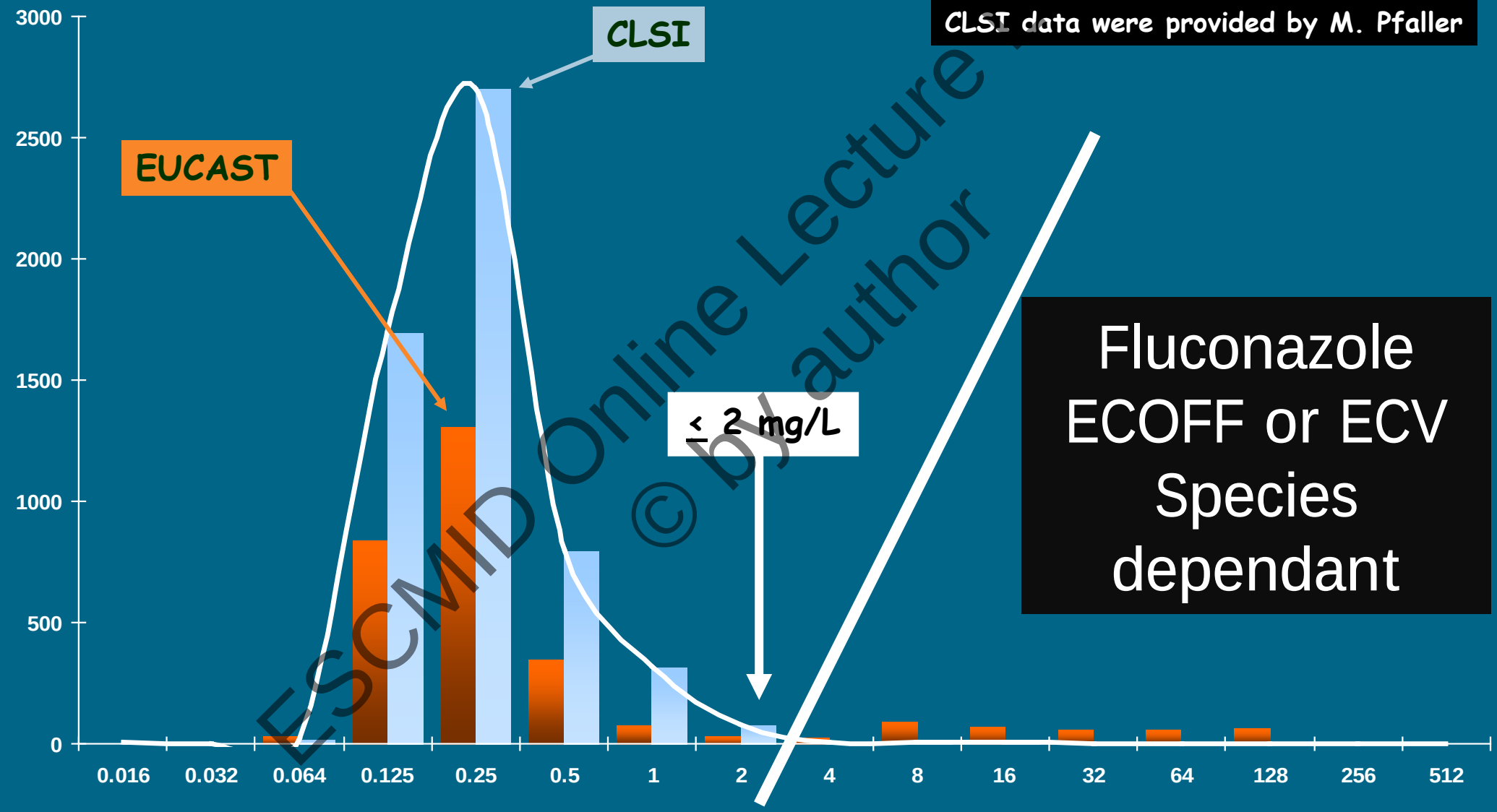
Reference methods, dilution

- Standardization process
- Reproducibility
- QC assurance
- Breakpoints setting process to interpret results

Breakpoints setting procedure

STEPS	EUCAST	CLSI
1	Identifying most common dosage used in each European country	Examining available microbiological data
2	Defining the wild type population for each target microorganism at the species level and determining the epidemiological cut-offs	Knowing resistance mechanisms and their relation to MIC values and in vivo outcomes
3	Describing the pharmacokinetics of the drug	Examining pertinent pharmacokinetic parameters
4	Examining the pharmacodynamics including Monte Carlo simulations;	Examining pharmacodynamic parameters
5	Exploring the correlation of MIC values with clinical outcome of patients treated with the drug	Analyzing clinical outcome data

