Instituto de Salud Carlos III

Antifungal susceptibility testing methods. Recommendation for a daily practice

Manuel Cuenca-Estrella

Antifungal Resistance and its Challenges in the Management of Invasive Fungal Infections, ESCMID Postgraduate Education Course
20 - 22 June 2013, Sibiu, Romania

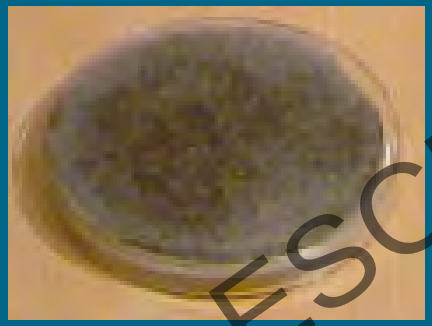
Conflict of interest disclosure

- 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 rate of growth

Mucorales



18-24 h

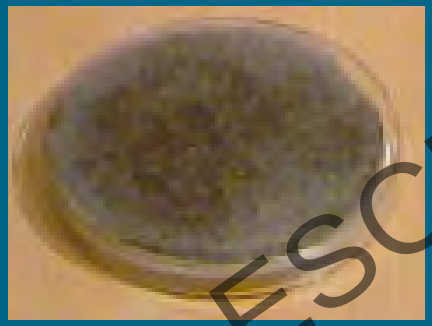
Yeasts

24-48 h

Problems for AST of Fungi

Different rate of growth

Mucorales

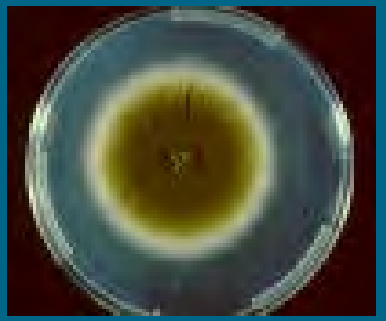


18-24 h

Yeasts

24-48 h

Aspergillus

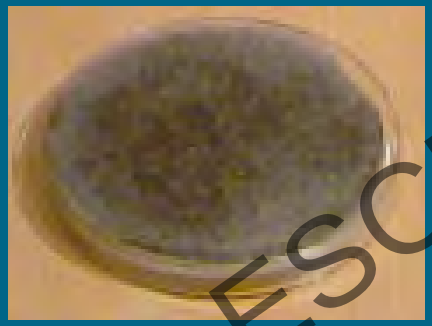


48-72 h

Problems for AST of Fungi

Different rate of growth

Mucorales



18-24 h

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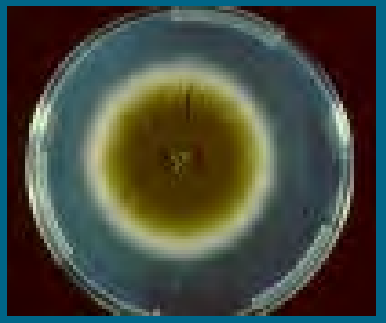
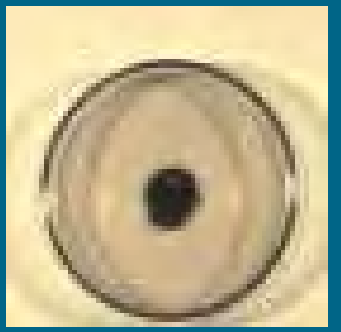


48-72 h

Black Yeasts



Several days

Problems for AST of Fungi

Different respiratory behaviour

Fermentative yeasts as →  *Candida*

Problems for AST of Fungi

Different respiratory behaviour

Fermentative yeasts as →  *Candida*

Non-Fermentative yeasts as

 *Cryptococcus*

 *Trichosporon*

 *Rhodotorula*

HOW AST?

