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Resistance mechanisms in fungal infections - *in Denmark*

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Disclosures

GILEAD

ESCMID Research Grants 2013

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Talk divided in 2 parts

1. **Resistance in *Candida*:** Preliminary results from a multicentre study

2. **Resistance in *Aspergillus*:** Overview of 4 recent clinical cases in DK

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Post treatment antifungal resistance

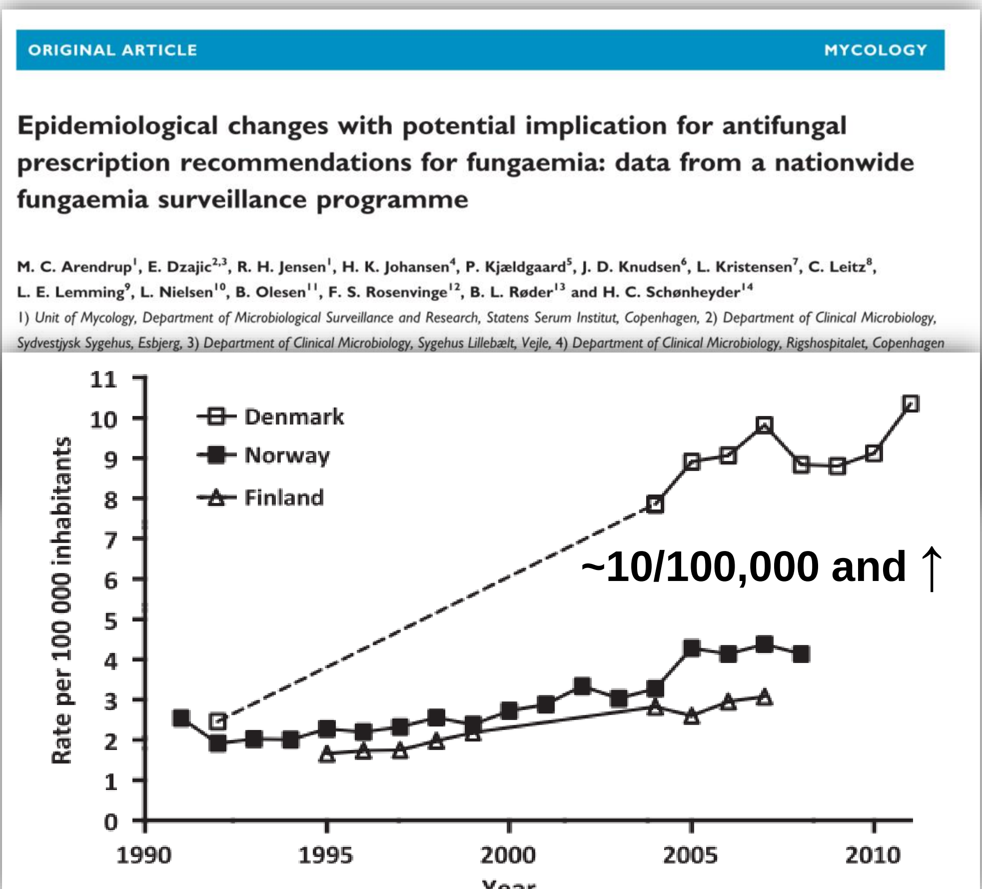
among colonising *Candida* isolates in candidaemia patients:

preliminary results

Candidaemia and resistance

Numbers from Denmark

Increasing prevalence



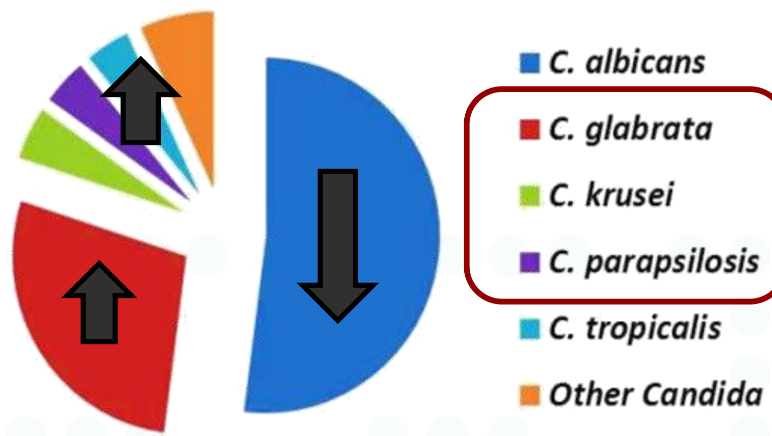
ORIGINAL ARTICLE MYCOLOGY

Epidemiological changes with potential implication for antifungal prescription recommendations for fungaemia: data from a nationwide fungaemia surveillance programme