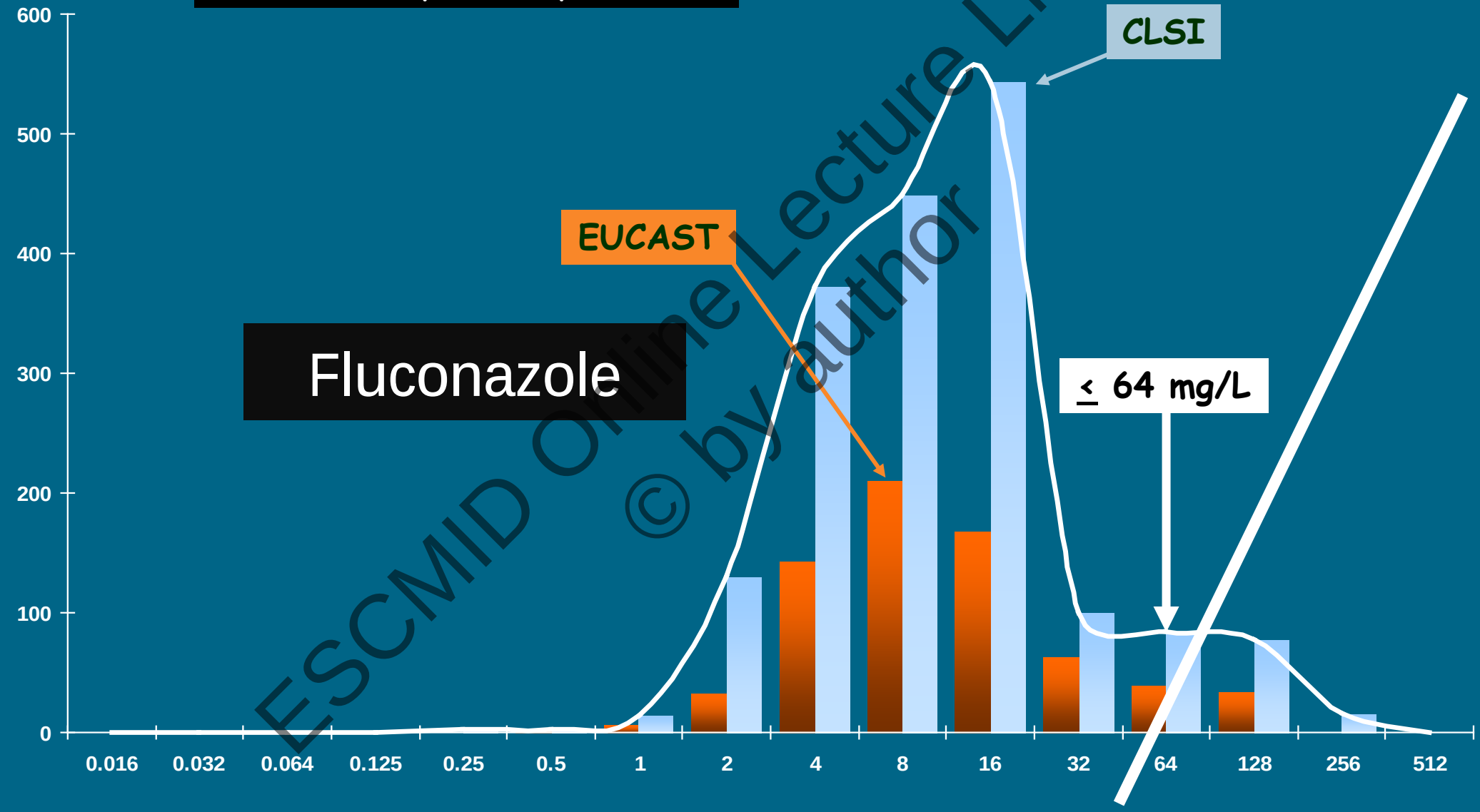
Epidemiological BPs for *C. albicans*



Fluconazole
ECOFF or ECV
Species
dependant

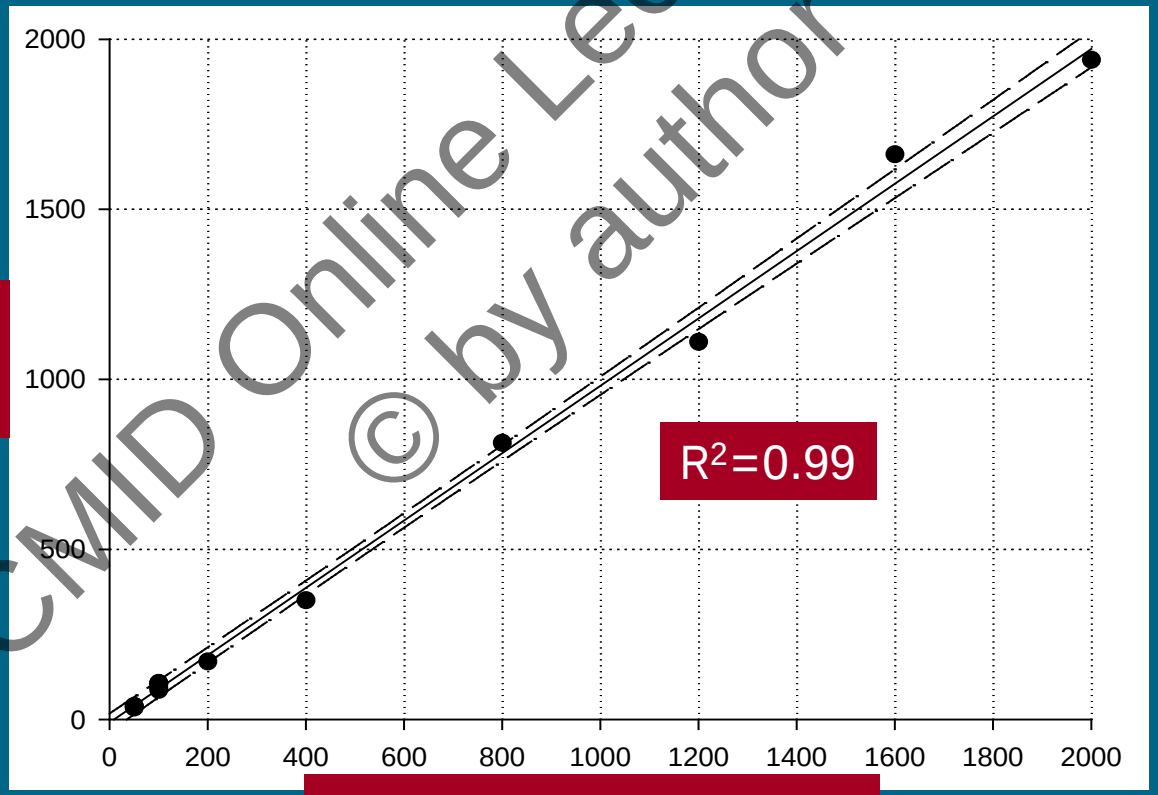
Epidemiological BPs for *C. glabrata*

CLSI data was provided by M. Pfaller



PK/PD - AUC/MIC

AUC = Dose



Antimicrobials for *Candida* infections - EUCAST and CLSI clinical MIC breakpoints