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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 7.2 by EUCAST (including *Cryptococcus*)
- M44 A2 by CLSI, diffusion****

HOW 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 9.2 by EUCAST, Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia-forming moulds

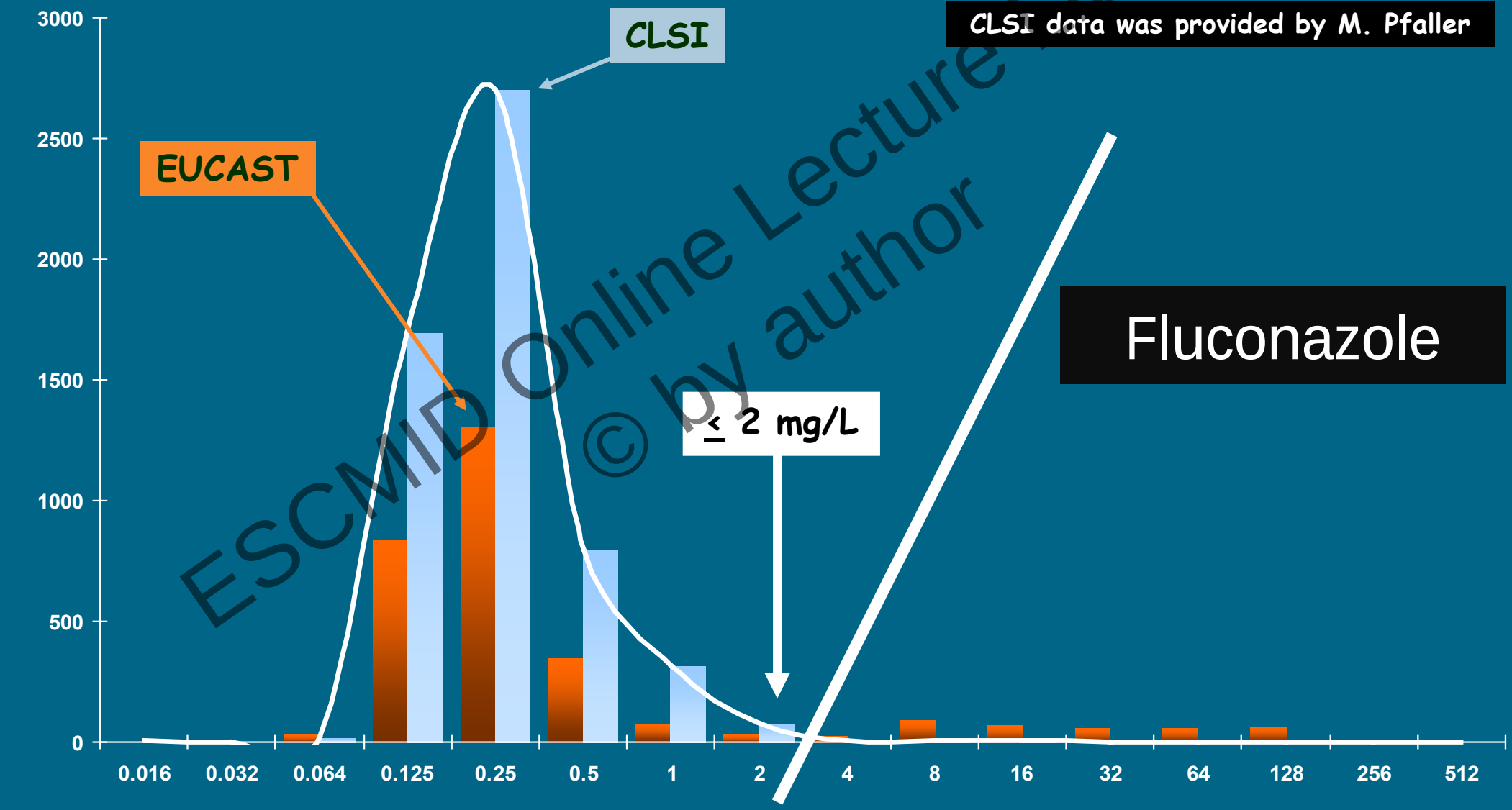
Reference methods, dilution

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