M. C. Arendrup[1], E. Dzajic[2,3], R. H. Jensen[1], H. K. Johansen[4], P. Kjældgaard[5], J. D. Knudsen[6], L. Kristensen[7], C. Leitz[8], L. E. Lemming[9], L. Nielsen[10], B. Olesen[11], F. S. Rosenvinge[12], B. L. Røder[13] and H. C. Schønheyder[14]
1) Unit of Mycology, Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, 2) Department of Clinical Microbiology, Sydvestjysk Sygehus, Esbjerg, 3) Department of Clinical Microbiology, Sygehus Lillebælt, Vejle, 4) Department of Clinical Microbiology, Rigshospitalet, Copenhagen

Antifungal resistance?

Intrinsically resistant species

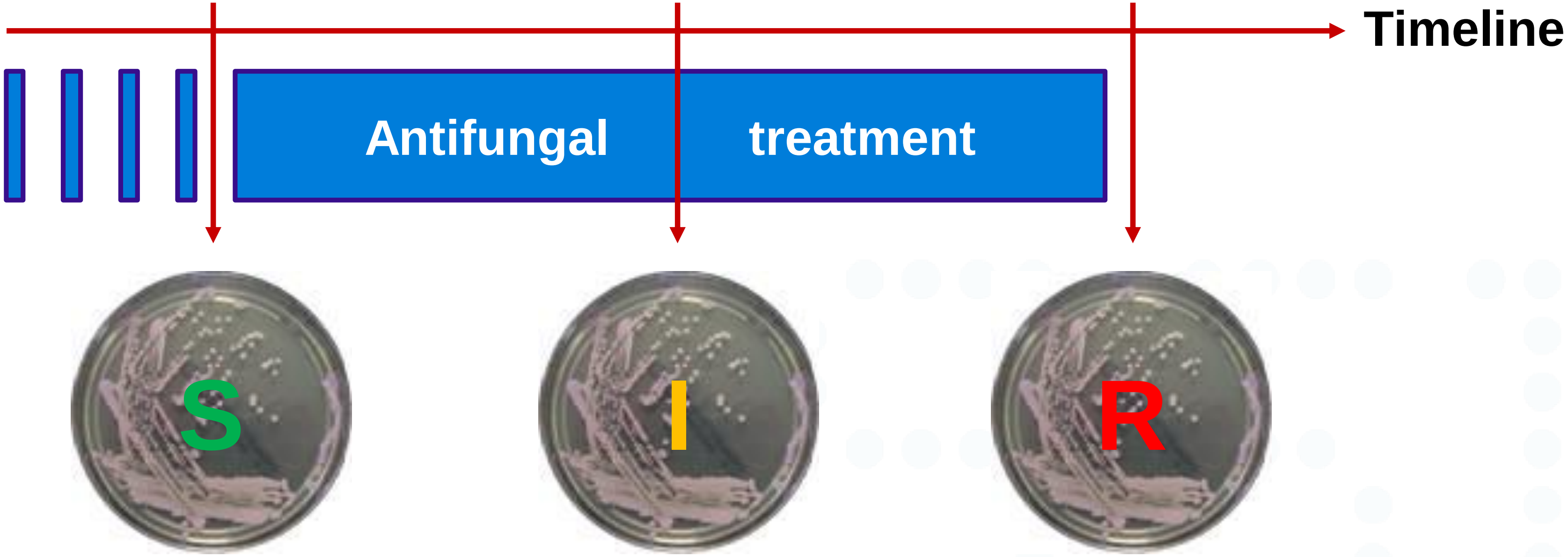


But what about acquired resistance?

Only a few cases in the recent study

Resistance is selected by treatment

Acquired resistance developed during treatment



A challenge in surveillance studies?

