Instituto de Salud Carlos III

2nd ESCMID Conference on Invasive Fungal Infections

Antifungal resistance: from bench to bedside

Antifungal drug resistance mechanisms in pathogenic fungi

Manuel Cuenca-Estrella

ROME, 2013

Steps of Development of AST

- Reliable reference procedures (CLSI, EUCAST)
- Development of breakpoints
- Commercial methods
- Spreading of AST
- Description of resistance mechanisms and prevalence
- Prevention and control

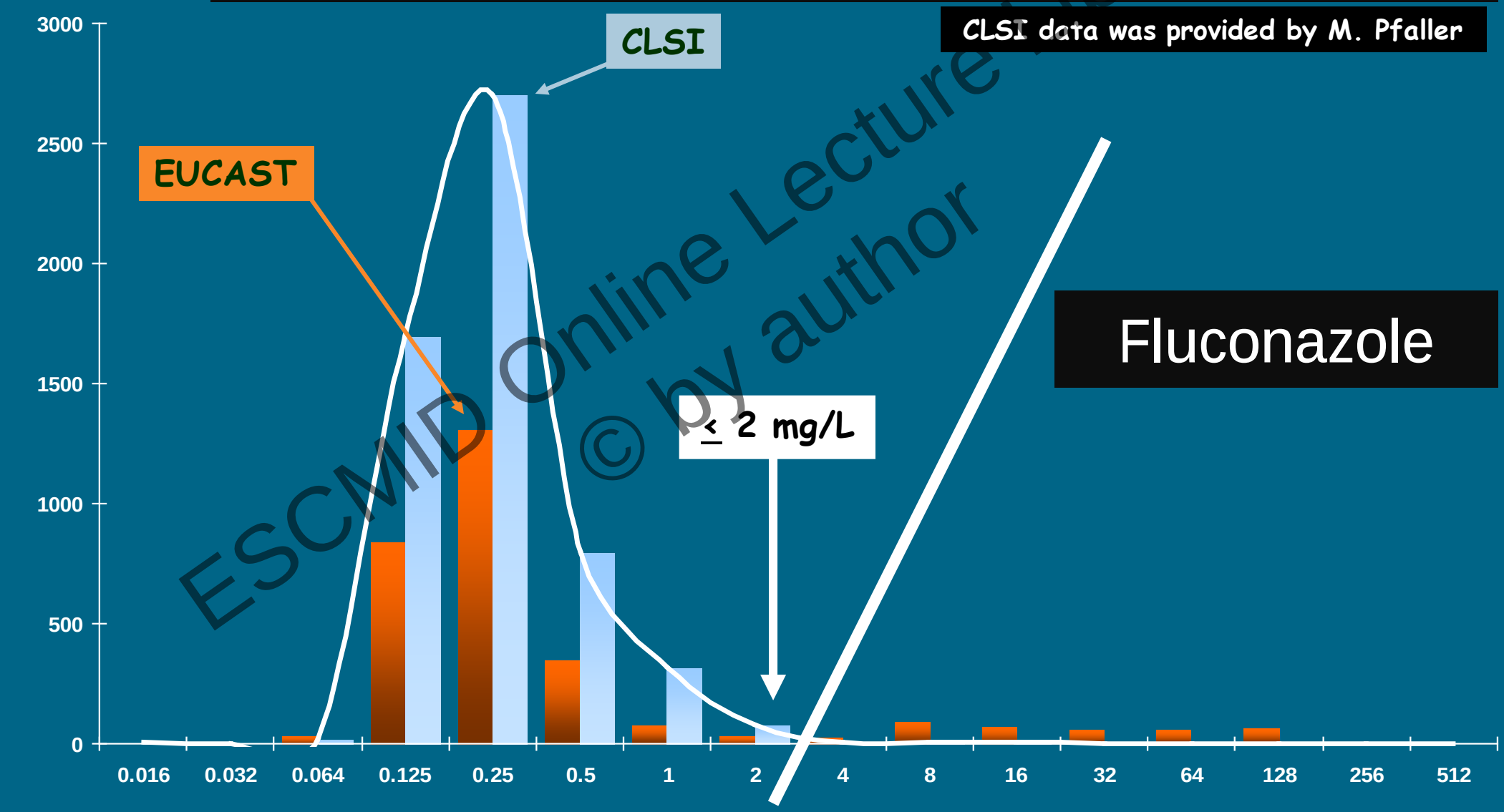
Reference methods of AST

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

Breakpoints setting procedure

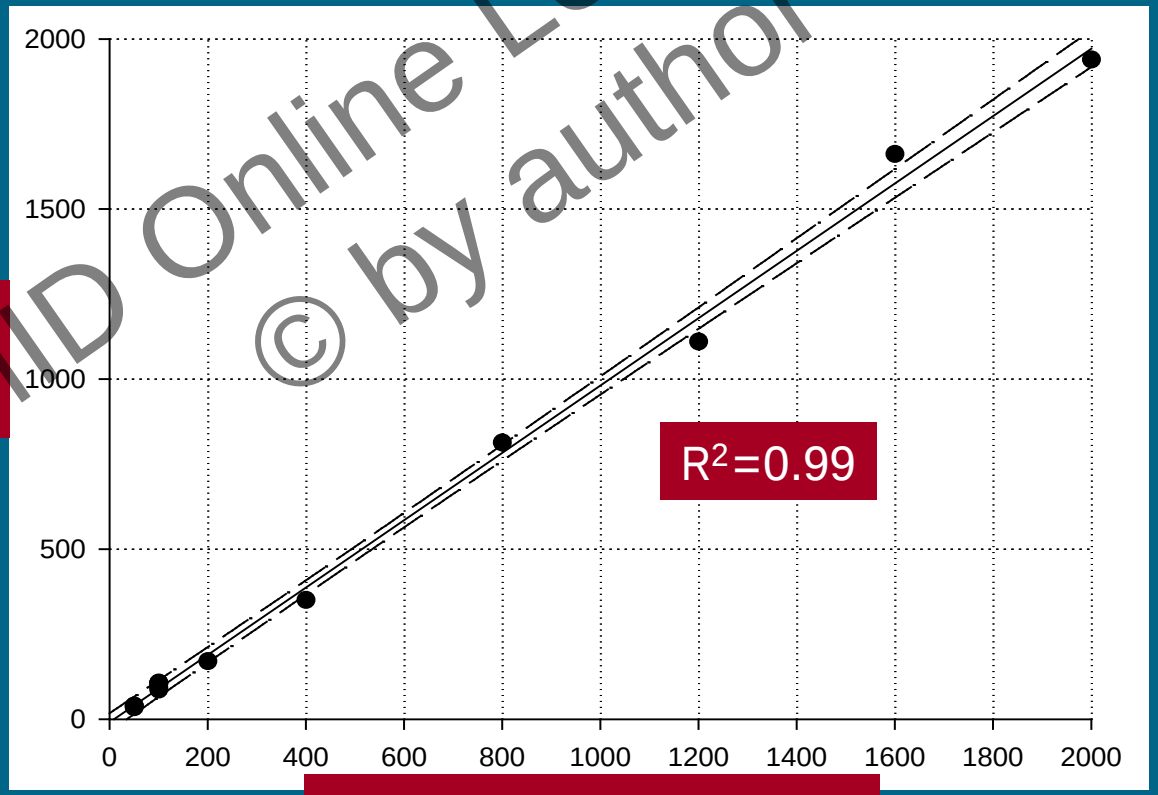
STEPS	EUCAST	CLSI
1	Identifying most common dosage used in each European country	Examining available microbiological data
2	Defining the wild type population for each target microorganism at the species level and determining the epidemiological cut-offs	Knowing resistance mechanisms and their relation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



PK/PD - AUC/MIC

AUC = Dose



BPs set already

- *Candida*:
  - Amphotericin B, azoles and echinocandins
- *Aspergillus*:
  - Amphotericin B and azoles
- Other species: not yet

Steps of Development of AST

- Reliable reference procedures (CLSI, EUCAST)
- Development of breakpoints
- Commercial methods
- Spreading of AST
- Description of resistance mechanisms and prevalence
- Prevention and control

Description of resistance mechanisms and prevalence

- MIC value determination as phenotypic screening:

1. Is it reliable?

- Determining of resistance mechanisms at molecular level:

1. Clinical relevance??
2. Reliable molecular tools?
3. Could be more reliable that MIC determination for prevention and control?

Current situation of antifungal resistance. Summary

- Amphotericin B:
  - Resistance is an uncommon phenomenon (*Trischosporon* spp., *A. terreus*)
- Azole agents:
  - Main concern in this field (*Candida glabrata*, some isolates of *Aspergillus*)
- Echinocandins:
  - Some *Candida* isolates
  - Non reliable method of AST for moulds yet