Antifungal	Species	EUCAST			CLSI			
		Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
Itraconazole	*C. albicans*	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. glabrata	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. krusei	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. parapsilosis	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. tropicalis	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
Fluconazole	***C. albicans***	**≤ 2**	**4**	**> 4**	**≤ 2**	**4**	**--**	**≥ 8**
	C. glabrata	**IE**	**IE**	**IE**	**--**	**≤ 32**	**--**	**≥ 64**
	C. krusei	**PT**	**PT**	**PT**	**PT**	**PT**	**PT**	**PT**
	C. parapsilosis	**≤ 2**	**4**	**> 4**	**≤ 2**	**4**	**--**	**≥ 8**
	C. tropicalis	**≤ 2**	**4**	**> 4**	**≤ 2**	**4**	**--**	**≥ 8**
Voriconazole	*C. albicans*	≤ 0.125	---	> 0.125	≤ 0.12	--	0.25-0.50	≥ 1
	C. glabrata	IE	IE	IE	IE	IE	IE	IE
	C. krusei	IE	IE	IE	≤ 0.50	IE	1	≥ 2
	C. parapsilosis	≤ 0.125	---	> 0.125	≤ 0.12	--	0.25-0.50	≥ 1
	C. tropicalis	≤ 0.125	---	> 0.125	≤ 0.12	--	0.25-0.50	≥ 1
Posaconazole	*C. albicans*	≤ 0.06	---	> 0.06	NEY	NEY	NEY	NEY
	C. glabrata	IE	IE	IE	NEY	NEY	NEY	NEY
	C. krusei	IE	IE	IE	NEY	NEY	NEY	NEY
	C. parapsilosis	≤ 0.06	---	> 0.06	NEY	NEY	NEY	NEY
	C. tropicalis	≤ 0.06	---	> 0.06	NEY	NEY	NEY	NEY

Antimicrobials for *Candida* infections - EUCAST and CLSI clinical MIC breakpoints

Antifungal	Species	EUCAST			CLSI			
		Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
Itraconazole	*C. albicans*	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. glabrata	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. krusei	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. parapsilosis	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
	C. tropicalis	NEY	NEY	NEY	≤ 0.12	0.25-0.50	---	≥ 1
Fluconazole	*C. albicans*	≤ 2	4	> 4	≤ 2	4	--	≥ 8
	C. glabrata	IE	IE	IE	--	≤ 32	--	≥ 64
	C. krusei	PT	PT	PT	PT	PT	PT	PT
	C. parapsilosis	≤ 2	4	> 4	≤ 2	4	--	≥ 8
	C. tropicalis	≤ 2	4	> 4	≤ 2	4	--	≥ 8
Voriconazole	*C. albicans*	≤ 0.125	---	> 0.125	≤ 0.12	--	0.25-0.50	≥ 1
	C. glabrata	IE	IE	IE	IE	IE	IE	IE
	C. krusei	IE	IE	IE	≤ 0.50	IE	1	≥ 2
	C. parapsilosis	≤ 0.125	---	> 0.125	≤ 0.12	--	0.25-0.50	≥ 1
	C. tropicalis	≤ 0.125	---	> 0.125	≤ 0.12	--	0.25-0.50	≥ 1
Posaconazole	*C. albicans*	≤ 0.06	---	> 0.06	NEY	NEY	NEY	NEY
	C. glabrata	IE	IE	IE	NEY	NEY	NEY	NEY
	C. krusei	IE	IE	IE	NEY	NEY	NEY	NEY
	C. parapsilosis	≤ 0.06	---	> 0.06	NEY	NEY	NEY	NEY
	C. tropicalis	≤ 0.06	---	> 0.06	NEY	NEY	NEY	NEY

Antimicrobials for *Candida* infections - EUCAST and CLSI clinical MIC breakpoints

Antifungal	Species	EUCAST			CLSI			
		Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
AMB	*C. albicans*	≤ 1	---	>1	NEY	NEY	NEY	NEY
	C. glabrata	≤ 1	---	>1	NEY	NEY	NEY	NEY
	C. krusei	≤ 1	---	>1	NEY	NEY	NEY	NEY
	C. parapsilosis	≤ 1	---	>1	NEY	NEY	NEY	NEY
	C. tropicalis	≤ 1	---	>1	NEY	NEY	NEY	NEY

Antimicrobials for *Candida* infections - EUCAST and CLSI clinical MIC breakpoints

Antifungal	Species	EUCAST			CLSI			
		Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
Caspofungin	*C. albicans*	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
	C. glabrata	NEY	NEY	NEY	≤ 0.12	---	0.25	≥ 0.50
	C. krusei	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
	C. parapsilosis	NEY	NEY	NEY	≤ 2	---	4	≥ 8
	C. tropicalis	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
Micafungin	*C. albicans*	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
	C. glabrata	NEY	NEY	NEY	≤ 0.06	---	0.12	≥ 0.25
	C. krusei	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
	C. parapsilosis	NEY	NEY	NEY	≤ 2	---	4	≥ 8
	C. tropicalis	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
Anidulafungin	*C. albicans*	≤ 0.03	---	> 0.03	≤ 0.25	---	0.50	≥ 1
	C. glabrata	≤ 0.06	---	> 0.06	≤ 0.12	---	0.25	≥ 0.50
	C. krusei	≤ 0.06	---	> 0.06	≤ 0.25	---	0.50	≥ 1
	C. parapsilosis	PT	PT	PT	≤ 2	---	4	≥ 8
	C. tropicalis	≤ 0.06	---	> 0.06	≤ 0.25	---	0.50	≥ 1

