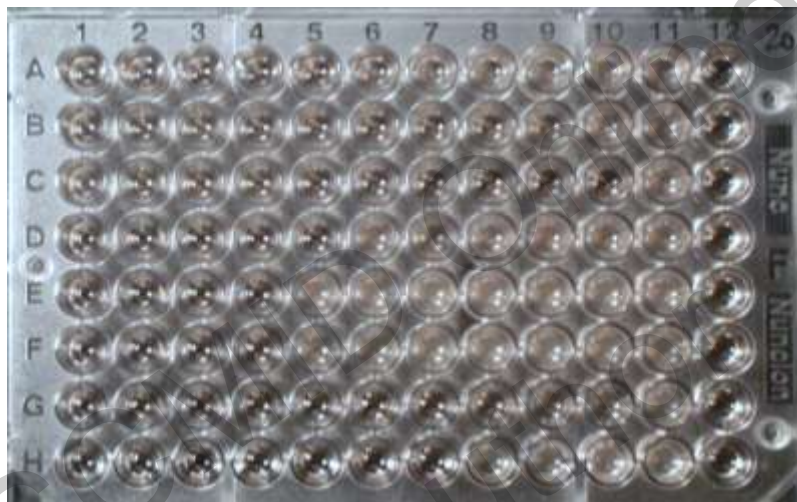




Does antifungal resistance occur everywhere?



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Disclosures last 5 years:

Research grants or Speaker: Astellas, Basilea, Gilead, MSD & Pfizer;

Advisory board: MSD, Pcoverly, Pfizer; Acted as consultant for: Alcimed, Astellas, Gilead & Pfizer

Chair(wo)man for EUCAST-AFST; Advisor for CLSI 2012-14

❖ Intrinsic resistance vs Acquired resistance

- species
- mechanisms

❖ Size of the problem

- *Candida*
- *Aspergillus*

❖ Conclusion

Antifungals spectrum: Yeasts

	<i>Candida</i>				<i>Trichosporon</i> & <i>Geotrichum</i>	Cidal Biofilm
	<i>albicans/tropicalis</i>	<i>glabrata</i>	<i>krusei</i>	<i>parapsilosis</i>		
Amphotericin	+	+	+	+	+/?	+
Anidulafungin	+	+	+	+/?	-	+
Caspofungin	+	+	+	+/?	-	+
Micafungin	+	+	+	+/?	-	+
Fluconazole	+	+/-	-	+	+/-	-
Itraconazole	+	+/-	+/-	+	+/-	-
Posaconazole	+	+/?	+/?	+	+	-
Voriconazole	+	+/?	+/?	+	+	-
5-FC	+	+	-	+	-	+/-

Antifungals spectrum: Yeasts

	<i>Candida</i>				<i>Trichosporon</i> & <i>Geotrichum</i>	Cidal Biofilm
	<i>albicans/tr</i>	<i>glabrata</i>	<i>krusei</i>	<i>parapsilosis</i>		
Amphotericin		+/- <i>C. lusitaniae, C. ciferrii</i>			+/?	+
Anidulafungin	+	+	+	+/?	-	+
Caspofungin	+	+	+	+/?	-	+
Micafungin	+	+	+	+/?	-	+
Fluconazole	+	+/-	-	+	+/-	-
Itraconazole	+	+/-	+/-	+	+/-	-
Posaconazole	+	+/?	+/?	+	+	-
Voriconazole	+	+/?	+/?	+	+	-
5-FC	+	+	-	+	-	+/-

Antifungals spectrum: Yeasts

Antifungal	<i>Candida</i>				Trichosporon & <i>Geotrichum</i>	Cidal Biofilm
	<i>albicans/tr</i>	<i>glabrata</i>	<i>krusei</i>	<i>parvula</i>		
Amphotericin	+/-	+/-	-	-	+/?	+
Anidulafungin	+/-	+/-	-	-	-	+
Caspofungin	+/-	+/-	-	-	-	+
Micafungin	+/-	+/-	-	-	-	+
Fluconazole	+	+/-	-	+	+/-	-
Itraconazole	+	+/-	+/-	+	+/-	-
Posaconazole	+	+/?	+/?	+	+	-
Voriconazole	+	+/?	+/?	+	+	-
5-FC	+	+	-	+	-	+/-

Antifungals spectrum: Yeasts

	<i>Candida</i>				<i>Trichosporon</i> & <i>Geotrichum</i>	Cidal Biofilm
	<i>albicans/tr</i>	<i>glabrata</i>	<i>krusei</i>	<i>parapsilosis</i>		
Amphotericin	+/- <i>C. lusitaniae, C. ciferrii</i>				+/?	+
Anidulafungin					-	+
Caspofungin	+/- <i>C. fermentati, C. guilliermondii</i>				-	+
Micafungin				<i>C. metapsilosis and C. orthopsilosis</i>	-	+
Fluconazole	+/-				+/-	-
Itraconazole				<i>C. auris, C. ciferrii, C. fermentati,</i>	+/-	-
Posaconazole				<i>C. guilliermondii, C. humicola,</i>	+	-
Voriconazole				<i>C. inconspicua, C. lambica, C. lipolytica,</i> <i>C. norvegensis, C. palmiophila,</i> <i>C. rugosa and C. valida</i>	+	-
5-FC	+	+	-	+	-	+/-