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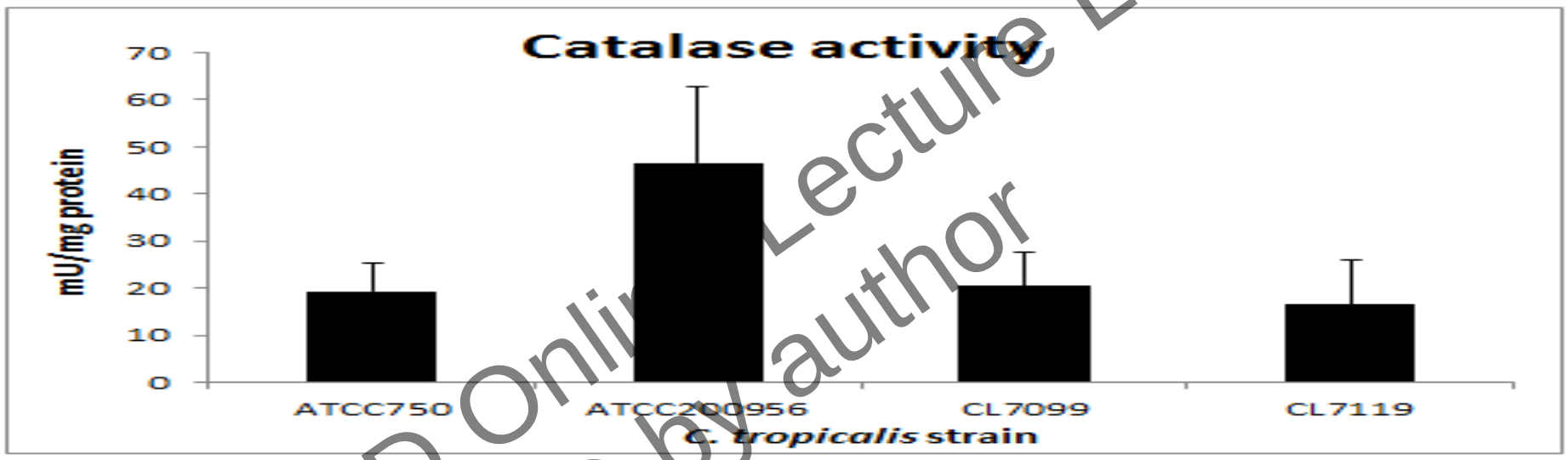
Resistance to amphotericin B

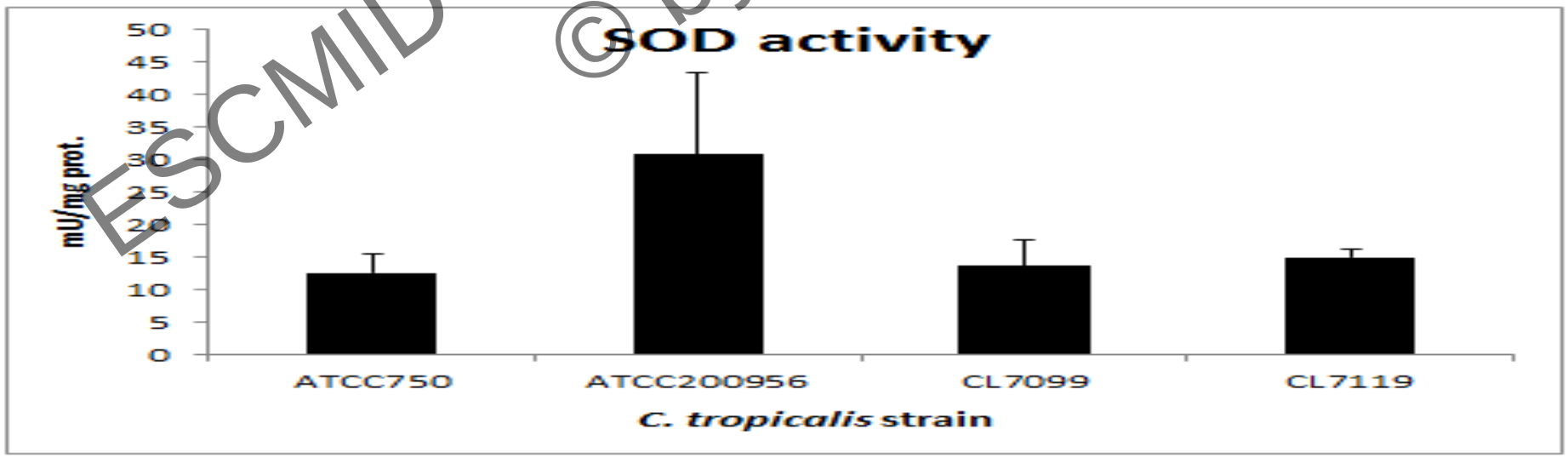
Resistance to amphotericin B

- MIC determination is able to detect resistant strains (*). BPs availability
- Resistance mechanisms:
  - Mutants with low ergosterol membrane
  - AmB induces oxidative stress in fungal cells and resistant isolates have higher levels of antioxydative enzymes and/or alterations in the production of free radicals

C. tropicalis, MIC AmB=8 mg/L

Spanish Reference Lab, preliminary results





Resistance to amphotericin B II

Determining molecular resistance mechanisms:

1. **Clinical relevance??**

Uncommon phenomenon

2. **Reliable molecular tools?**

No yet. Ergosterol quantification, catalase activity or free radical production are phenotypic and non standardized

3. **Could be more reliable that MIC determination for prevention and control?**

I don not think so. The fitness issue

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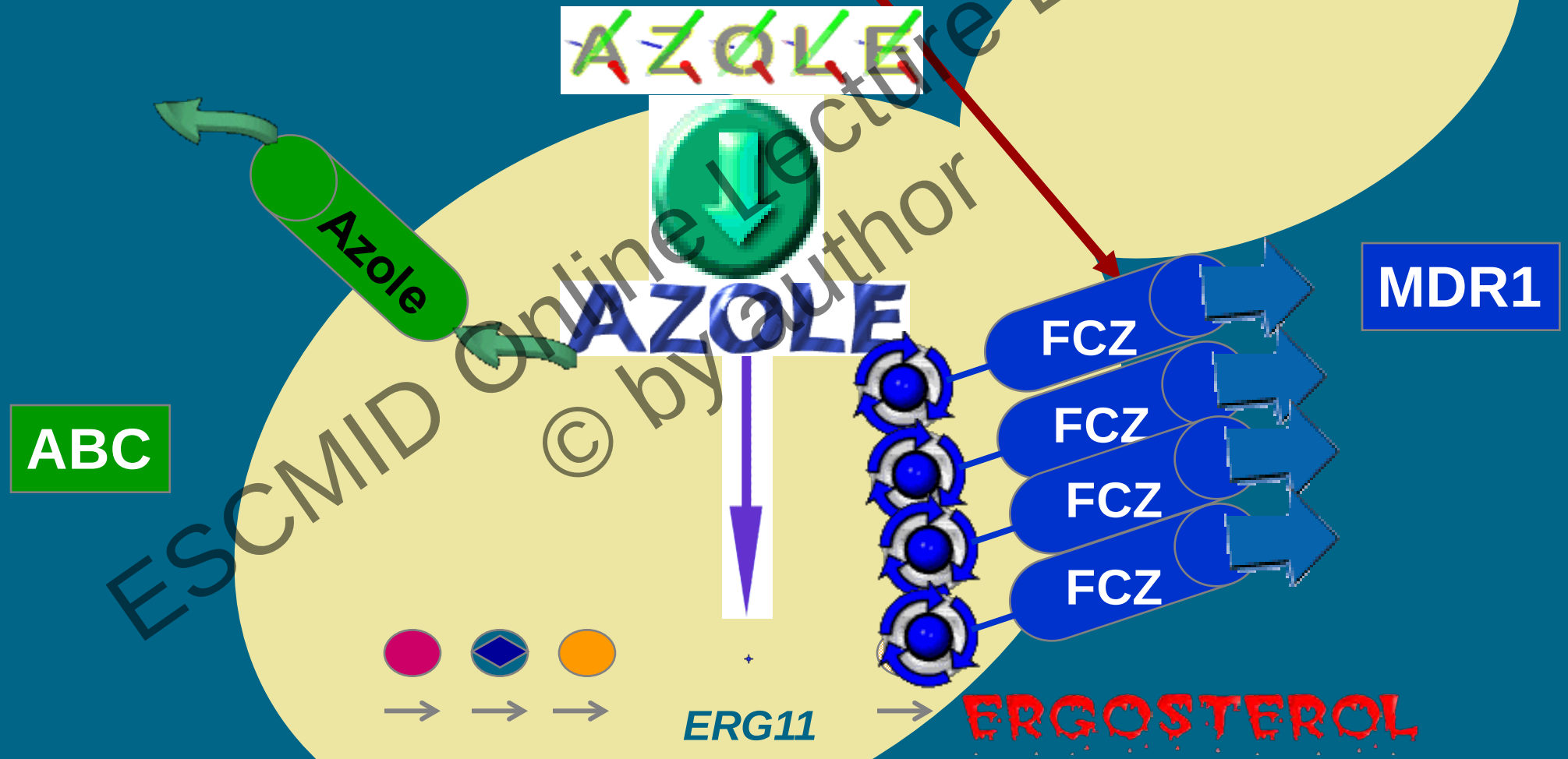
Resistance to azoles

Resistance to azoles

- MIC determination is able to detect resistant strains. BPs availability
- Resistance mechanisms:
  - Described in yeasts and moulds