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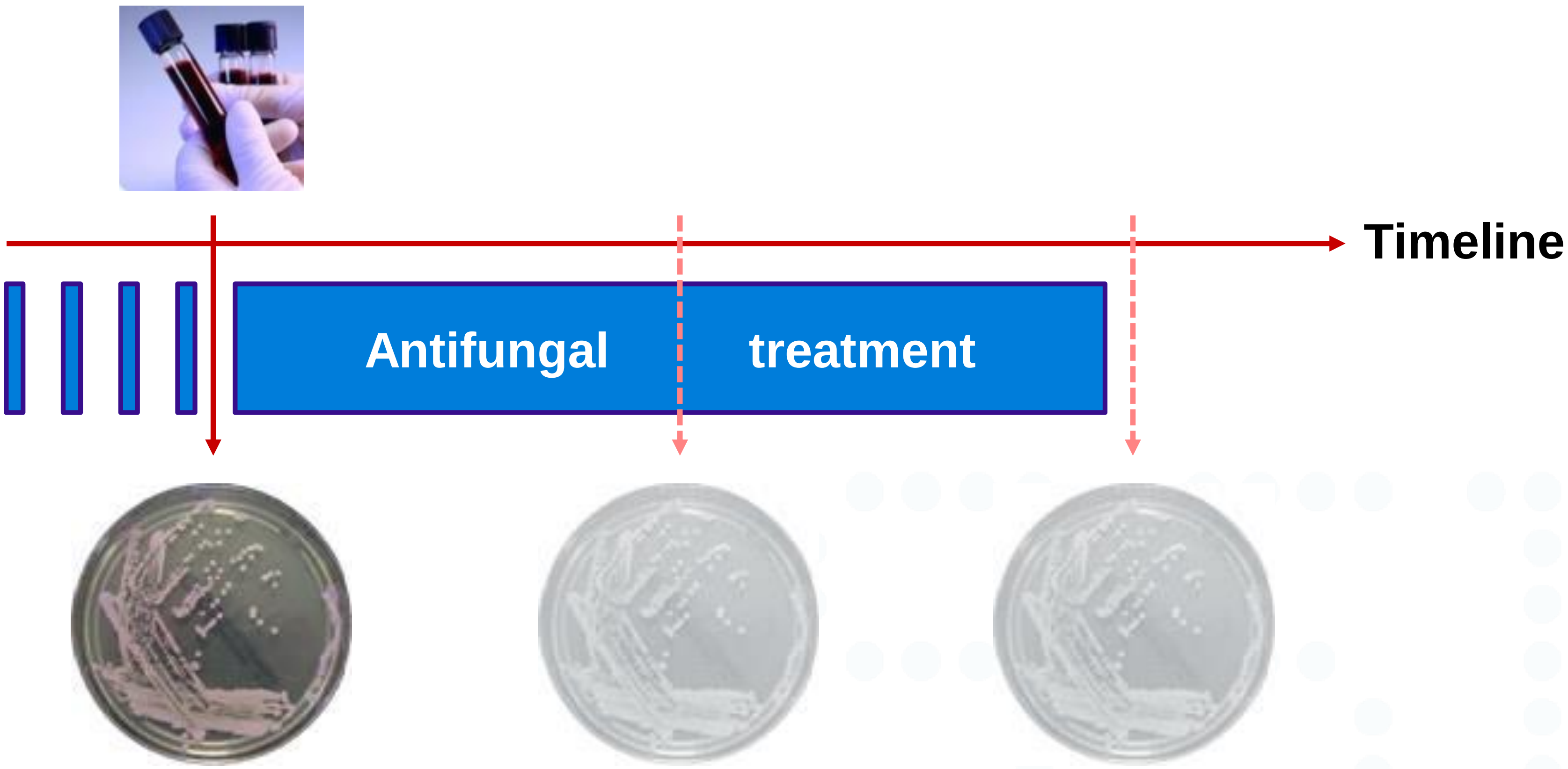
Surveillance studies only include the initial blood isolate



How can we address this?