Antifungals spectrum: Moulds

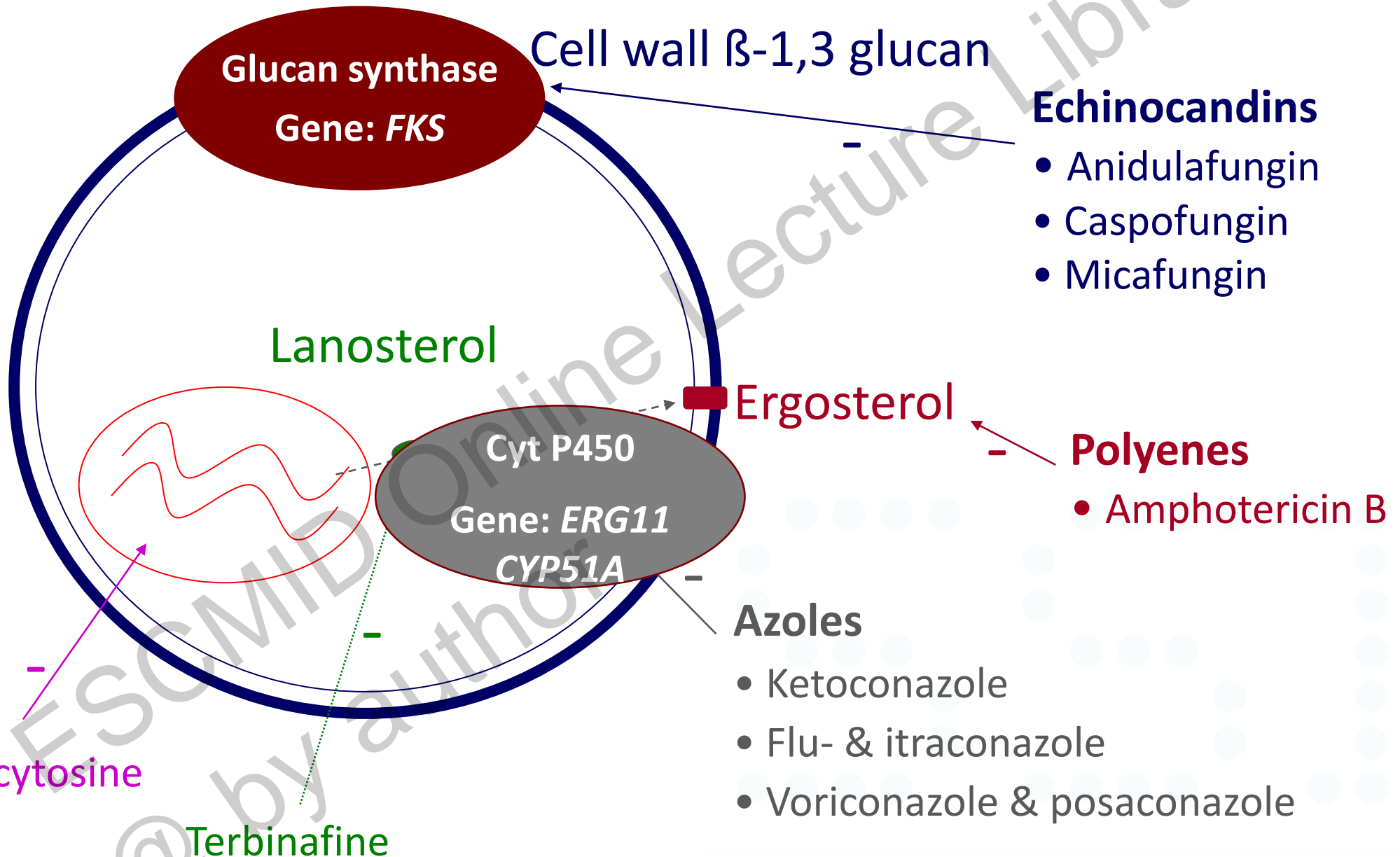
	<i>Aspergillus</i>				<i>Aspergillus</i> Cidal?	<i>Fusarium</i>	Zygo- mycetes
	<i>fumigatus</i>	<i>terreus</i>	<i>flavus</i>	<i>niger</i>			
Amph. B	+	-	+/-	+	+	(+)	(+)
Anidula	+	+	+	+	-	-	-
Caspo	+	+	+	+	-	-	-
Mica	+	+	+	+	-	-	-
Fluco	-	-	-	-	-	-	-
Itra	+	+	+	+/-	-	-	-
Posa	+	+	+	+	?	+	+/-
Vori	+	+	+	+	+	+/-	-
5-FC	-	-	-	-	-	-	-

Intrinsic and Primary resistance

Intrinsic resistance: ✓ Variable susceptibility (✓)

	AMB	Azoles	Echinocandins
<i>Aspergillus</i> section <i>fumigati</i>			
<i>A. fumigatiaffinis</i>	✓	✓	
<i>A. lentulus</i>	✓	✓	(✓)
<i>N. pseudofischeri</i>	(✓)	✓	
<i>A. viridinutans</i>	✓	✓	
<i>N. udagawae</i>	✓	✓ (vor)	
<hr/>			
<i>A. terreus</i> (and <i>A. alabamensis</i>)	✓		
<i>A. flavus</i>	✓	(✓)	(✓)
<i>A. versicolor</i> (and <i>A. sydowi</i>)	✓	(✓)	
<hr/>			
<i>A. calidoustus</i>		✓	(✓)
<i>A. allilaceus</i>	(✓)		(✓)

Systemic Antifungals: Mode of Action



Acquired Resistance in *Candida*

Compound	Azoles	Echinocandins	Amphotericin B
Target	P450 demethylase	Glucan synthase	Ergosterol
Target gene mutation	<i>ERG11</i> → less binding	<i>FKS1</i> <i>FKS2</i> → less binding	<i>ERG2</i> 3, 5 and 11 (C a) <i>ERG1</i> , 2, 6 and 11 (C g) → less ergosterol
Target up-regulation	<i>ERG11</i> - Promotor		
Efflux pumps	<i>MDR</i> , <i>CDR</i> <i>CgSNQ2</i>		
	Found in DK isolates		

Acquired Resistance in *Aspergillus*

Compound	Azoles	Echinocandins	Amphotericin B
Target	P450 demethylase	Glucan synthase	Ergosterol
Target gene mutation	<i>CYP51A</i>	<i>FSK1</i> *	
Target up-regulation	<i>CYP51A+</i> Promotor or P88L in <i>HapE</i>	✓	
Efflux pumps	ABC & MF		
	Found in DK isolates	* only laboratory engineered strains	