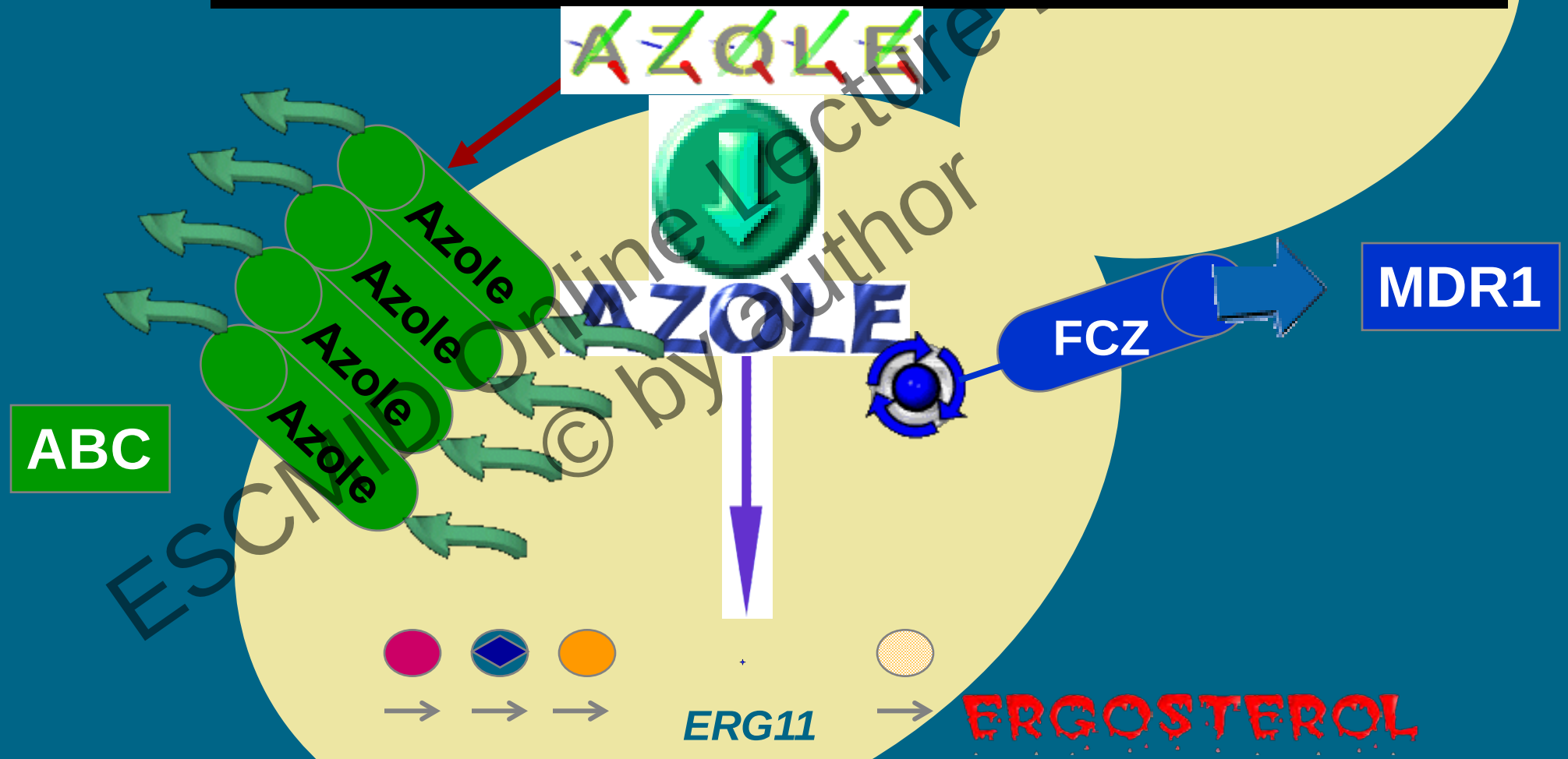
Up regulation of Major facilitator genes