Breakpoints setting procedure

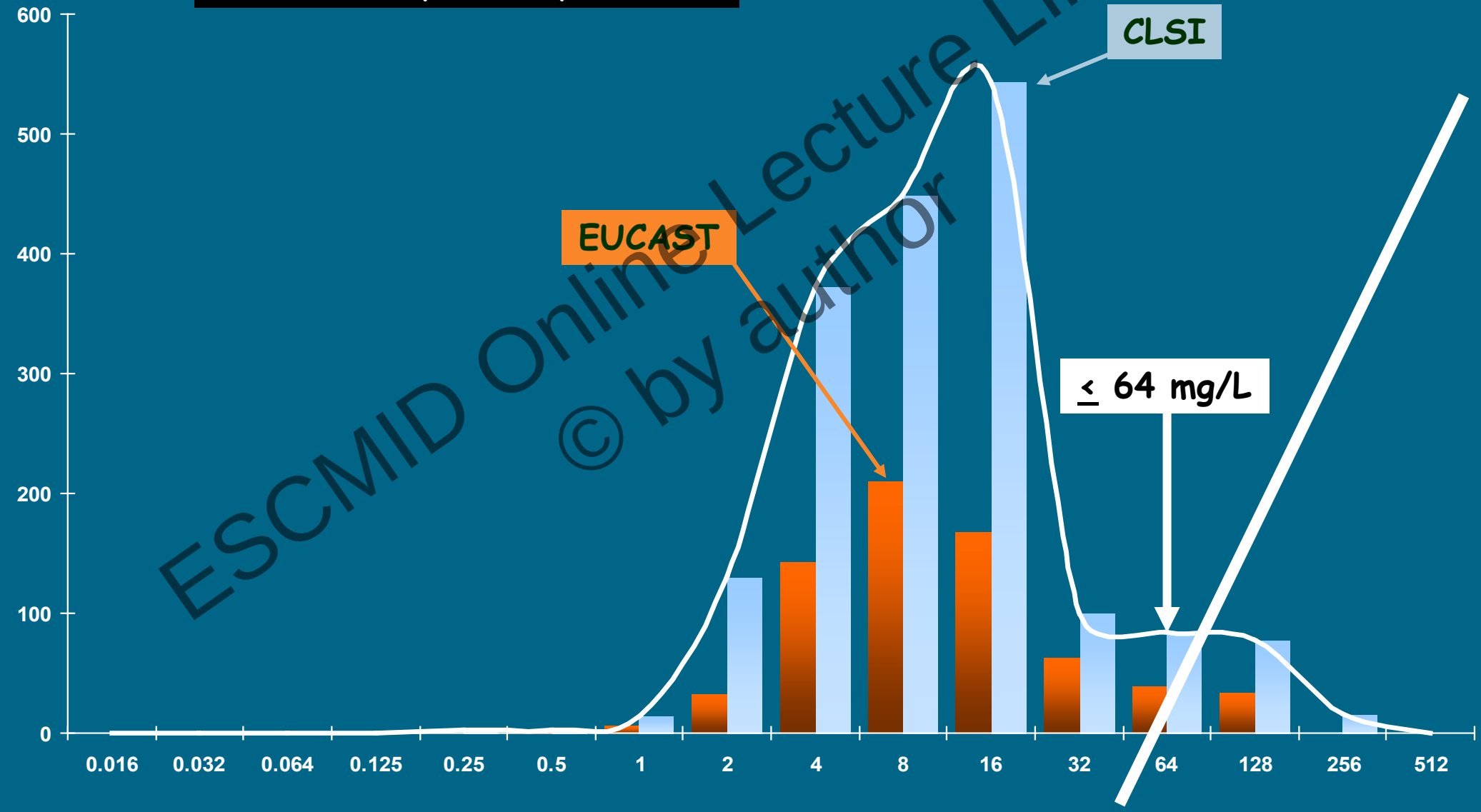
STEPS	EUCAST	CLSI
1	Identifying most common dosage used in each European country	Examining available microbiological data
2	Defining the wild type population for each target microorganism at the species level and determining the epidemiological cut-offs	Knowing resistance mechanisms and their relation 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*