Antimicrobials for *Candida* infections - EUCAST and CLSI clinical MIC breakpoints

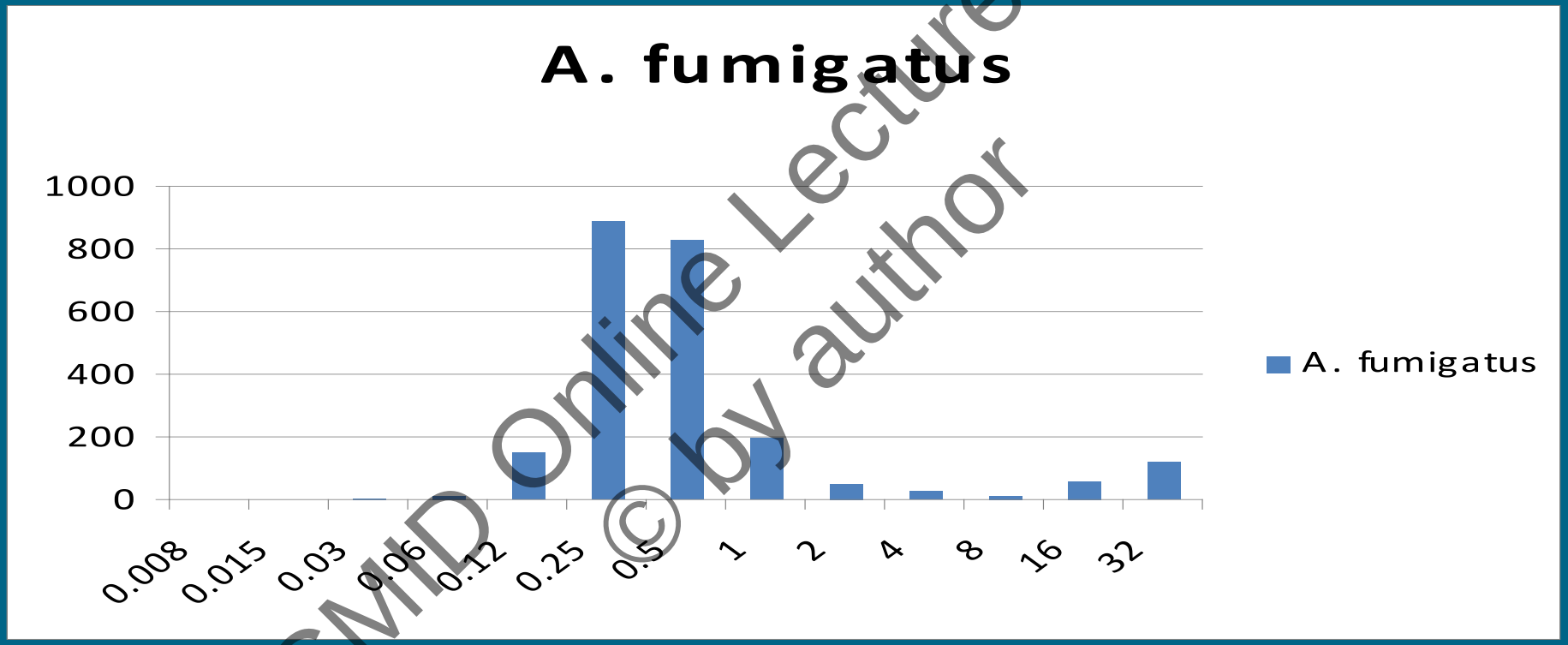
Antifungal	Species	EUCAST			CLSI			
		Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
Caspofungin	*C. albicans*	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
	C. glabrata	NEY	NEY	NEY	≤ 0.12	---	0.25	≥ 0.50
	C. krusei	NEY	NEY	NEY	≤ 0.25	---	0.50	≥ 1
	C. parapsilosis	NEY	NEY	NEY	≤ 2	---	4	≥ 8
	C. tropicalis							≥ 1
Micafungin	*C. albicans*							≥ 1
	C. glabrata							≥ 0.25
	C. krusei							≥ 1
	C. parapsilosis							≥ 8
	C. tropicalis							≥ 1
Anidulafungin	*C. albicans*							≥ 1
	C. glabrata	≤ 0.06	---	> 0.06	≤ 0.12	---	0.25	≥ 0.50
	C. krusei	≤ 0.06	---	> 0.06	≤ 0.25	---	0.50	≥ 1
	C. parapsilosis	PT	PT	PT	≤ 2	---	4	≥ 8
	C. tropicalis	≤ 0.06	---	> 0.06	≤ 0.25	---	0.50	≥ 1

Echinocandin BPs, under review
Resistance mechanisms at molecular level

Candida resistance to echinocandins

Aminoácido	641 a 649	690 a 701	1357 a 1364
	HOT SPOT 1	HOT SPOT 3	HOT SPOT 2
FKS 1			
Secuencia	FSTLSLRDP		DWIRRYTL
	HOT SPOT 1		HOT SPOT 2
FKS 2			
	HOT SPOT 1		HOT SPOT 2
FKS 3			

Epidemiological BPs for *A. fumigatus* and itraconazole



Labs: 12. Nb isolates: 2,389

Mutations in *Cyp51A*

ABC

Strain with high MIC

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2003, p. 1120–1224 Vol. 47, No. 3
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.3.1120–1124.2003