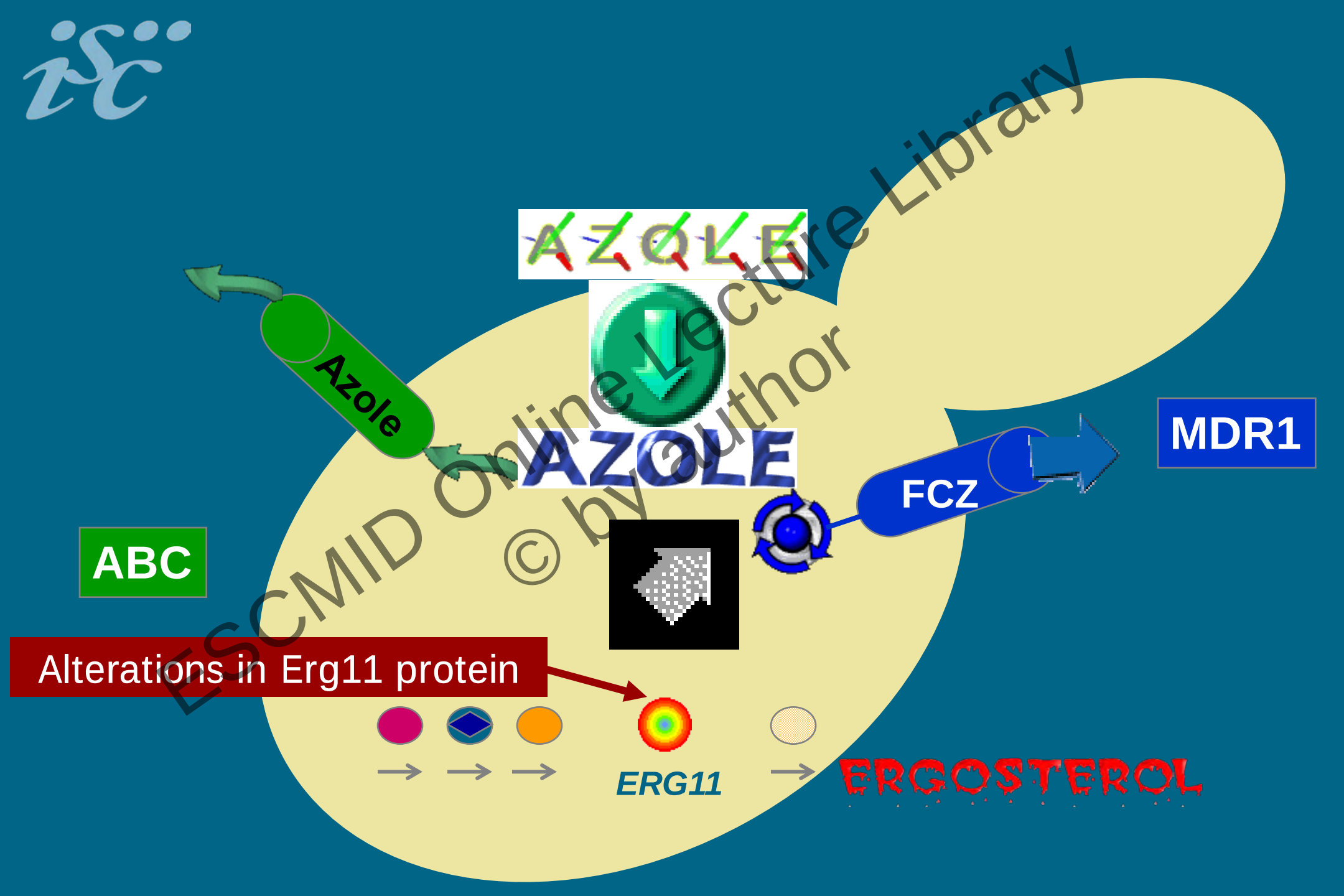
Resistance only to FZ

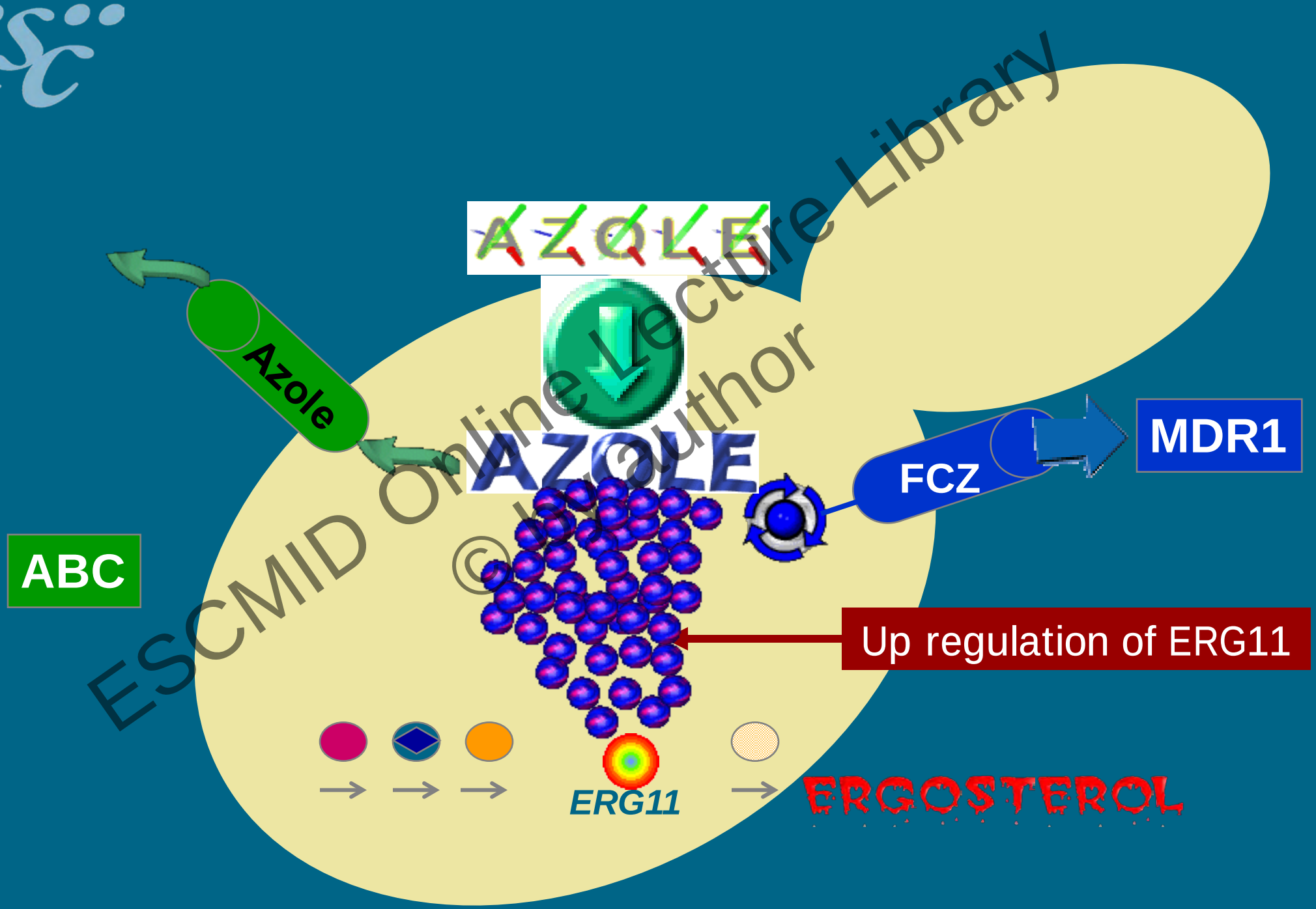


Up regulation of ATP binding cassette transporters genes

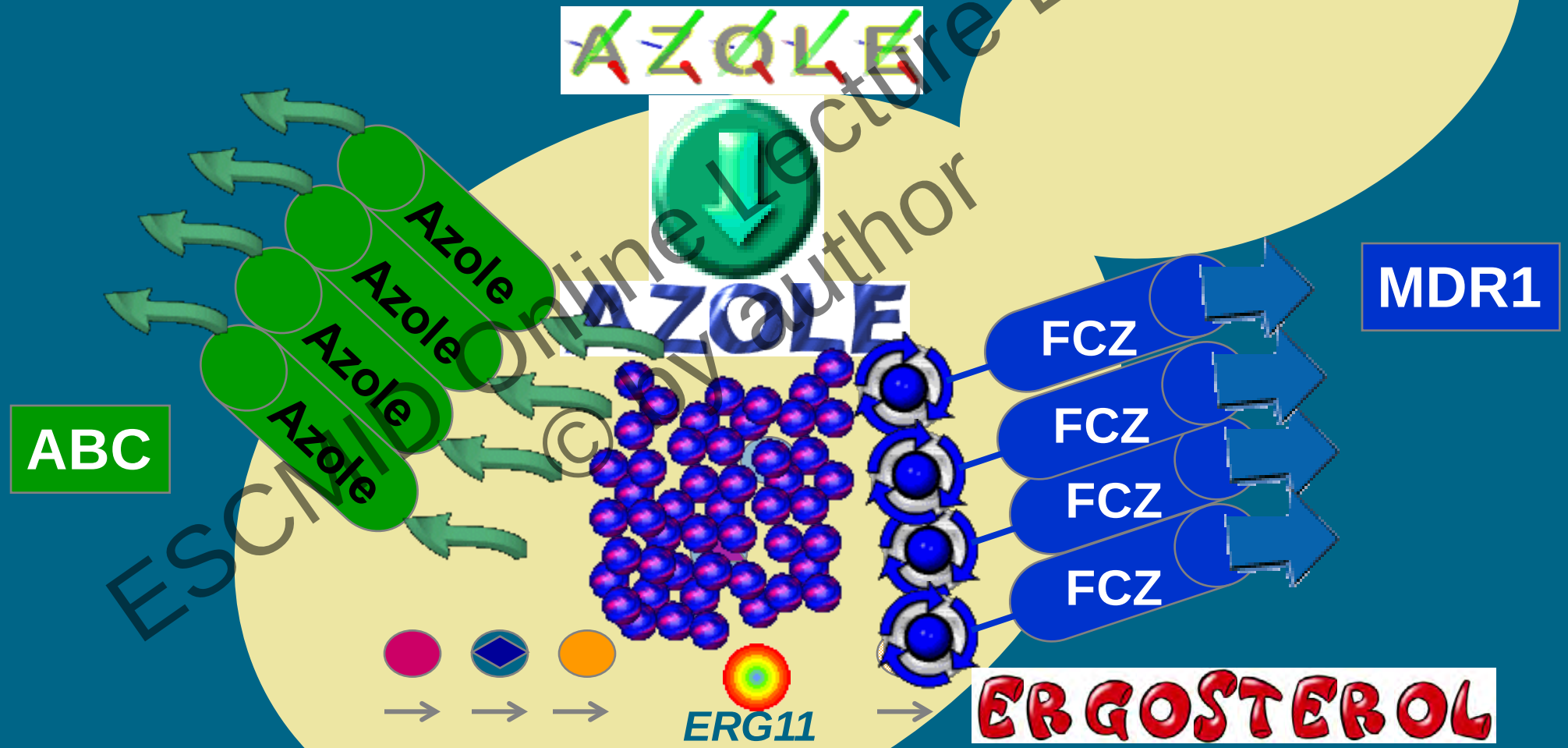
Cross resistance to all azoles







All mechanisms can coexist



All mechanisms can coexist

Description of mutations in *ERG11*

Alterations in promotor region

Sanglard and others

ABC

JOURNAL OF CLINICAL MICROBIOLOGY, July 2001, p. 2431–2438
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.7.2431–2438.2001

Vol. 39, No. 7

Identification of Two Different 14-α Sterol Demethylase-Related Genes (*cyp51A* and *cyp51B*) in *Aspergillus fumigatus* and Other *Aspergillus* species

E. MELLADO,* T. M. DIAZ-GUERRA, M. CUENCA-ESTRELLA, AND J. L. RODRIGUEZ-TUDELA
Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

Azole

AZOLE

AZOLE

Strain with low MIC

Cyp51A Cyp51B

ERGOSTEROL

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. Garcia-Effron, 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

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

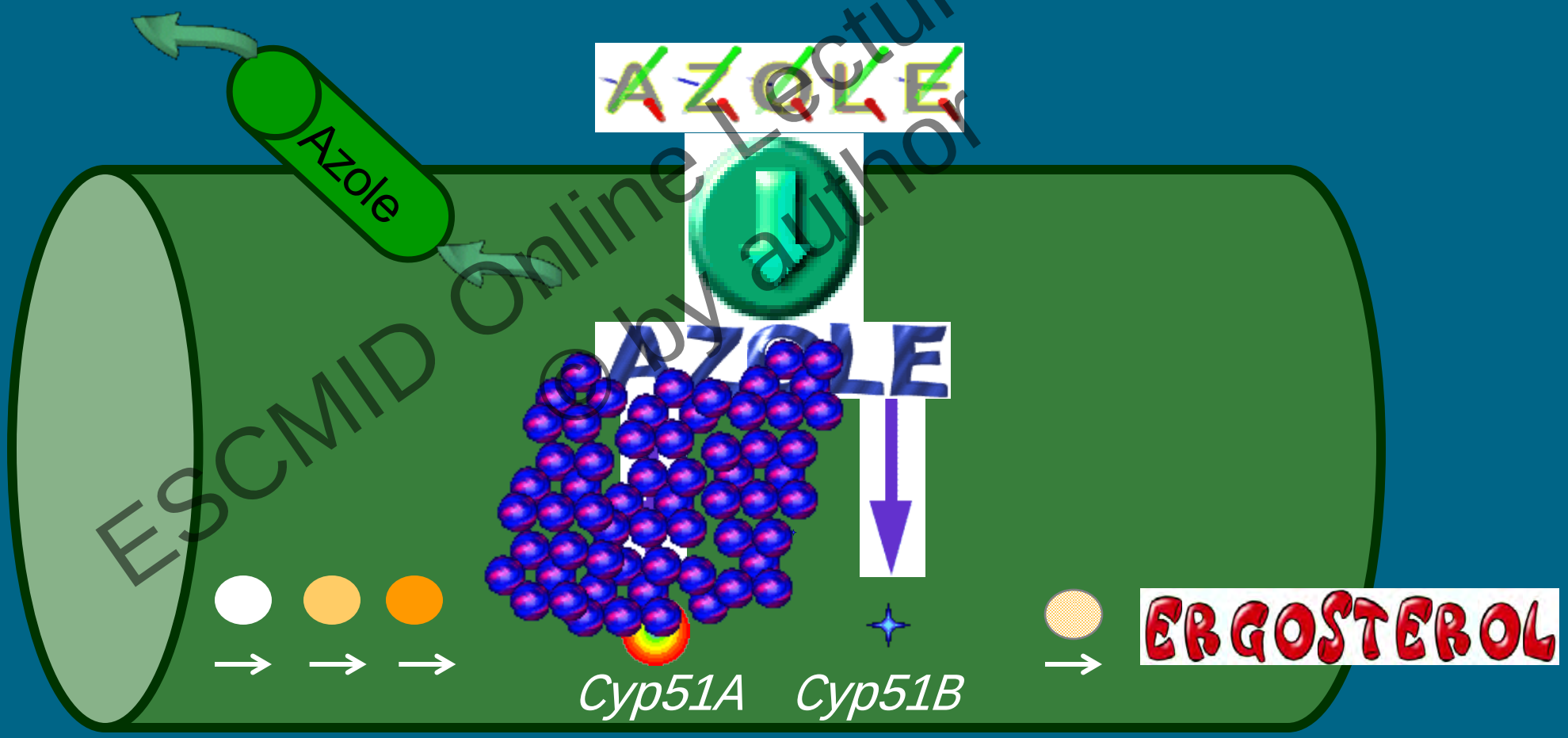
E. Mellado,* G. Garcia-Effron, M. J. Buitrago, L. Alcazar-Fuoli, M. Cuenca-Estrella, and J. L. Rodriguez-Tudela