❖ Intrinsic resistance vs Acquired resistance

- species
- mechanisms

❖ Size of the problem

- *Candida*
- *Aspergillus*

❖ Conclusion

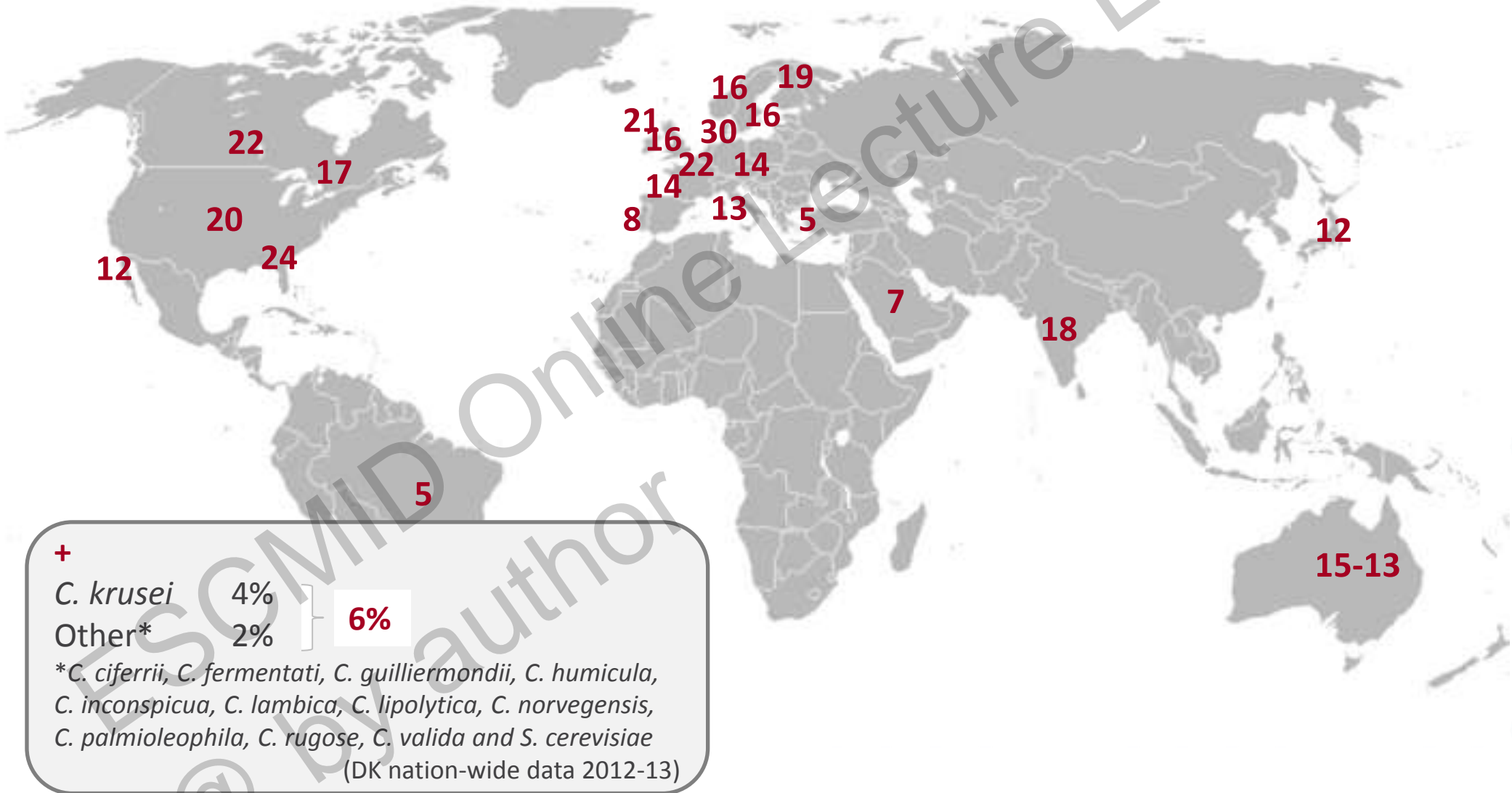
Intrinsic Azoles Resistance

C. glabrata proportion



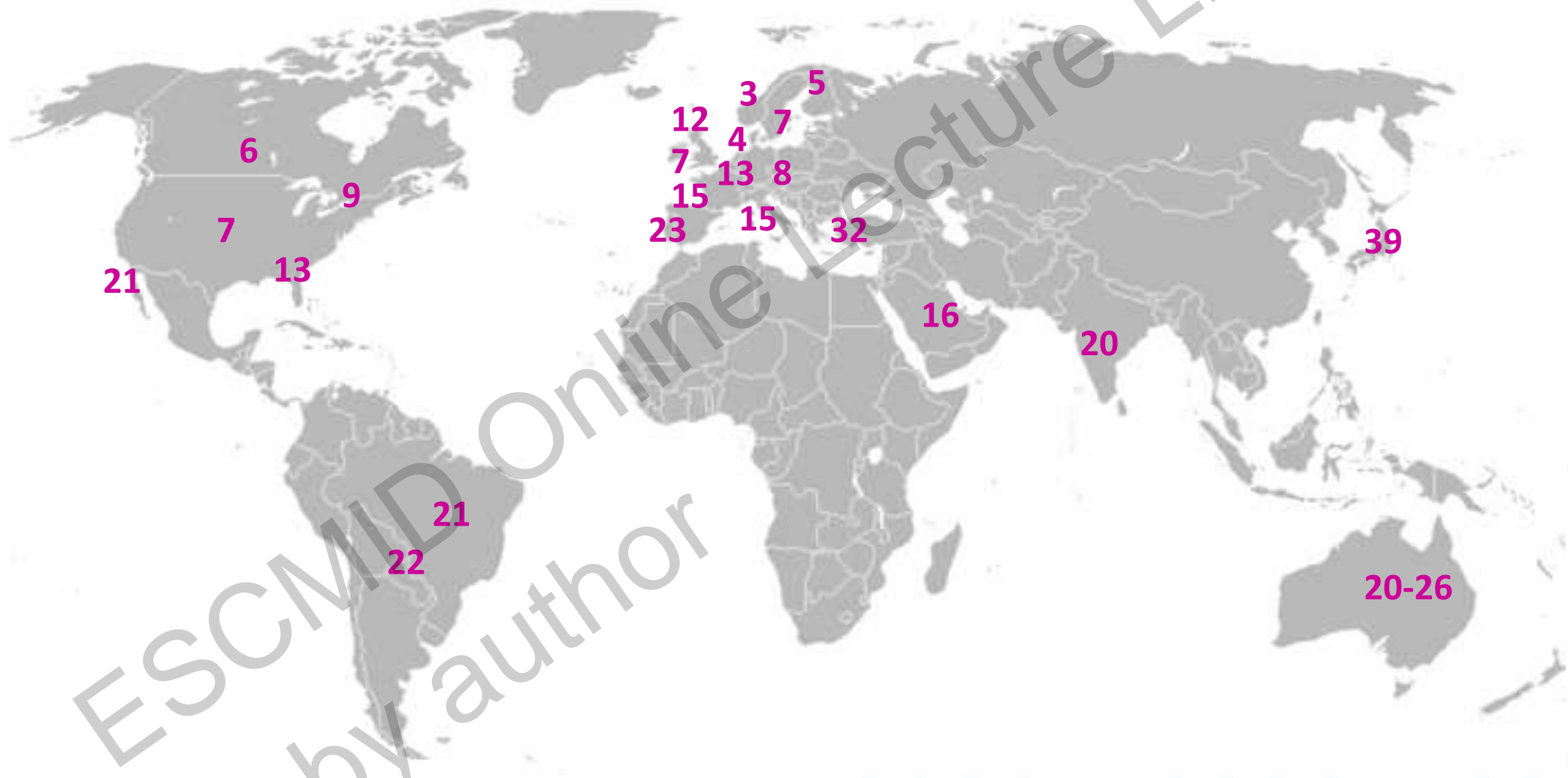
Intrinsic Azoles Resistance

C. glabrata proportion



Intrinsic Echinocandins “resistance”

C. parapsilosis proportion



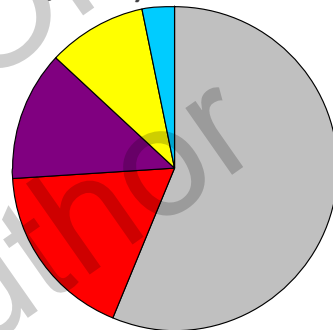
Prior AF exposure & intrinsic resistant candidaemia

❖ *C. glabrata*, *C. krusei* or *S. cerevisiae*

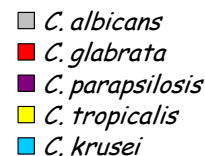
- 57.1% (16/28) ≥ 1 week antifungal exposure
- 28.6% (6/21) < 1 week exposure
- 28.3% (73/258) No exposure

❖ Paris observation

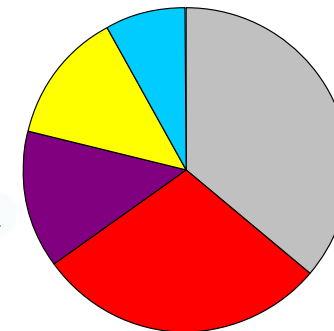
No fluconazole
(n. 2,289)



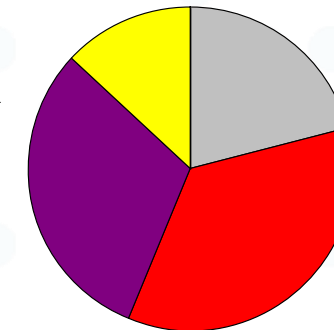
No caspofungin
(n. 2,387)



Fluconazole (159)



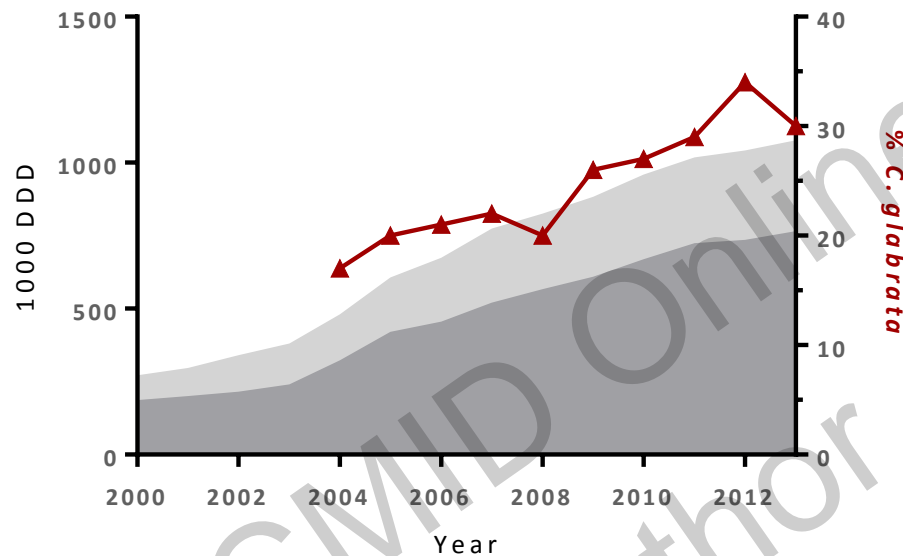
Caspofungin (61)