A Point Mutation in the 14α-Sterol Demethylase Gene *cyp51A* Contributes to Itraconazole Resistance in *Aspergillus fumigatus*

T. M. Diaz-Guerra, E. Mellado,* M. Cuenca-Estrella, and J. L. Rodriguez-Tudela

Unidad de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2004, p. 2747–2750 Vol. 48, No. 7
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.7.2747–2750.2004

Substitutions at Methionine 220 in the 14α-Sterol Demethylase (Cyp51A) of *Aspergillus fumigatus* Are Responsible for Resistance In Vitro to Azole Antifungal Drugs

E. Mellado,* G. García-Effron, L. Alcázar-Fuoli, M. Cuenca-Estrella, and J. L. Rodríguez-Tudela

Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2005, p. 2536–2538 Vol. 49, No. 6
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.6.2536–2538.2005

Targeted Gene Disruption of the 14-α Sterol Demethylase (*cyp51A*) in *Aspergillus fumigatus* and Its Role in Azole Drug Susceptibility

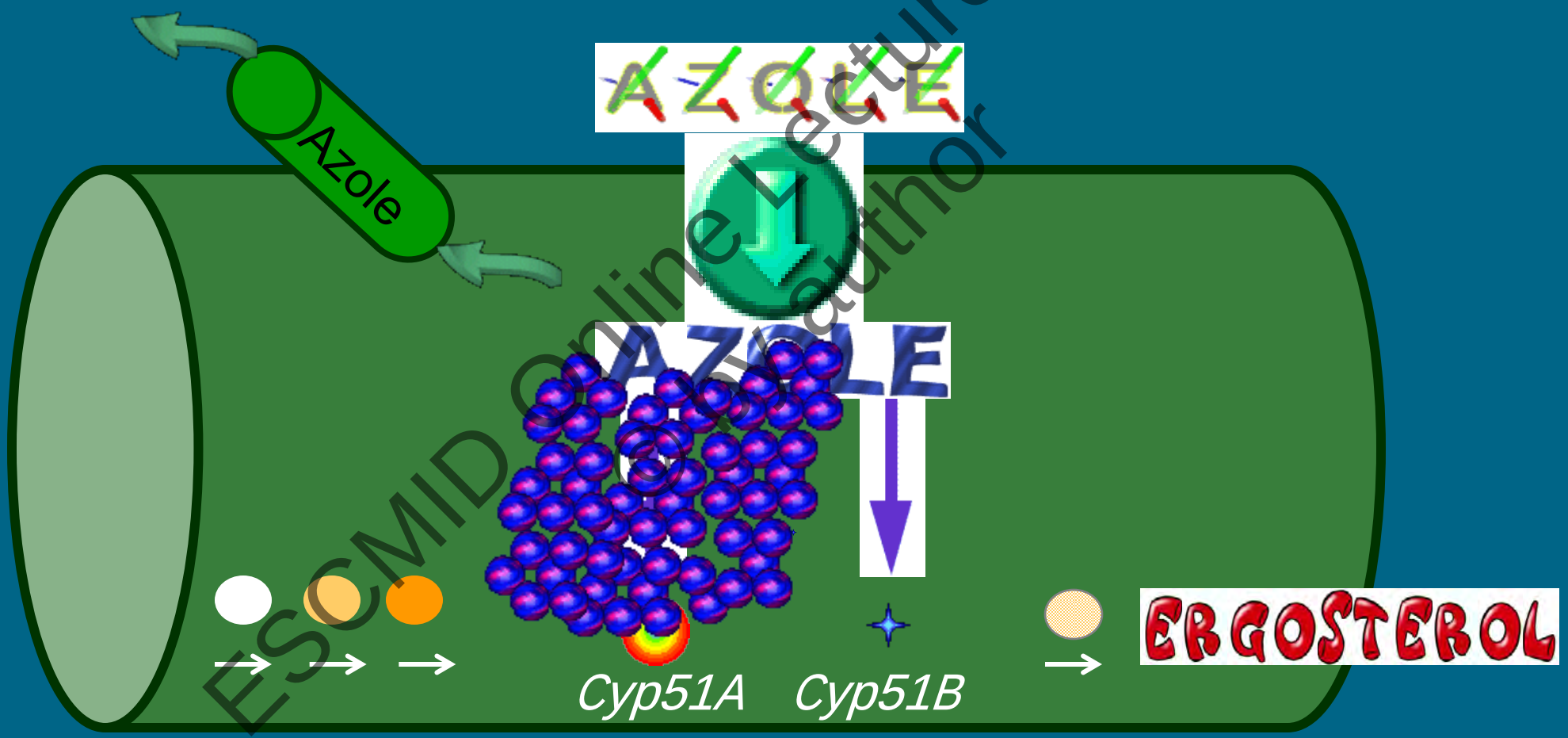
E. Mellado,* G. Garcia-Effron, M. J. Buitrago, L. Alcazar-Fuoli, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela

Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

UP regulation of *Cyp*51A

ABC

Strain with high MIC



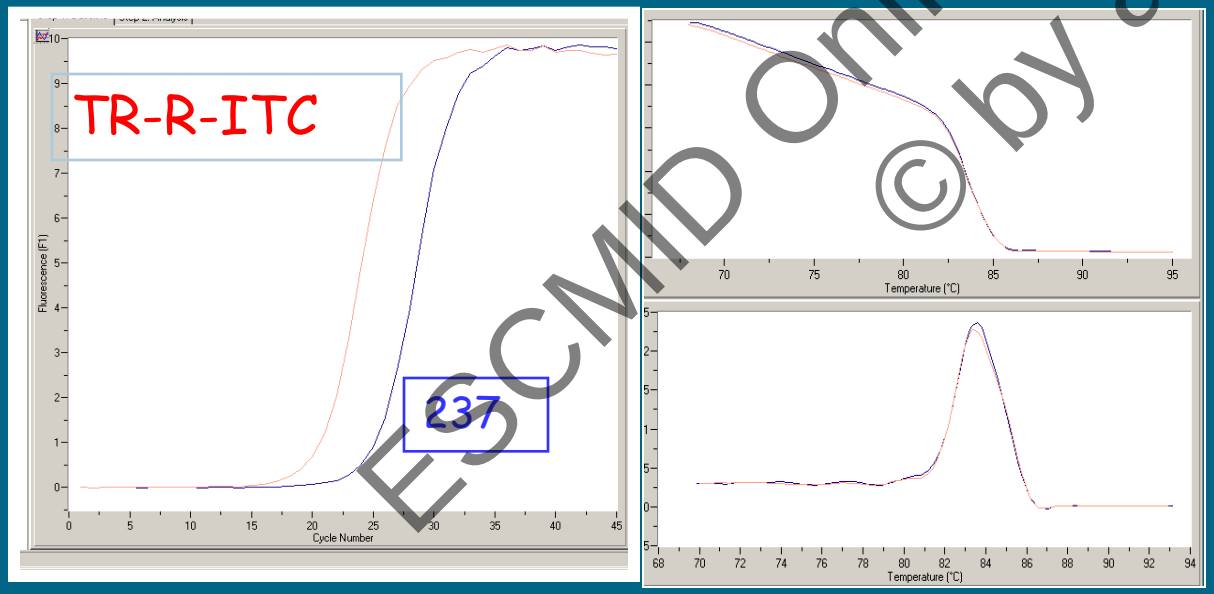
Gen Cyp51A in a resistant strain

Duplication of 34-bp sequence in the promoter

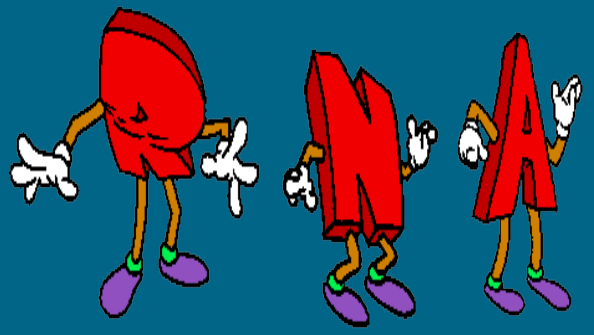
GAATCACGCGG GAATCACGCGG

Cyp51A

L98H



x 8



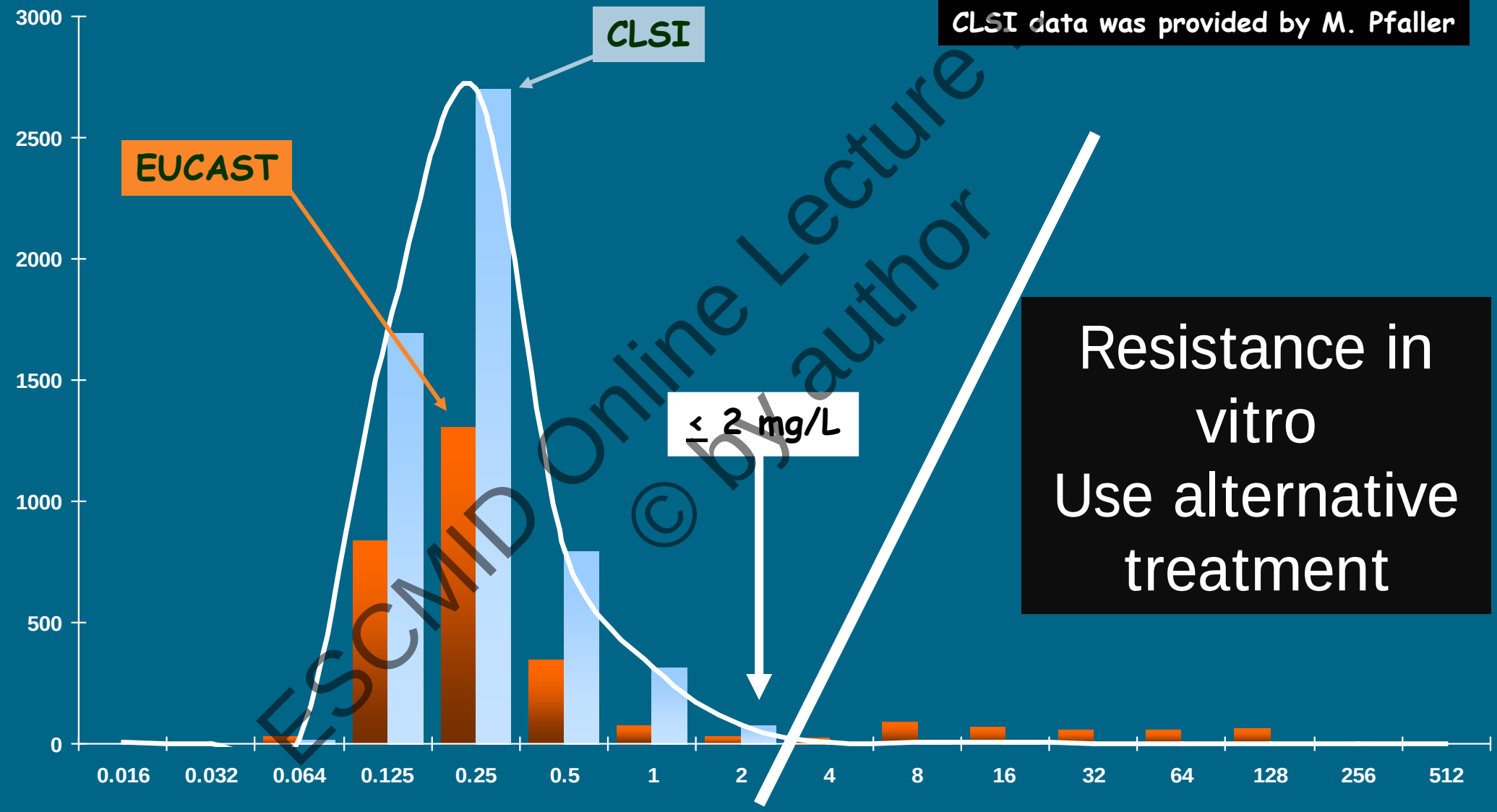
EUCAST BPs of ITRACONAZOLE for *Aspergillus*