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



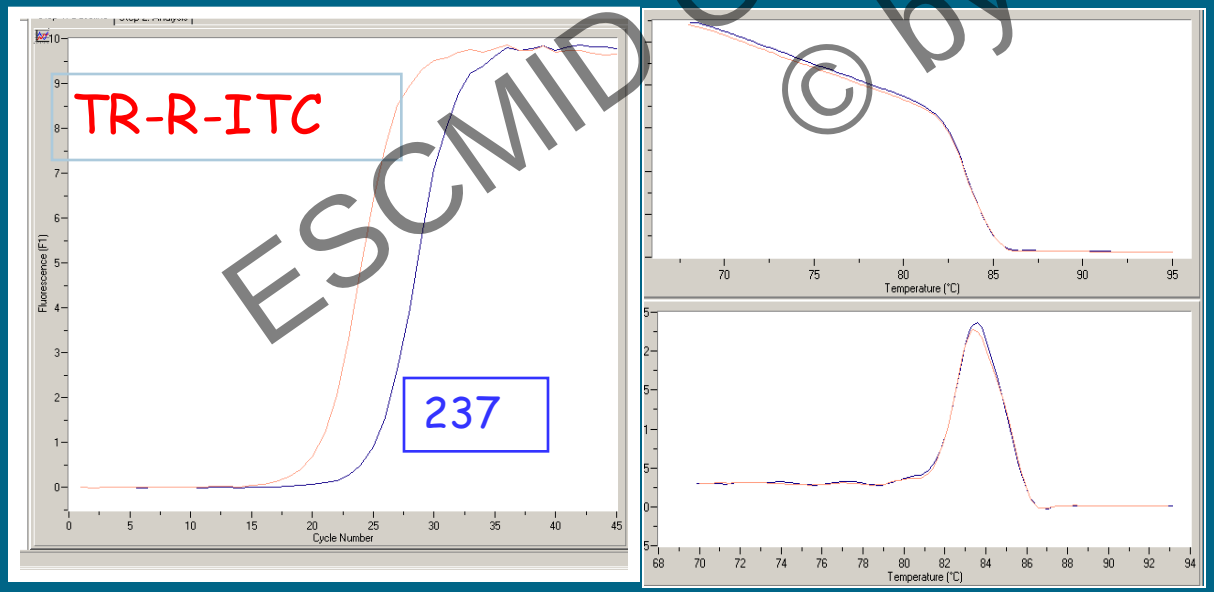
Gen Cyp51A in a resistant strain

Duplication of
34-bp sequence in
the promoter

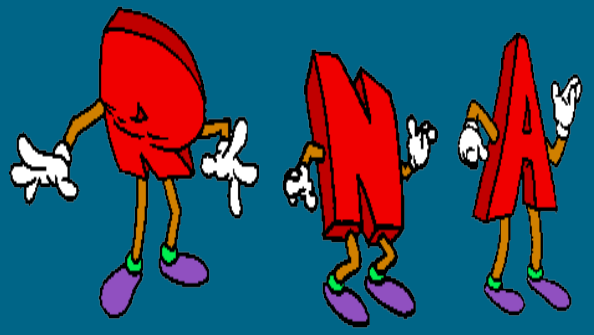
GAATCACGCGG GAATCACGCGG

Cyp51A

L98H



x 8

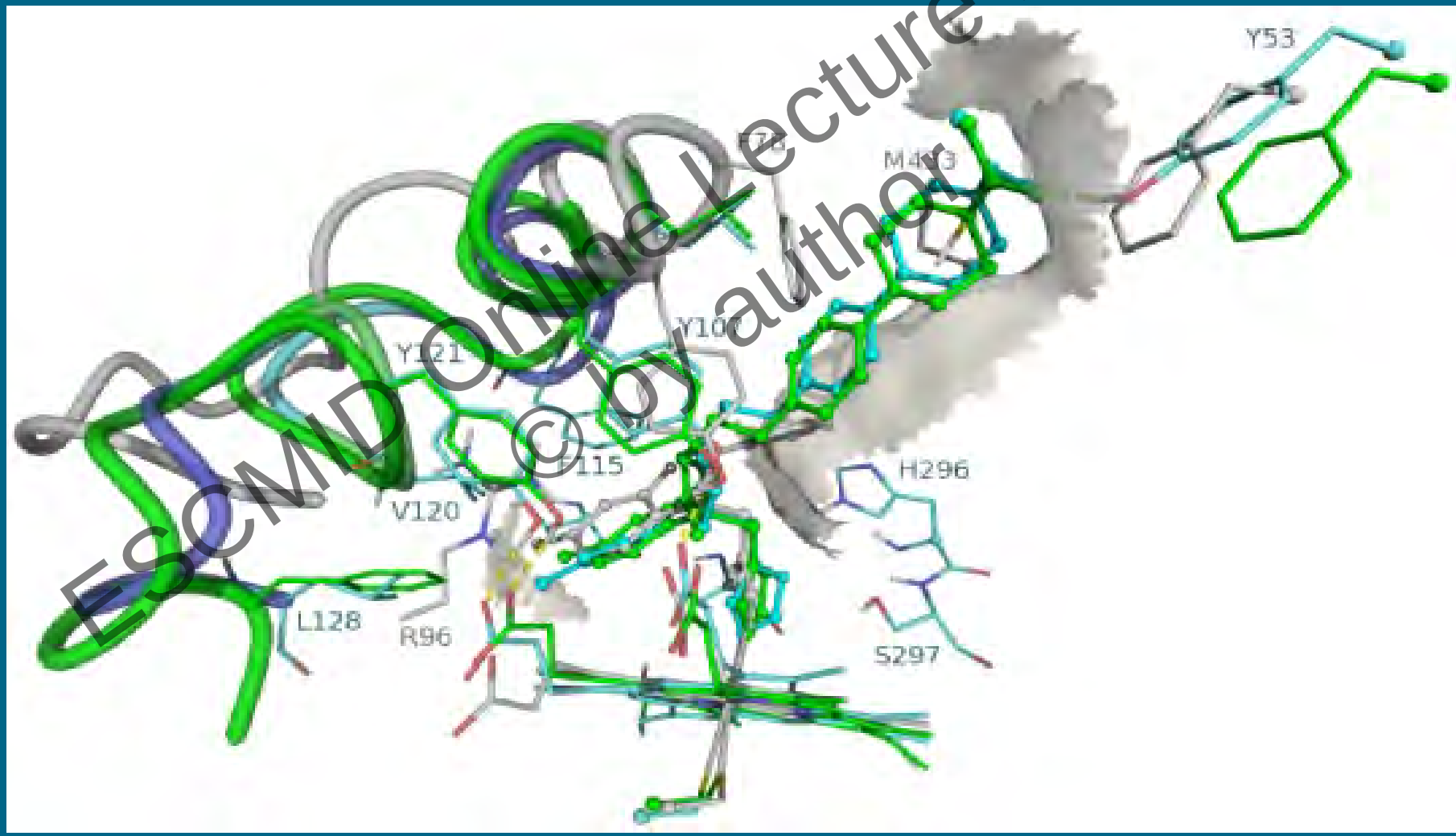


New mechanisms of resistance in *Aspergillus*

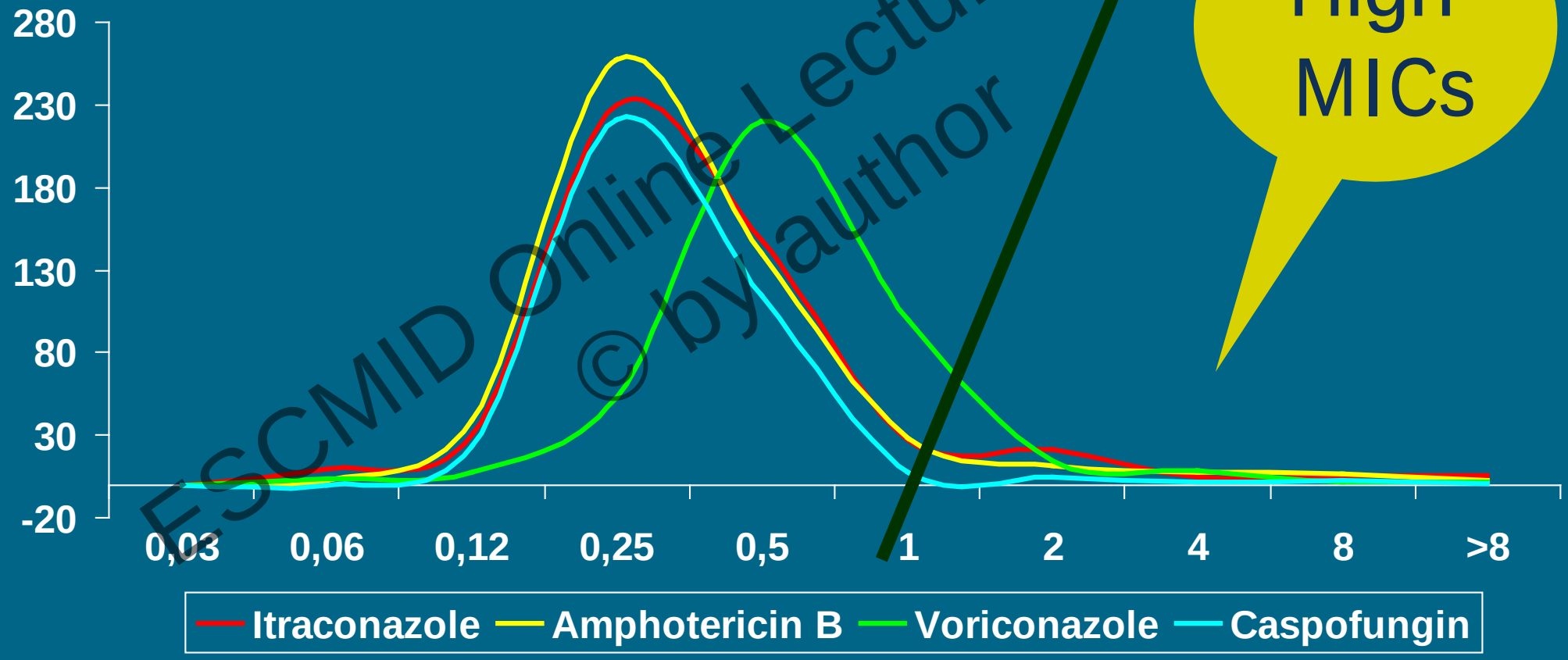
- The azole target cyp51A is a hotspot for mutations that confer phenotypic resistance, but in an increasing number of resistant isolates the underlying mechanism remains unknown.
- Increase of expression and mutations related to efflux pumps
- Mutation in the CCAAT-binding transcription factor complex subunit HapE. A P88L substitution in HapE. *Camps et al Plos One 2012*