Increasing AF usage and intrinsic resistance

❖ Nation-wide data Denmark

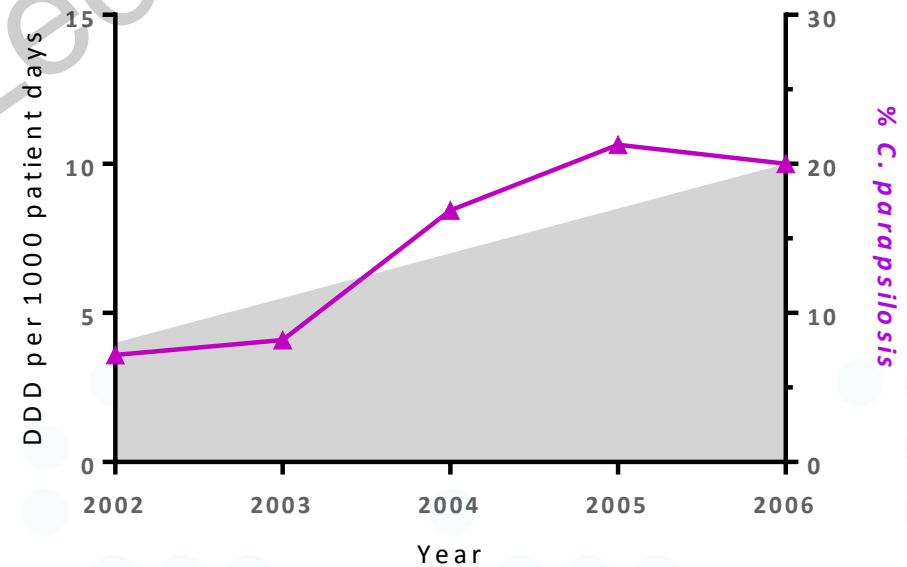
- 2004 → 2013
- 5008 blood isolates



▲ % *C. glabrata*
■ Hospital use of fluconazole
■ GP use of fluconazole

❖ Single Tertiary Centre US

- 2002 → 2006
- 469 candidaemia cases



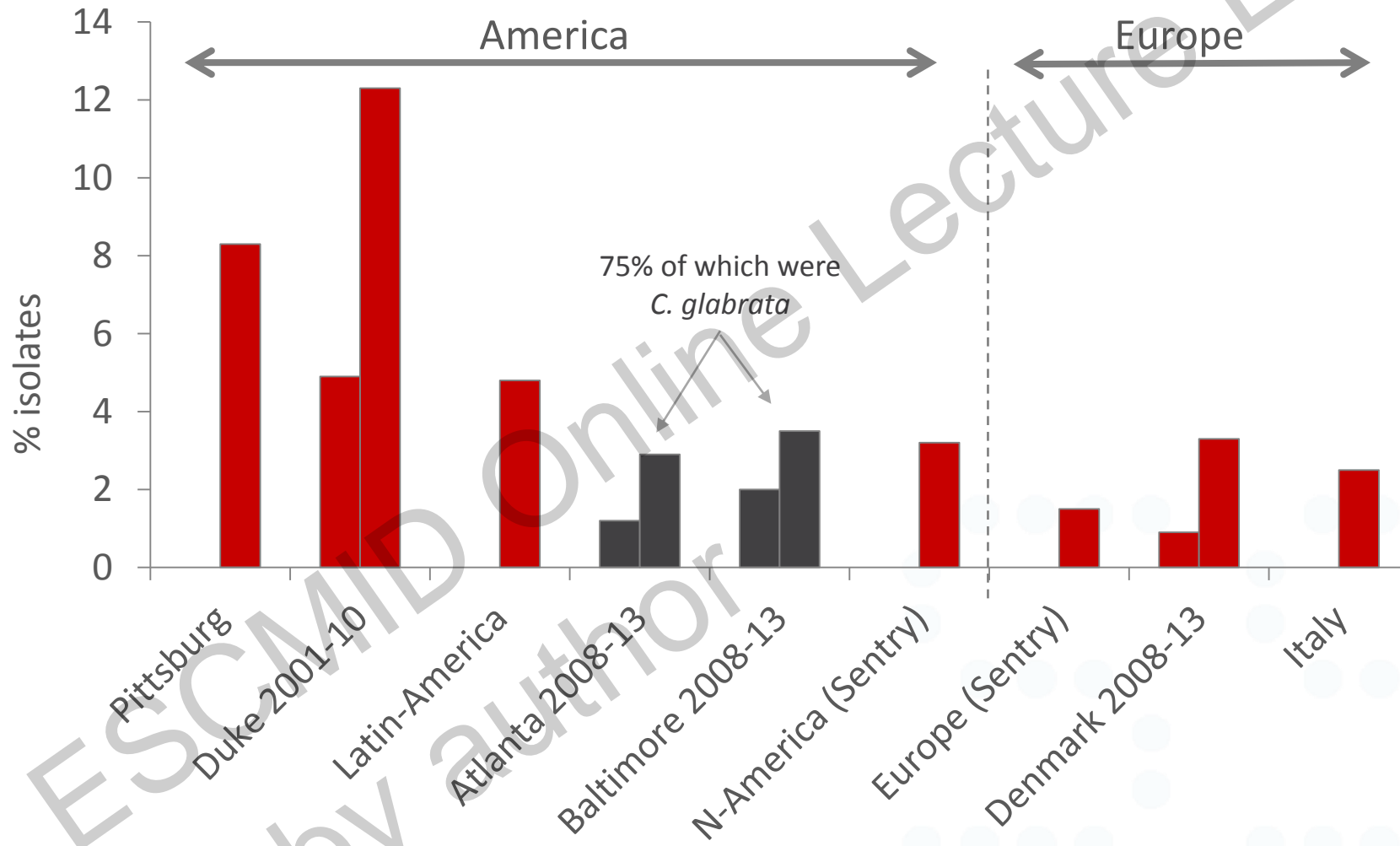
▲ % *C. parapsilosis*
■ Echinocandin use