Study design

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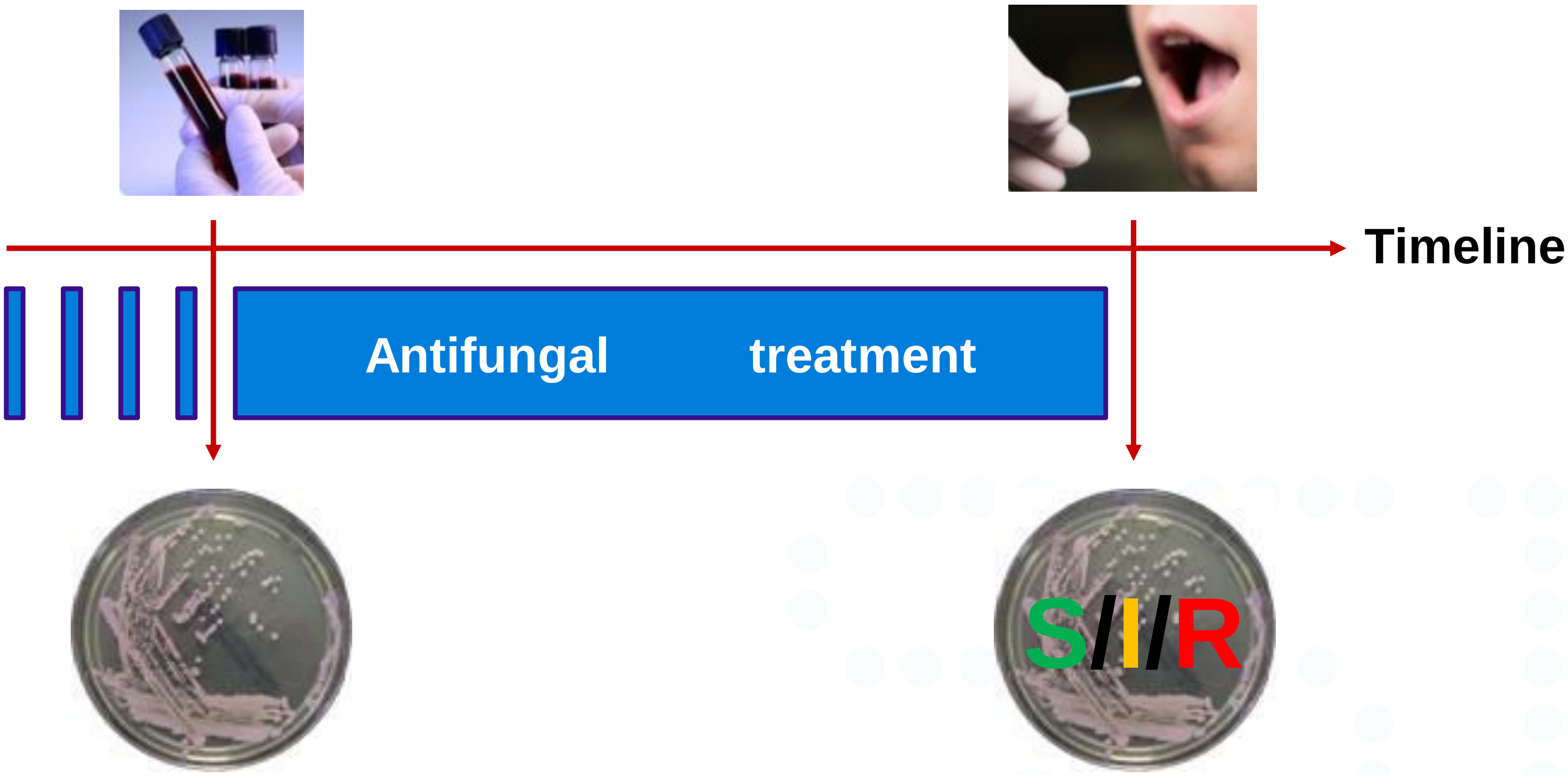
We introduce a mouth swab post treatment



Colonisation linked to invasion and *vice versa*

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Scandinavian Journal of Infectious Diseases, 2010; 42:109–113

informa healthcare

ORIGINAL ARTICLE

Typing of Candida isolates from patients with invasive infection and concomitant colonization

ANNA BRILLOWSKA-DABROWSKA[1,2], OLAV BERGMANN[3], IRENE MOELLER JENSEN[4], JENS OTTO JARLØV[4] & MAIKEN C. ARENDRUP[1]