Arendrup et al, Denning et al, Mellado et al, Verweij et al

3D Representation of three aligned structures of CYP51 with the ligands in their active site, constructed by using the Yasara software. *Snelders Plos One 2012*



EUCAST *A. fumigatus* MICs distribution



Data obtained from Mycology Reference Laboratory. Spain

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*

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Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism

Eveline Snelders[1,2], Henrich A. L. van der Lee[1,2], Judith Kuijpers[1,2], Anthonius J. M. M. Rijs[1,2], János Varga[3,4], Robert A. Samson[3], Emilia Mellado[5], A. Rogier T. Donders[6], Willem J. G. Melchers[1,2], Paul E. Verweij[1,2*]

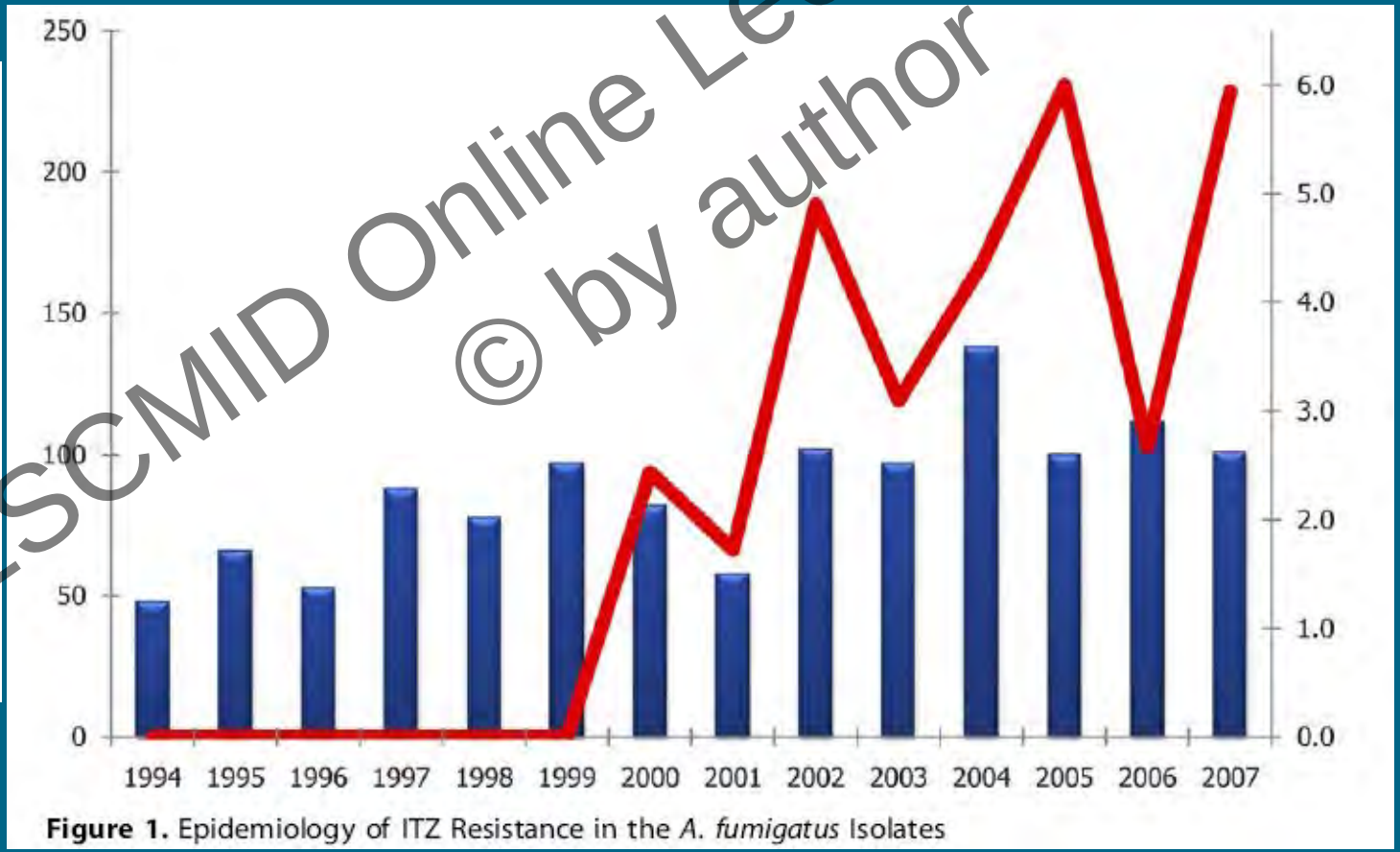

Figure 1. Epidemiology of ITZ Resistance in the *A. fumigatus* Isolates

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Emergence of Azole Resistance in *Aspergillus fumigatus* and Spread of a Single Resistance Mechanism

Eveline Snelders[1,2], Henrich A. L. van der Lee[1,2], Judith Kuijpers[1,2], Anthonius J. M. M. Rijs[1,2], János Varga[3,4], Robert A. Samson[3], Emilia Mellado[5], A. Rogier T. Donders[6], Willem J. G. Melchers[1,2], Paul E. Verweij[1,2*]

Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in *Aspergillus fumigatus*. *Plos One 2012*

Also in yeasts. *C. tropicalis. Yang et al. Plos One 2012*

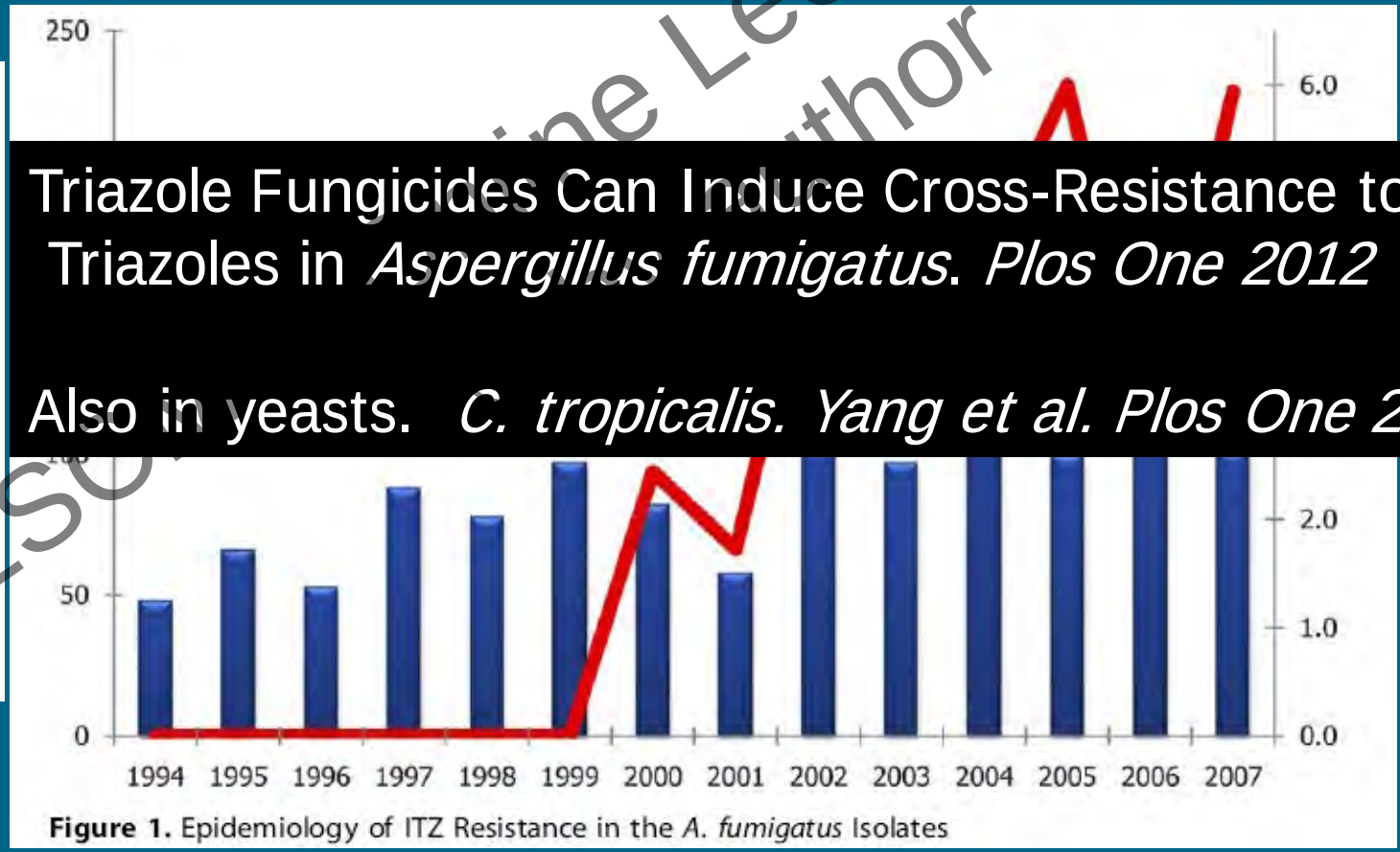

Figure 1. Epidemiology of ITZ Resistance in the *A. fumigatus* Isolates

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 15, No. 7, July 2009

Frequency and Evolution of Azole Resistance in *Aspergillus fumigatus* Associated with Treatment Failure[1]

Susan J. Howard, Dasa Cerar, Michael J. Anderson, Ahmed Albarrag, Matthew C. Fisher, Alessandro C. Pasqualotto, Michel Laverdiere, Maiken C. Arendrup, David S. Perlin, and David W. Denning