Online Lecture Library

**Slide withheld
at request of author**

Echinocandin R in *C. glabrata*

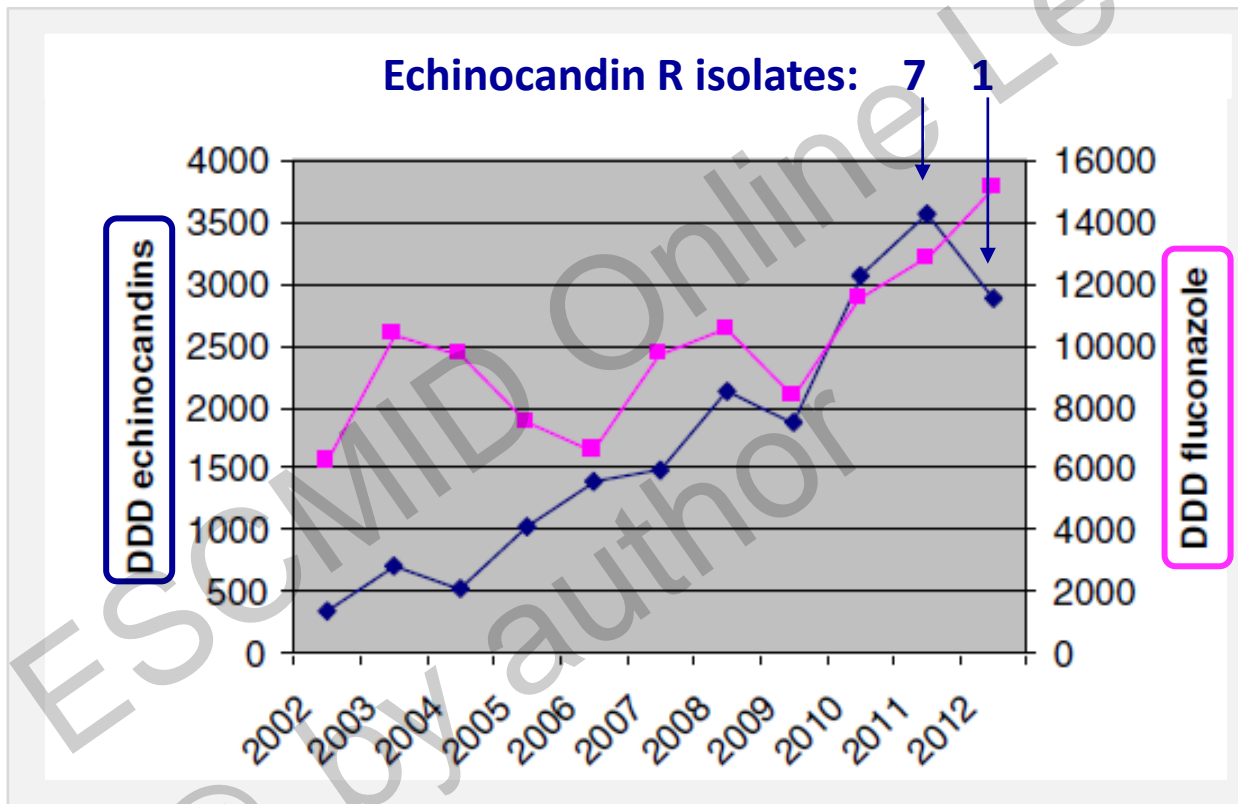


Echinocandin use and resistance

❖ Fluconazole & Echinocandin use (a Paris tertiary centre)

Year	Patients on echinocandins	DDD/patient	Breakthrough rate
2011	213	16.7	3.3%
2012	216	13.3	0.5%

P: 0.03



Median exposure for patients with "R" isolates:

37 days

(8-58 days)