Under review

Aspergillus	Susceptible	Intermediate	Resistant
A. fumigatus	≤ 1	2	> 2
A flavus	≤ 1	2	> 2
A niger	≤ 1	2	> 2
A. terreus	≤ 1	2	> 2

AMB, POSA and VOR in progress

BPs of antifungal agents



Resistance in vitro
Use alternative treatment

The dilution standard reference procedures are unpractical for clinical laboratories since they recommend rather complex methods for susceptibility testing

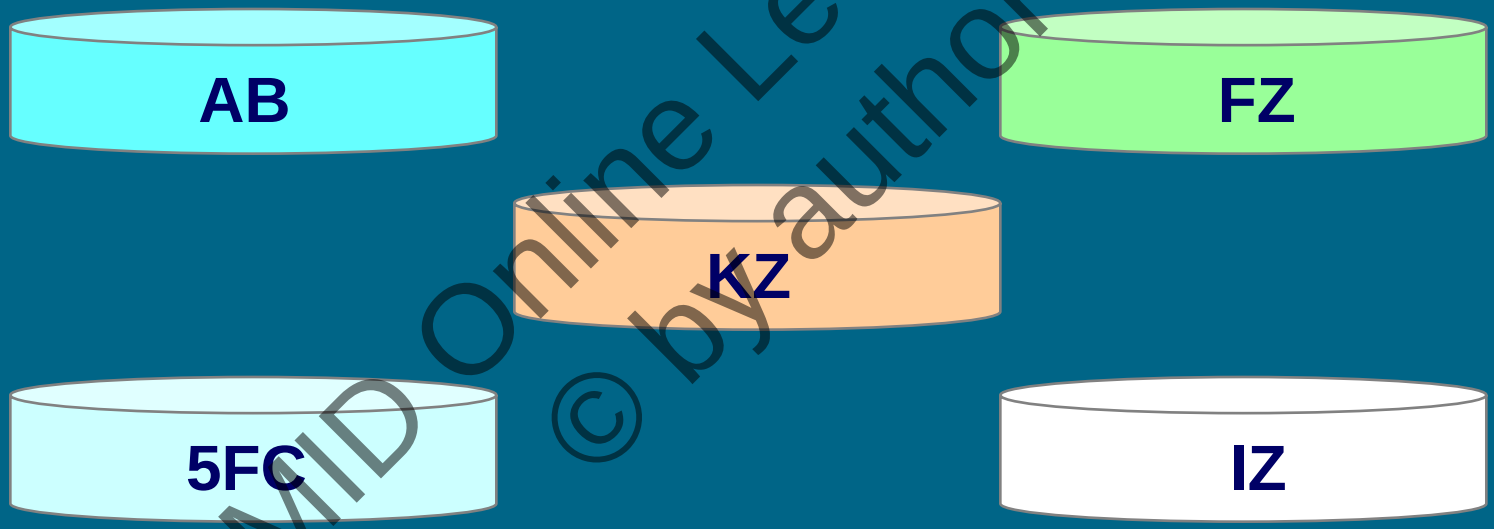
Cuenca-Estrella M and Rodriguez-Tudela JL. Expert Rev Anti Infect Ther 2010

Applicability of AST in clinical laboratory for patient management

- Ease of performance
- Economy
- More rapid results

Diffusion and commercial techniques

Disk Diffusion



Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-Year Analysis of Susceptibilities of *Candida* and Other Yeast Species to Fluconazole and Voriconazole by Standardized Disk Diffusion Testing

M. A. Pfaller,[1]* D. J. Diekema,[1] M. G. Rinaldi,[2] R. Barnes,[3] B. Hu,[4] A. V. Veselov,[5] N. Tiraboschi,[6] E. Nagy,[7] D. L. Gibbs,[8] and the Global Antifungal Surveillance Group

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 5848–5859

- **140,767 yeasts were collected from 127 participating**
- **39 countries from June 1997 through December 2003**

Categorical agreement rates were higher than 90%. Very major error rate was under 1%