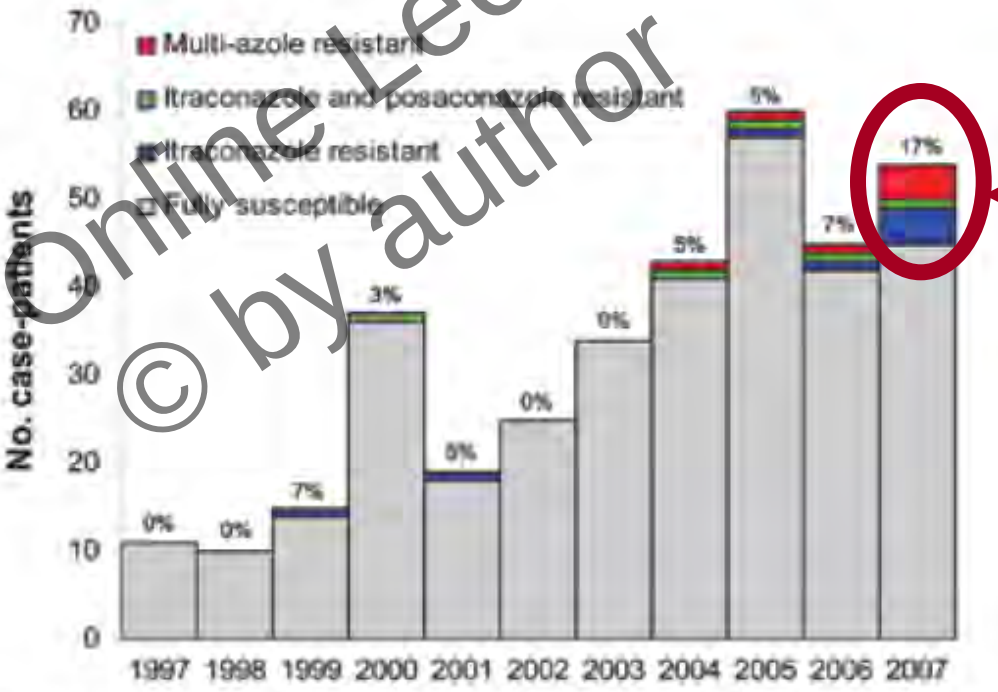

Figure 1. Azole resistance in clinical *Aspergillus fumigatus* isolates received in the Regional Mycology Laboratory Manchester, UK, 1997–2007. Overall azole resistance for each year is shown above each column as a percentage. Data do not include sequential isolates from the same patient.

Resistance to azoles

Determining molecular resistance mechanisms:

1. **Clinical relevance??**

Yes

2. **Reliable molecular tools?**

Yes, lack of standardization. Some resistance mechanism still unknown

3. **Could be more reliable that MIC determination for prevention and control?**

Not yet

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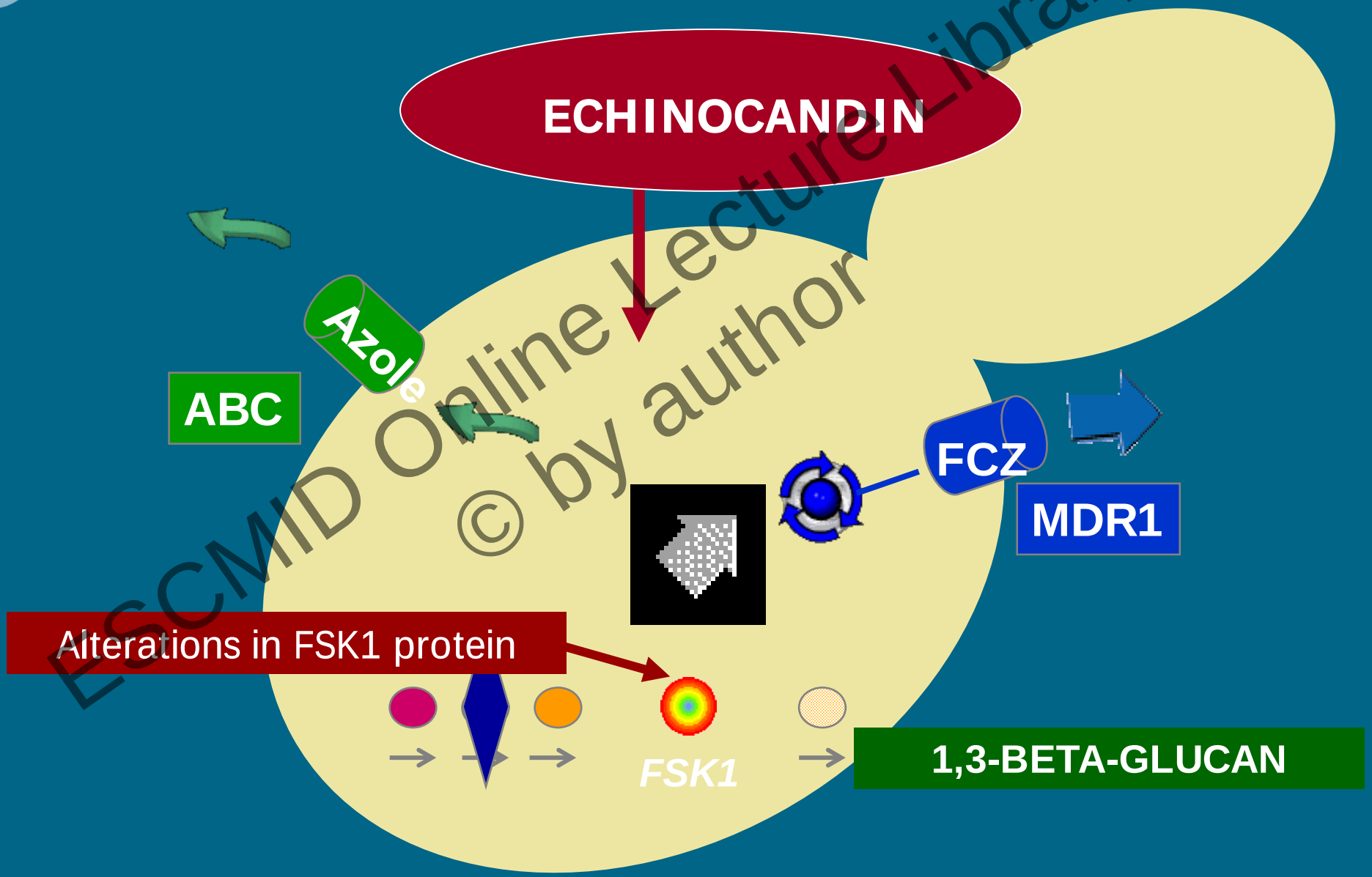
Resistance to echinocandins

Resistance to echinocandins

- **MIC determination is able to detect resistant yeast strains. BPs availability (*)**
- **Resistance mechanisms** *Beyda et al. Annals of Pharmacotherapy 2012*:
  - **Adaptive stress responses, which result in elevated cell wall chitin content and paradoxical growth in vitro**
  - **Acquired FKS mutations, which confer reduced glucan synthase sensitivity, elevated MICs, and are associated with clinical failure**
  - **Intrinsic FKS mutations, which are naturally occurring mutations in *C. parapsilosis* and *C. guilliermondii*, elevated MIC but a lower level of reduced glucan synthase sensitivity compared with acquired FKS mutations.**



Resistance to echinocandins

Perlin et al, Arendrup et al

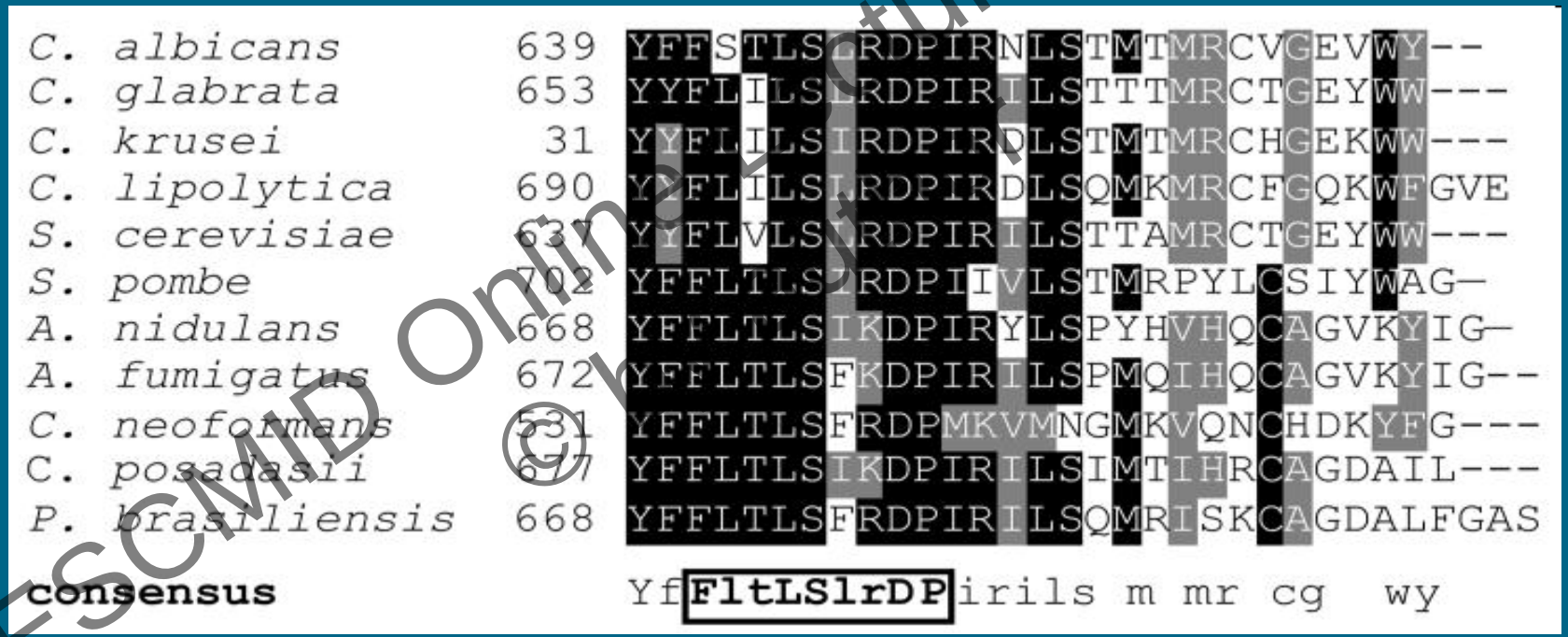
FKS1

Hotspot1 Hotspot2

Organism	Hot Spot1	Hot Spot 2
C. albicans	FLTLSLRDP	DWIRRYTL
C. krusei	FLILSIRDP	DWIRRYTL
C. glabrata	FLILSIRDP	DWIRRYTL
C. guilliermondii	FMALSIRDP	DWIRRYTL
C. tropicalis	FLTLSLRDP	DWIRRYTL
C. lypolytica	FLILSIRDP	DWIRRCVL
C. parapsilosis	FLTLSIRDA	DWIRRYTL
C. dubliniensis	FLTLSIRDP	DWIRRYTL