Week 1: 0

Week 2: 2

Week 4: 1

Week 5: 2

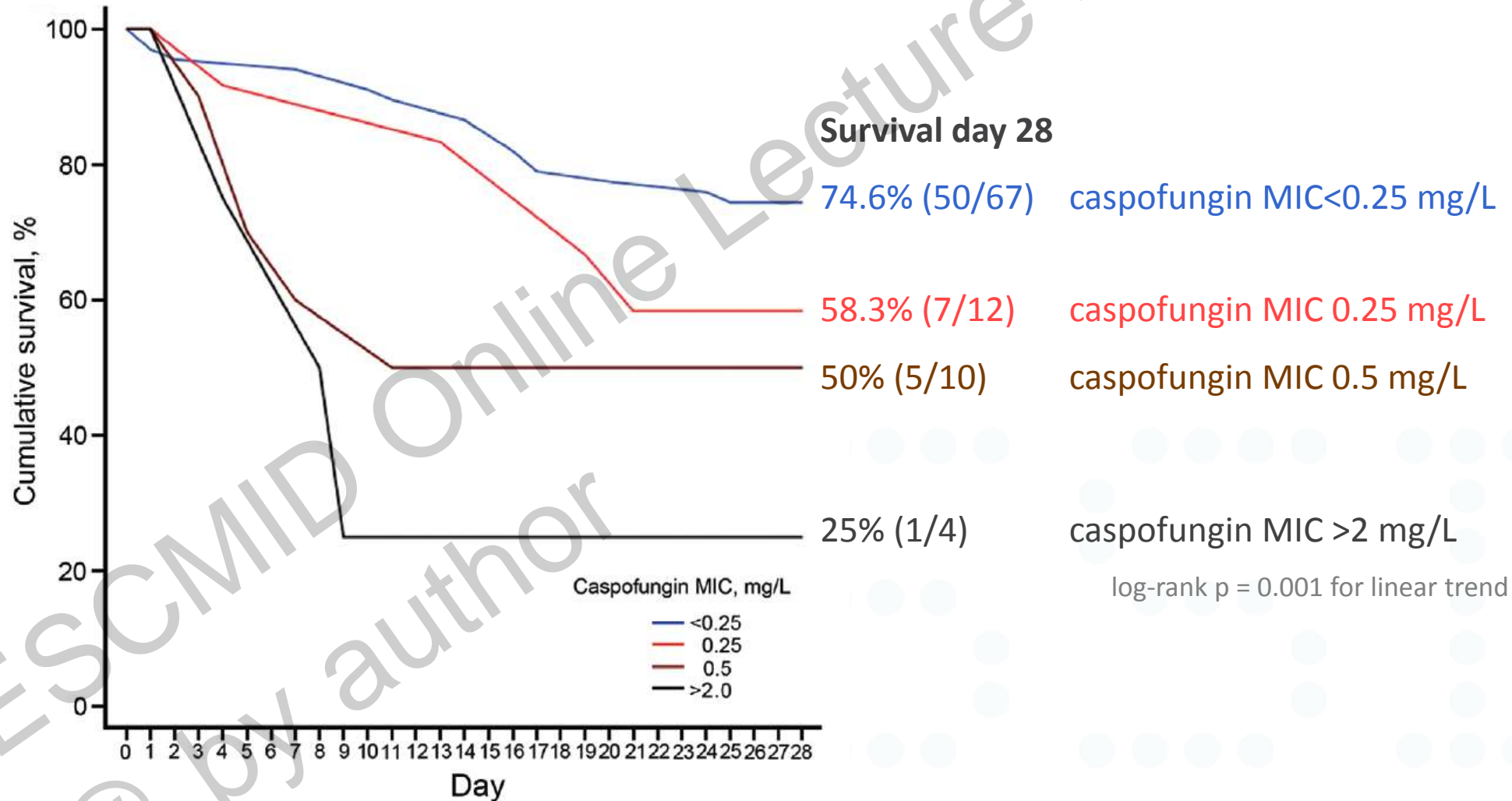
Week 6: 2

Week 8: 1

Week 9: 1

C. glabrata echinocandin MIC & outcome

❖ Cancer patients w 93 blood isolates in 2005-13

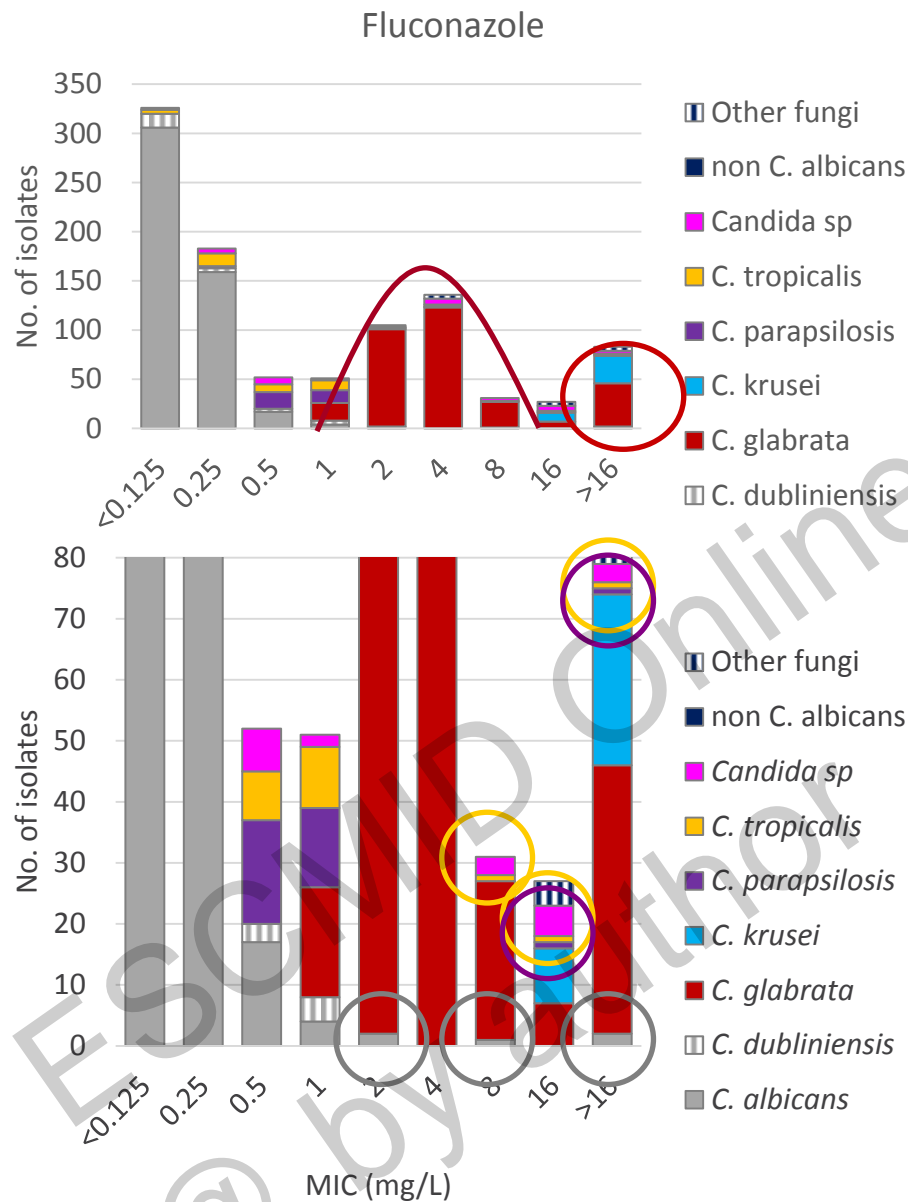


Acquired azole resistance in *Candida*

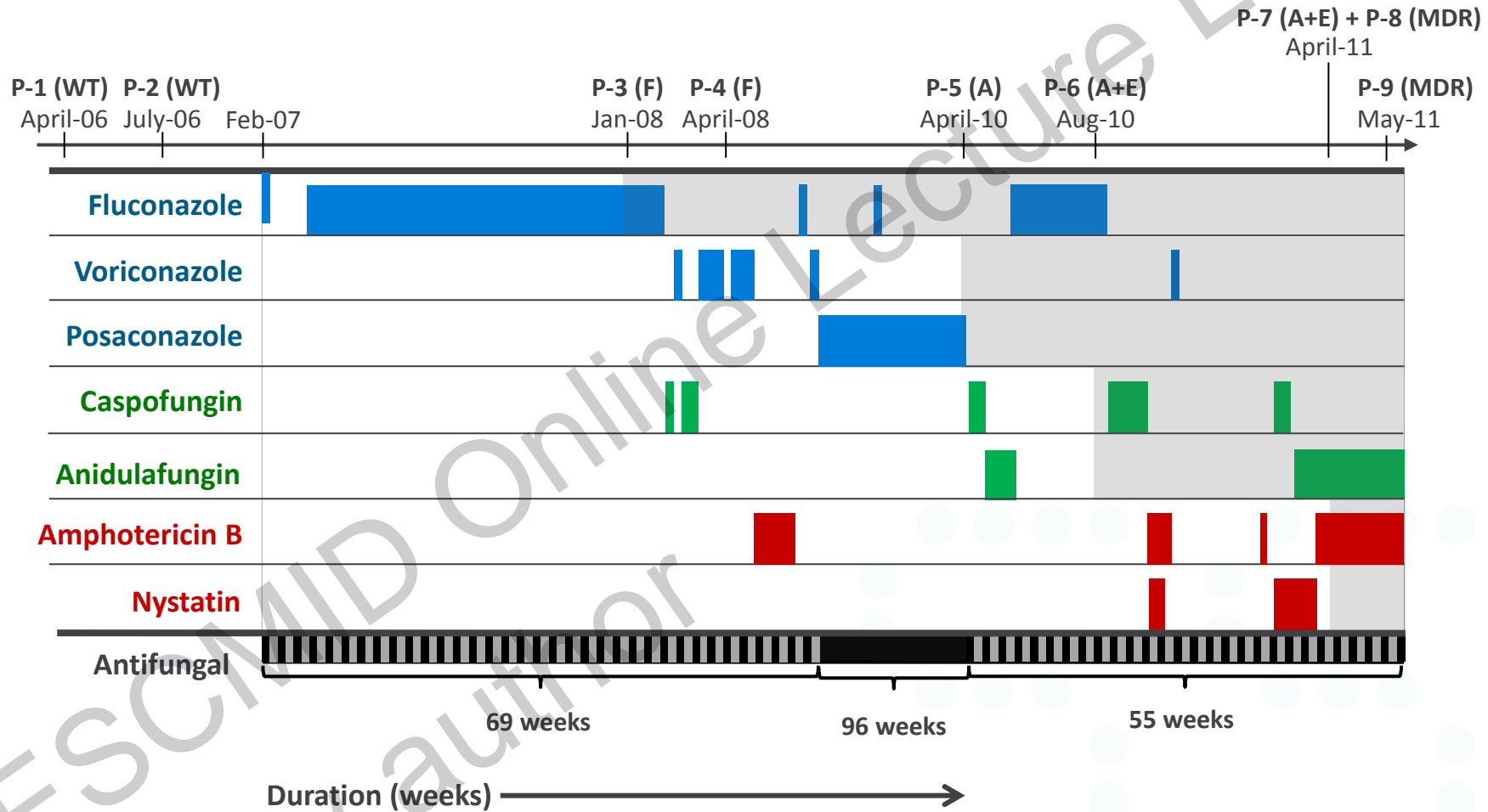
Nationwide surveillance of fungaemia DK
2012-13; 1026 isolates

**Acquired fluconazole resistance in
C. glabrata: 13.9% (444/317)**

**Acquired fluconazole resistance in
C. albicans: 0.6% (3/491)
C. parapsilosis: 8.6% (3/36)
C. tropicalis: 12.2% (5/41)**