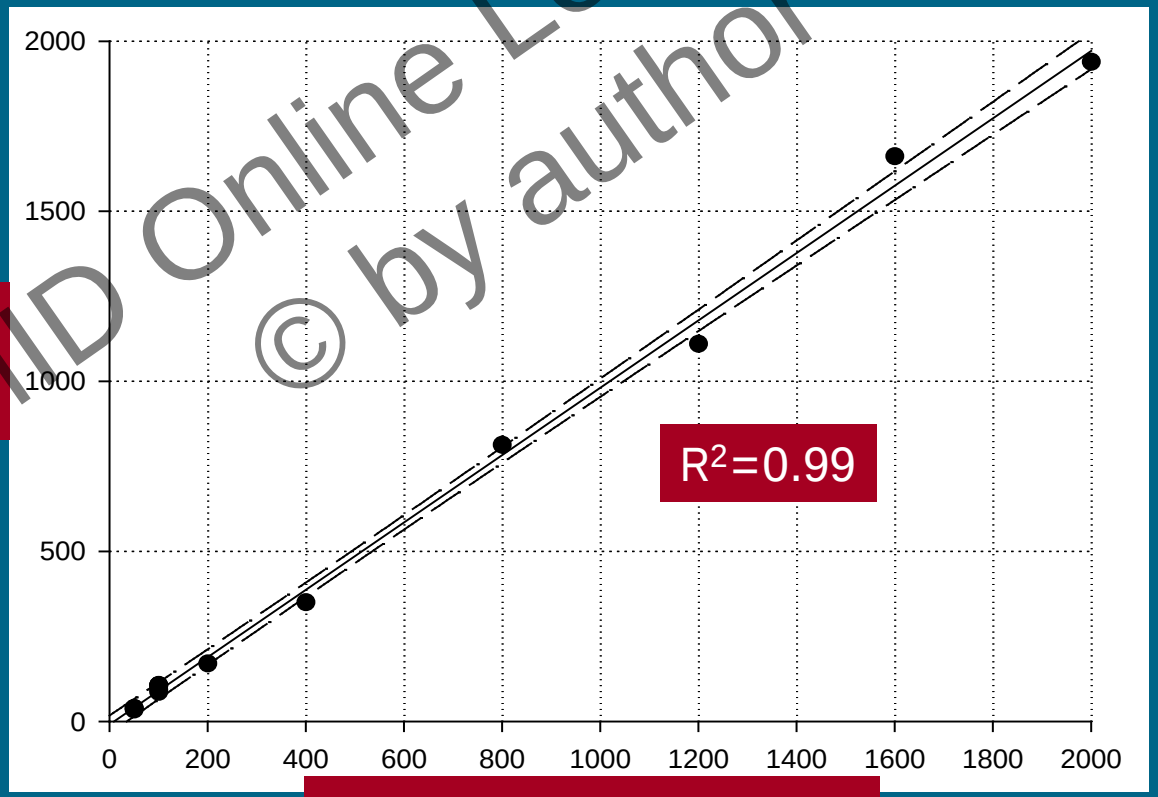
Epidemiological BPs for *C. glabrata*

CLSI data was provided by M. Pfaller



PK/PD - AUC/MIC

AUC = Dose



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

AMPHOTERICIN B

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
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

NEY: Not established yet

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

FLUCONAZOLE

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
C. albicans	≤ 2	4	> 4	≤ 2	4	--	≥ 8
C. glabrata	---	≤32	>32	--	≤ 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

IE: Insufficient evidences

PT: Poor target in vitro

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

ITRACONAZOLE

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
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

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

VORICONAZOLE

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
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

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

POSACONAZOLE

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
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

CASPOFUNGIN

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
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

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

MICAFUNGIN

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
C. albicans	≤0.016	---	>0.016	≤ 0.25	---	0.50	≥ 1
C. glabrata	≤ 0.03	---	>0.03	≤ 0.06	---	0.12	≥ 0.25
C. krusei	IE	IE	IE	≤ 0.25	---	0.50	≥ 1
C. parapsilosis	---	≤2	>2	≤ 2	---	4	≥ 8
C. tropicalis	≤ 0.03	---	>0.03	≤ 0.25	---	0.50	≥ 1