Resistant phenotype No phenotype Weak Resistance

Sequencing and analysis



Clinical failures

Table 3. Summary of 17 reported echinocandin recipients with fungal isolates demonstrating reduced susceptibility or resistance to echinocandins

Reference	Hosts	Indications[a]	Recur[b]	Multiple courses[c]	Total (days)[d]	Isolate	MIC[e]	MIC I (days)[f]	MIC II (days)[g]	MIC III (days)[h]	Amino acid substitution[i]	Failure[j]
[55]	AIDS (6 cells/μL[k])	Oesophagitis	Yes	Yes	25	*C. albicans*	E	0.25 (0)	2 (23)	2 (23)	F641S	Yes
[58]	AIDS (32 cells/μL[k])	Oesophagitis	Yes	Yes	88	*C. albicans*	C	0.25 (0)	>64 (88)	>64 (88)	N/A	Yes
[60]	AIDS (5 cells/μL[k])	Oesophagitis	Yes	No	>300	*C. albicans*	C	0.06 (0)	2 (42)	2 (42)	S645F, R1361H	Yes
[61]	AIDS (19 cells/μL[k])	Oesophagitis	Yes	No	450	*C. albicans*	C	8 (450)	N/A	N/A	S645P	Yes
[63]	Neutropenia	Febrile neutropenia	No	No	14	*C. albicans*	C	0.5 (0)	>8 (14)	>8 (14)	S645F, S645P	Yes
[63]	Neutropenia	Febrile neutropenia	No	No	39	*C. albicans*	C	0.5 (4)	4 (27)	4 (27)	S645F	Yes
[63]	Neutropenia	Febrile neutropenia	No	No	27	*C. krusei*	C	32 (17)	N/A	N/A	R1361G	Yes
[6]	Abdominal OP	Candidaemia	Yes	No	35	*C. albicans*	E, Et	0.25 (0)	0.5 (13)	32 (28)	S645P	Yes
[59]	Abdominal OP	Candidaemia	Yes	No	136	*C. glabrata*	C	0.5 (N/A)	8 (N/A)	>8 (126)	N/A	N/A
[64]	Abdominal OP	Candidaemia	No	No	16	*C. krusei*	C	2 (0)	N/A	N/A	N/A	Yes
[57] and [68]	AML	Candidaemia	No	No	17	*C. krusei*	C	0.25 (0)	1 (17)	1 (17)	F655C	Yes
						C. fermentati	C	>16 (17)	N/A	N/A	N/A	
[18]	AML	Fever and otitis	No	No	17	*C. tropicalis*	C	4 (17)	N/A	N/A	N/A	Yes
[62]	AVR/aortic repair	Candidaemia	Yes	Yes	52	*C. parapsilosis*	C	2 (0)	>16 (42)	>16 (42)	N/A	Yes
[66]	HSCT recipient	Candidaemia	Yes	No	N/A	*C. glabrata*	C	0.12 (0)	>8 (N/A)	>8 (N/A)	N/A	Yes
[67]	OLTx recipient	Candidaemia	Yes	No	61	*C. glabrata*	C	0.25 (0)	2 (40)	2 (40)	F659V	Yes
[65]	Crohn's disease	Candidaemia	Yes	Yes	67	*C. glabrata*	C	0.5 (0)	8 (21)	>16 (39)	N/A	Yes
[56]	Diabetes mellitus	Candidaemia	Yes	Yes	>25	*C. glabrata*	C	0.06 (0)	>2 (14)	>2 (14)	D632E	N/A

International Journal of Antimicrobial Agents 35 (2010) 211–218

Characterisation of breakthrough invasive mycoses in echinocandin recipients: an evidence-based review

Hsin-Yun Sun[a,b], Nina Singh[a,c,*]

Minimal Effective Concentration and Echinocandins. BPs?

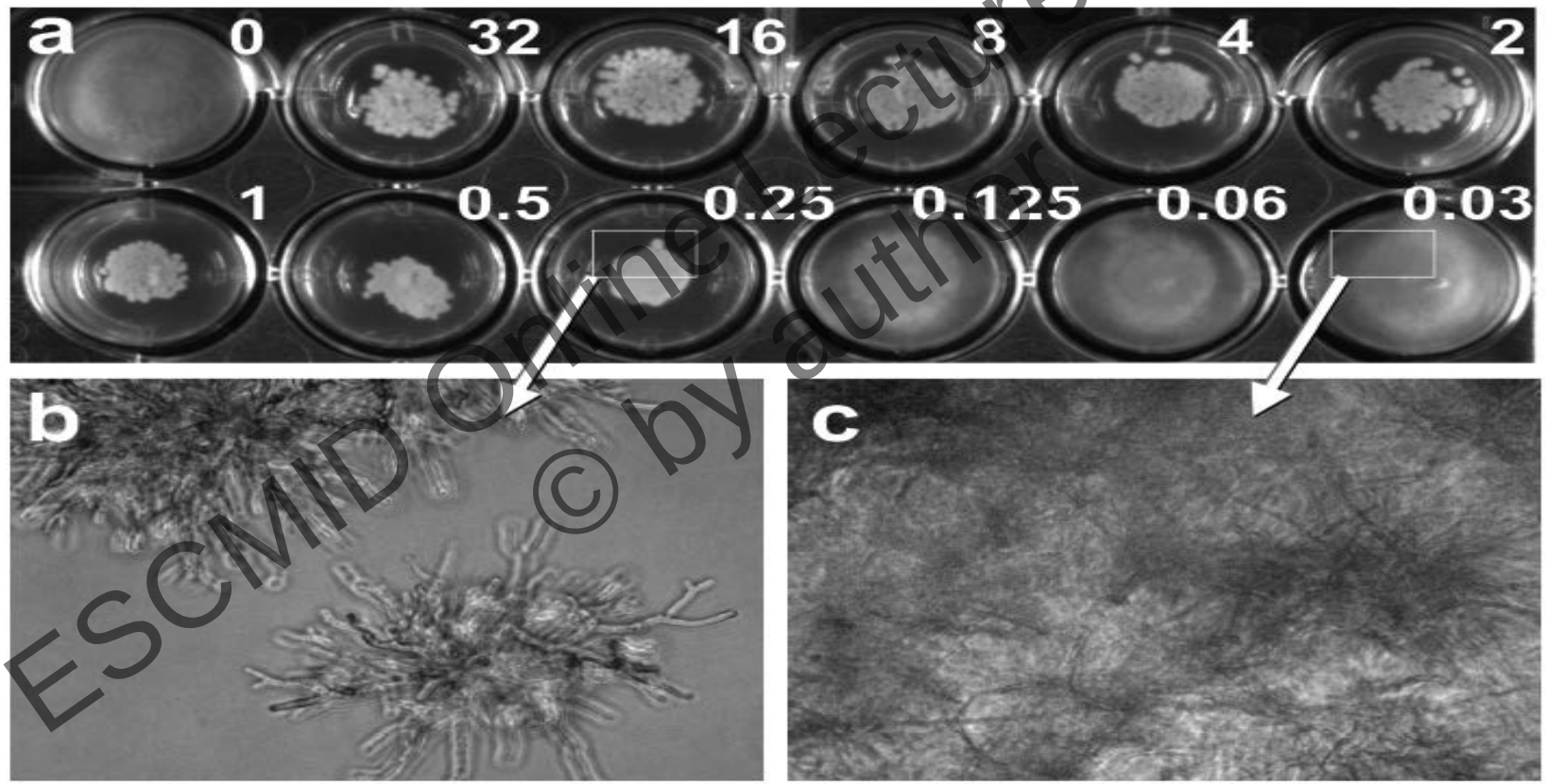


FIG. 1. Agar dilution method. (a) Well 1 is a positive control (without CAS), and wells 2 to 12 contain drug concentrations ranging from 32 to 0.03 μg/ml (as shown). The MIC is 0.25 μg/ml, interpreted as the lowest drug concentration showing compact granular microcolonies compared to the radial filamentous colonies of the growth control and wells containing lower drug concentrations (0.125 to 0.03 μg/ml). The corresponding microscopic appearance of colonies at effective concentrations and subinhibitory concentrations of drug are shown in panels b and c, respectively.

Resistance to echinocandins

Determining molecular resistance mechanisms:

1. **Clinical relevance??**

Uncommon phenomenon, but…

2. **Reliable molecular tools?**

Yes, lack of standardization. Some resistance mechanism still unknown

3. **Could be more reliable that MIC determination for prevention and control?**

Not yet. The mould issue

Conclusions

Antifungal resistance has clinical relevance. Resistance to azoles in *Candida* and, in some countries, *Aspergillus*

We have reliable phenotypic methods for ID of resistant isolates. Efforts for culture isolates and periodical surveillance studies

Description of resistance mechanisms

New sequencing techniques could change AST soon