Drug exposure in a patient with an MDR isolate



Multi drug resistant *C. albicans*

Isolate	EUCAST MIC (mg/L)					Etest (mg/L)		Sequence profiles of relevant genes			
	FLU	ITR	POS	VOR	ANI	CAS	AMB	<i>ERG11</i>	<i>CDR2</i>	<i>FKS1</i>	<i>ERG2</i>
ATCC 10231	1	≤ 0.125	≤ 0.03	≤ 0.03	0.08	0.25	0.25	WT	1.18	WT	WT
Pt #1	0.125	≤ 0.03	-	≤ 0.03	-	0.06	0.25	-	NA	-	-
Pt #2	0.25	≤ 0.03	-	≤ 0.03	-	0.25	0.5	-	NA	-	-
Pt #3	16	0.03/4*	0.03/4*	0.03/4*	-	0.25	0.38	-	NA	-	-
Pt #4	8	≤ 0.125	≤ 0.03	≤ 0.03	0.015	0.25	0.5	E266D, G307S, G450E, V488I	69.2	-	WT
Pt #5	> 16	16	> 4	1	0.015	0.50	0.5	A61E, E266D, G307S, G450E, V488I	868.1	-	WT
Pt #7	> 16	4	> 4	0.5	0.25	> 32	0.5	A61E, E266D, G307S, G450E, V488I	194.8	S645S/P	Het F105Stop
Pt #8	> 16	16	4	0.125	1	> 32	> 32	A61E, E266D, G307S, G450E, V488I	132.5	S645S/P	Hom F105Stop
Pt #9	16	> 16	0.5/4*	0.125	0.5	> 32	> 32	A61E, E266D, G307S, G450E, V488I	14.5	S645S/P	F105Stop

*Trailing phenotype with ~50% growth inhibition in the concentration range 0.03-4 µg/ml.

❖ Intrinsic resistance vs Acquired resistance

- species
- mechanisms

❖ Size of the problem

- *Candida*
- *Aspergillus*

❖ Conclusion

❖ *A. terreus* high “endemic” centres

- Houston, Texas, 1993-2012:
 - 18.7% (96/513) with *A. terreus* alone
 - 16.0% (82/513) with *A. terreus* in mixed infections
- Innsbruck, Austria, 1994-2004:
 - 47.8% (32/67 IPA cases in haematological patients)

❖ *A. flavus*

- Developing countries
 - 50 and 80% of allergic fungal rhinosinusitis cases in India and the Middle East

❖ *A. fumigatiaffinis*, *A. lentulus*, *N. pseudofischeri*, *A. viridinutans*, *N. udagawae*, *A. calidoustus*, *A. allilaceus*

- RARE

PATH alliance study (960 cancer pts US)

<i>A. fumigatus</i>	72.6%
<i>A. flavus</i>	9.9%
<i>A. niger</i>	8.7%
<i>A. terreus</i>	4.3%

Fungicide use & Azole “R” *A. fumigatus* reports

Global market share of fungicide use in agriculture;

TR₃₄/L98H *A. fumigatus* ●

TR₄₆/Y121F/T289A *A. fumigatus* ●

9% N-America

37%

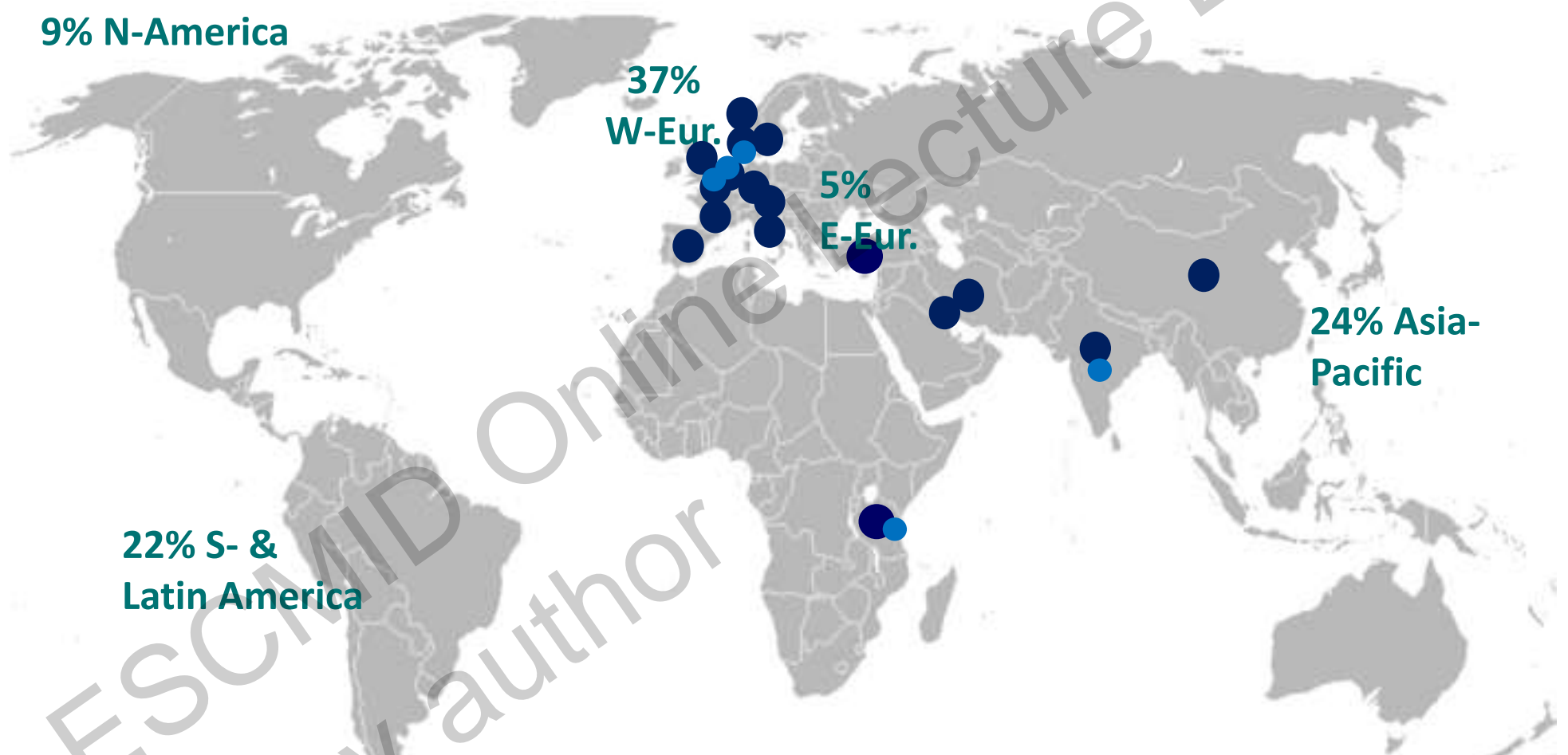
W-Eur.