Etest

Etest

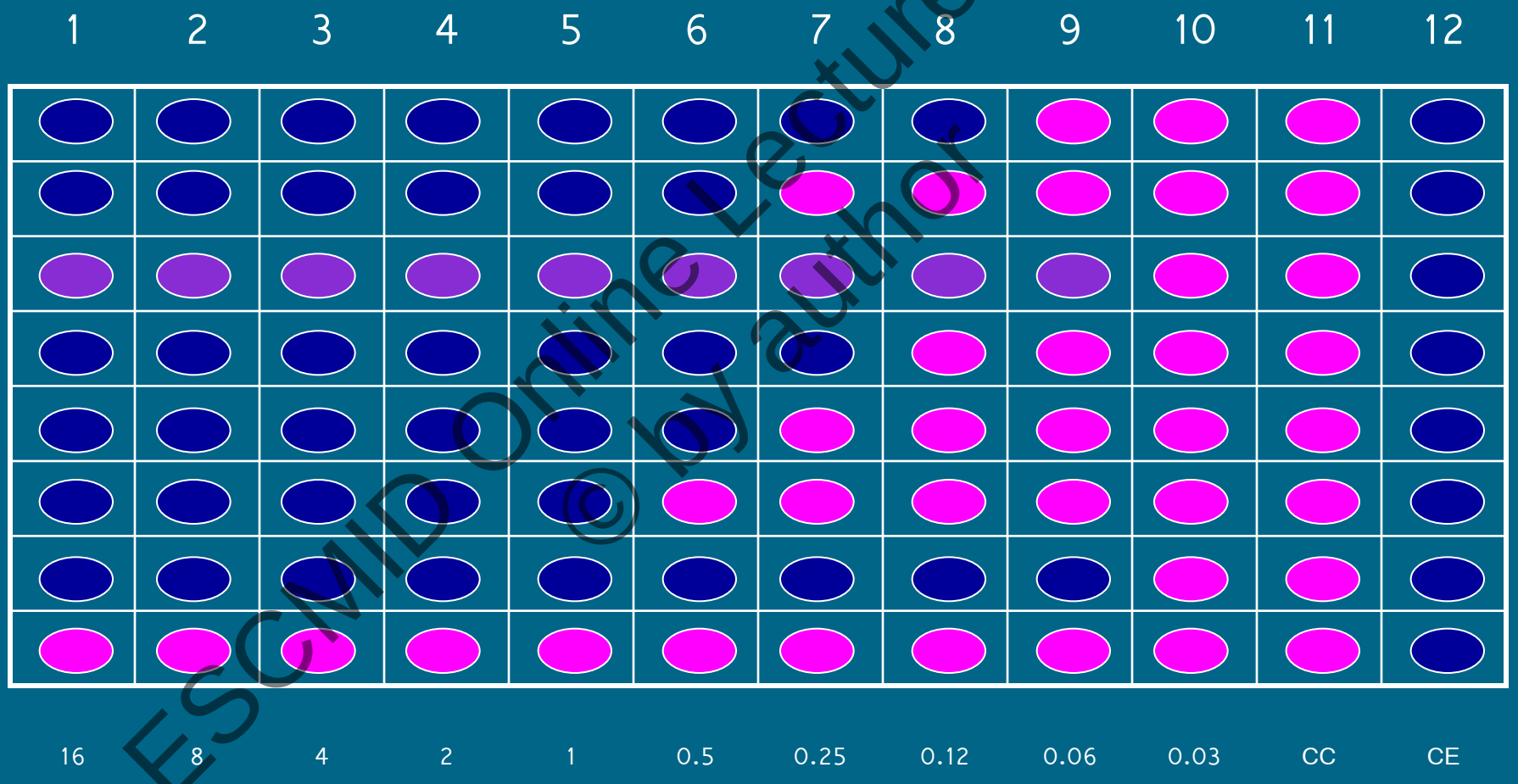
Strip with decreasing concentration of the antimicrobial agent

Etest meniscus

Etest

Double meniscus

Sensititre



Results by AF for Sensititre

Agent	Correlation	Agreement
AMB	0.29	50.5%
FLU	0.81	72%
FLC	0.93	90%
ITC	0.85	81%
VOR	0.82	84%
ECHINO	0.83	83%

Other microdilution techniques. Colored or simplified

- ASTY
- Fungitest
- ATB Fungus
- Mycostandard
- Mycototal
- Candifast
- Integral Systems Yeasts

Summary of commercial methods

(i) Disk-diffusion, Etest or sensititre are useful for clinical labs and patients management

(ii) Diffusion is rapid and cheap and useful for screening (FLC and VRC)

(iii) Sensititre is more expensive, and detects resistance in vitro to azole agents. PITFALLS (AMB)

(iv) Etest is expensive, detects azole. echino and AmB resistance. Some problems with trailing

(v) Other methods could be useful too, but validation is mandatory

(vi) CAUTION: Their own interpretative BPs!!!!!

AST Recommendations

ESCMID Diagnostic & Management Guideline for Candida Diseases 2011 (*Under review*)

Authors: Murat Akova, Maiken Arendrup, Sevtap Arikan-Akdagli, Matteo Bassetti, Jacque Bille, Thierry Calandra, Elio Castagnola, **Oliver A. Cornely**, **Manuel Cuenca-Estrella**, Peter Donnelly, Jorge Garbino , Andreas Groll, Raoul Herbrecht, **William Hope,** Henrik Elvang Jensen, Bart-Jan Kullberg, Cornelia Lass-Flörl, **Olivier Lortholary,** Wouter Meersseman, Georgios Petrikkos, Malcolm Richardson, Emmanuel Roilides, **Andrew J. Ullmann, Paul Verweij**, Claudio Viscoli

Main Coordinator: Andrew J. Ullmann

When are AST recommended for patient management and when for epidemiological reasons?

Isolated from	FOR patient management	FOR Epidemiology
Blood and other deep sites	All isolates and particularly: 1. Strains from patients exposed to antifungal agents 2. Clinical failures *(validated commercial method)* Rare and emerging species Species that are known to be resistant or less susceptible to antifungal drug(s) in clinical use	• All isolates should be tested using a reference method
Superficial sites	• Failed to respond or relapsing infection • Surveillance cultures from patients exposed to antifungal agents *(validated commercial method)*	• Periodical epidemiological studies should be done