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

ANIDULAFUNGIN

Species	EUCAST			CLSI			
	Susceptible	Intermediate	Resistant	Susceptible	S-DD	Intermediate	Resistant
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	---	≤ 4	≤ 4	≤ 2	---	4	≥ 8
C. tropicalis	≤ 0.06	---	> 0.06	≤ 0.25	---	0.50	≥ 1

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

BP values are still under discussion

Antifungal Agent	Breakpoint (> is resistant)	Species (complexes)
Amphotericin B	>2 mg/L	*fumigatus* and *niger*
Itraconazole	>2 mg/L	*fumigatus*, *flavus*, *terreus* and *nidulans*
Voriconazole	>2 mg/L	*fumigatus*
Posaconazole	> 0.25 mg/L	*fumigatus*

The dilution standard reference procedures are unpractical for routine daily practice 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

Etest.
Results by species

Species	Correlation	Agreement
C. albicans	0.86	92%
C. parapsilosis	0.80	88%
C. tropicalis	0.81	85%
*C. glabrata**	0.76	80%
C. krusei	0.88	89%

Dannaoui et al. Clin Microbiol Infect 2010. Cuenca-Estrella et al. J Clin Microbiol. 2010. Arendrup MC et al. Antimicrob Agents Chemother.

Sensititre
Results by species

Species	Correlation	Agreement
C. albicans	0.91	90 %
C. parapsilosis	0.88	88 %
C. tropicalis	0.88	80 %
C. glabrata	0.76	69 %
C. krusei	0.88	74 %

Alexander et al. J Clin Microbiol 2007. Cuenca-Estrella et al. Clin Microbiol Infect 2005. Cuenca-Estrella et al. J Clin Microbiol. 2010

But, reference methods, dilution

Development of interpretative breakpoints of antifungal agents

Continued surveillance to monitor trends in antifungal resistance

Re-testing clinical isolates with borderline/resistant MIC values by commercial or screening methods

Provide a standard basis from which new AFST techniques can be developed and compared

Evaluation of activity profile of new antifungal agents

Susceptibility testing of uncommon and emerging species

AST Recommendations

ESCMID Diagnostic & Management Guideline for Candida Diseases 2012

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 3. Rare and emerging species 4. 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	• Periodical epidemiological studies should be done

Population-Based Prospective Surveillance on Candidemia in Spain (CANDIPOP study):

Barcelona-Bilbao-Madrid-Sevilla-Valencia (29 hospitales)

- A prospective multicenter population-based surveillance program on *Candida* BSI was implemented in 29 hospitals from 5 areas in Spain (population 7,237,228) from May 2010 to April 2011

CANDIPOP, results

- 767 cases with yeast BSI were detected. 14 cases had two different species of yeasts, resulting in 781 isolates
- Annual incidences were:
  - 10.7 h/100,000 population (Barcelona study 2002, 4.2/10,000, geo differences)
  - 0.78/1,000 admissions
  - 1.2/10,000 patient-days

CANDIPOP, results

Especie	Species distribution
C. albicans	45 %
C. parapsilosis	24 %
C. tropicalis	7.5 %
C. glabrata	13 %
C. krusei	2 %
C. guilliermondii	1.7 %
C. lusitaniae	1.3 %
Otras especies	5.5 %

BPs to interpret AST

Species	Antifungal agent						
	ANF	FC	FLC	ITC	VRC	POS	ECHIN
Candida albicans	S	S	S	S	S	S	S
Candida parapsilosis	S	S	S	S	S	S	S-I
Candida tropicalis	S	S	S	S	S	S	S
Candida glabrata	S	S	I-R	S-I-R*	S-I-R*	S-I-R*	S
Candida krusei	S	R	R	S-I-R**	S-I-R**	S-I-R**	S
Candida guilliermondii	S	S	I-R	S	S	S	S-I
Candida lusitaniae	S	S	S	S	S	S	S
Cryptococcus spp.	S	S-I	S-I-R**	S	S	S	R
Trichosporon spp.	S-I-R**	R	I-R	S-I-R**	S-I-R**	S-I-R**	R