5%

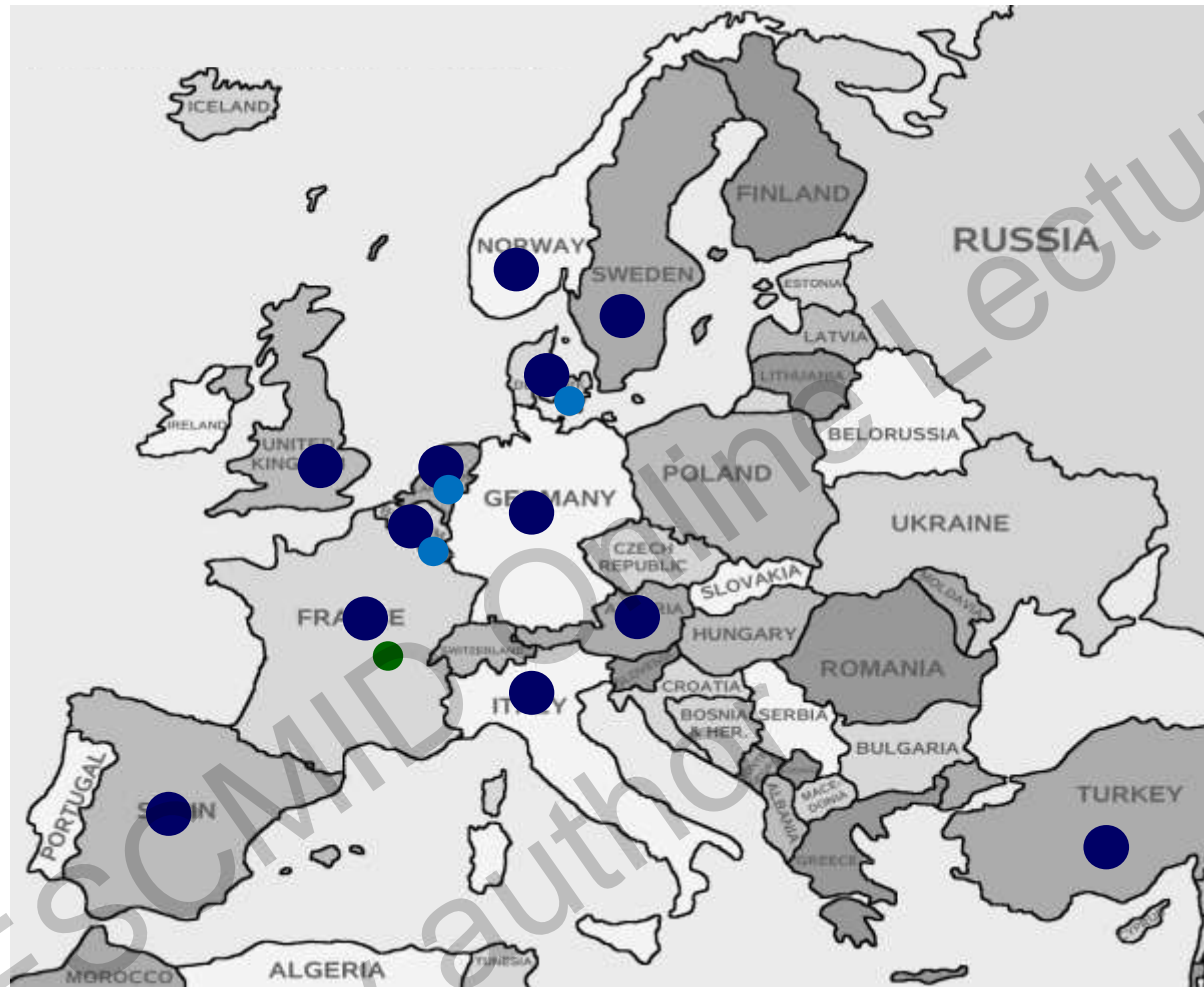
E-Eur.

24% Asia-Pacific

22% S- &
Latin America



Azole resistant *A. fumigatus* in azole naïve patients in Europe



● TR₃₄/L98H detected

“New” azole resistance mechanisms detected in azole naïve patients or in the environment

NL:
● TR₄₆/Y121F/T289A

France:
● G432S

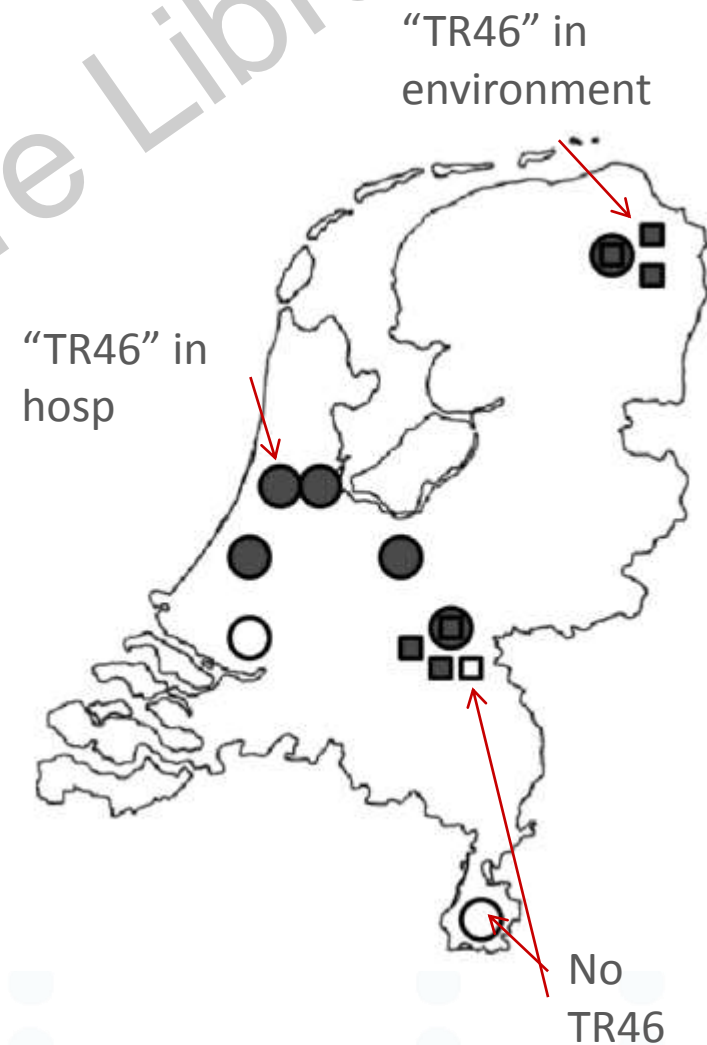
Voriconazole “R” in the NL

- ❖ December 2009 - January 2011
- ❖ 1315 *A. fumigatus* isolates from 921 patients

- 63 patients w azole resistance 6.8%
 - 47 patients with the TR34/L98H (74.6%)
 - 13 patients with the TR46/Y121F/T289A (20.6%)
 - 3 patients with no *CYP51A* mutations (4.7%)

- ❖ Environmental sampling (140 azole R samples)

- 10% TR₄₆/Y121F/T289A → found 6/9 sites
- 90% TR₃₄/L98H → found at 9/9 sites



Azole resistant *Aspergillus* – resistance profiles



❖ Intrinsic resistance vs A

- species
- mechanisms

YES
**Intrinsic resistance in *Candida*
occurs everywhere**

❖ Size of the problem

- *Candida*
- *Aspergillus*

YES
**Acquired Echinocandin
resistance in *Candida* is
emerging in exposed patients
everywhere**

No
**Acquired resistance in *Candida*
does not occur in naïve
patients “anywhere”**

❖ Conclusion

YES
**Acquired resistance in
Aspergillus occurs everywhere**

almost

Acknowledgements

(in alphabetic order):

The EUCAST Steering Committee

M Cuenca-Estrella
SJ Howard
C Lass-Flörl
J Meletiadis
J Mouton

The EUCAST General Committee

Other collaborators

Jensen RH
Astvad K
W Hope
BJ Kullberg
DS Perlin
M Pfaller
P Verweij

The Danish Fungaemia Study Group

Dzajic E
Rosenvinge FS
Johansen HK
Kjældgaard P
Knudsen JD
Kristensen L
Lemming LE
Nielsen L
Olesen B
Røder B
Thøger Gorm
Schønheyder HC

Thank you for your attention