CANDIPOP, results

Especie	Almirante et al 2003	Peman et al 2010	CANDIPOP 2012
C. albicans	51%	43%	45%
C. parapsilosis	23%	29%	24%
C. tropicalis	10%	10%	7,5%
C. glabrata	9%	8,5%	13%
C. krusei	4%	3%	2%
% R fluco	7,9%	6%	14%

CANDIPOP, results

Esp			2
C. a			
C. p			
C. t			
C.			
C. k			
% R fluco	7,9%	6%	14%

CANDIPOP:
Strains potentially resistant (FLU MIC> 4 plus all *C. glabrata*)

22% of decreased susceptibility to FLU

CANDIPOP, results

Species	FLC Resistance	Other resistances
C. albicans	2 %	Cross Resistance to VOR, POS and ITC in some cases. AMB R < 3 % ECHI R < 5 % (*non-parapsilosis*)
C. parapsilosis	5 %	
C. tropicalis	20 %	
C. glabrata	40 % (100 %)*	
C. krusei	100 %	
C. guilliermondii	60 %	
C. lusitaniae	15 %	
Otras especies	28 %	

ECCMID
Berlin, Germany
27 – 30 April 2013

ESCMID

The CANDIPOP Project

Impact of treatment strategy on outcome in patients with bloodstream infection due to *Candida parapsilosis*: results of a population-based surveillance in Spain

Mario Fernández-Ruiz[a], José María Aguado[a], Belén Padilla[b], Isabel Ruiz[c], José Garnacho-Montero[d], Manuel Cuenca-Estrella[e], on behalf of the CANDIPOP Project

[a]Hospital Universitario 12 de Octubre, Madrid; [b]Hospital General Universitario Gregorio Marañón, Madrid; [c]Hospital Universitari Vall d´Hebron, Barcelona; [d]Hospital Universitario Virgen del Rocío, Sevilla; [e]Centro Nacional de Microbiología, Madrid, Spain

Overall: 752 episodes of *Candida* BSI in 729 patients

Annual incidence: 8.1 episodes x 10^5 population

182 episodes of *C. parapsilosis* BSI (24.2%)

Annual incidence: 1.9 episodes x 10^5 population

Impact of treatment strategy on outcome of patients with bloodstream infection (BSI) due to *Candida parapsilosis*: results of a population-based surveillance in Spain

Outcomes

Mortality

Early (7-day) all-cause mortality	15 (8.2%)
Late (30-day) all-cause mortality	43 (23.6%)

Persistent BSI[a]

At 72 hours	39 (21.4%)
At 7 days	18 (9.9%)

Antifungal therapy (within the first 24 h)

Azole-based monotherapy	75 (41.2%)
Echinocandin-based monotherapy	49 (26.9%)

- *Antifungal therapy within the first 24 h*

	Azole-based regimen	Echinocandin-based regimen	*P*-value
At 72 hours	16 (31.4%)	8 (25.8%)	0.591
At 7 days	7 (11.3%)	5 (13.5%)	0.488

- *Antifungal therapy within the first 72 h*

	Azole-based regimen	Echinocandin-based regimen	*P*-value
At 72 hours	15 (31.2%)	8 (29.6%)	0.884
At 7 days	8 (14.3%)	5 (16.1%)	0.524

- *Antifungal therapy within the first 24 h*

	Azole-based regimen	Echinocandin-based regimen	*P*-value
Early mortality	3 (4.0%)	0 (0.0%)	0.218
Late mortality	14 (18.7%)	11 (22.4%)	0.608

- *Antifungal therapy within the first 72 h*

	Azole-based regimen	Echinocandin-based regimen	*P*-value
Early mortality	3 (4.3%)	0 (0.0%)	0.243
Late mortality	13 (18.8%)	11 (26.8%)	0.327

6th ADVANCES AGAINST ASPERGILLOSIS

Madrid, Spain

27 February – 1 March 2014

Melia Castilla Conference and Convention Centre

www.AAA2014.org