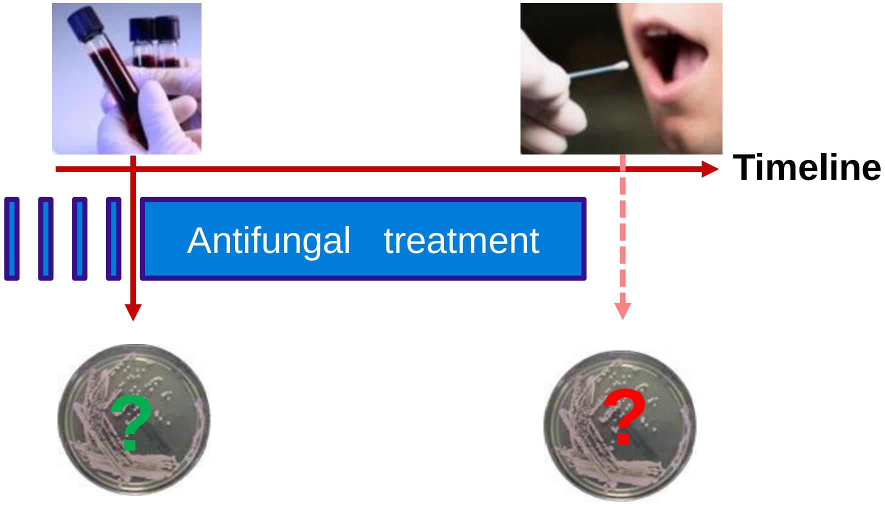
From the [1]Unit of Mycology and Parasitology, Statens Serum Institute, Copenhagen, Denmark, [2]Department of Microbiology, Gdansk University of Technology, Gdansk, Poland, [3]Department of Haematology, and [4]Department of Clinical Microbiology, Herlev University Hospital, Herlev, Denmark

11 candidaemia patients

50 isolates: 34 *C. albicans* (12 inv), 10 *C. glabrata* (3 inv.), 6 *C. krusei* (2 inv.)

Identical genotypes in invasive & colonising isolates, 11/11 patients (MLST + RAPD)

A prospective 1-year observational study



Inclusion criteria

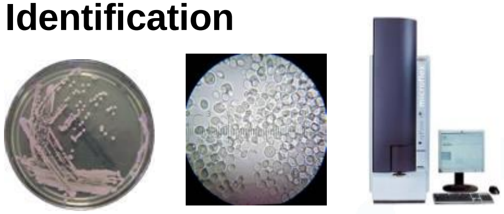
1. Patients with candidaemiae

2. Swab obtained (within 1 month)

Collaborating hospitals

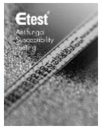
Rigshospitalet
Hvidovre
Slagelse
Århus
Odense
Ålborg
Herlev
Hillerød
Vejle
Viborg
Esbjerg
Sønderborg

Identification

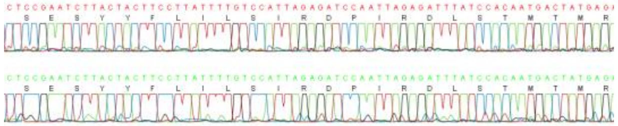


Susceptibility testing

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



DNA sequencing analyses:



Questions

Intrinsic resistance:

- Species distributions?

Acquired resistance:

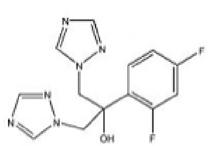
- Echinocandins/azoles

Overview, collected samples

Category	Number
Patients with BC+	415 (100 %)
Patients with swabs	172 (41 %)
Swabs positive *Candida* culture	116 (67 %)
Candida isolates in swab samples	175 (1.5 pr sample)

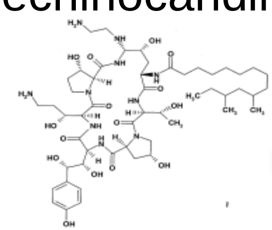
Patients grouped in 3 treatment arms

azoles



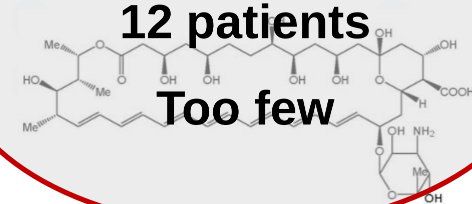
fluconazole

echinocandins



polyenes

12 patients



Too few

Azoles shift species distribution

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Species distribution in <u>59 patients</u> treated with azoles

Species	No. in blood samples (%)	No. in swab samples (%)
C. albicans	37 (62,7)	20 (33.9)^{PP}
C. glabrata	11 (18.6)	25 (42.4)^{PP}
C. krusei	4 (6.8)	4 (6.8)^{NS}
C. tropicalis	1 (1.7)	1 (1.7)^{NS}
C. parapsilosis	2 (3.4)	1 (1.7)^{NS}
S. cerevisiae	0 (0)	5 (8.5)$^{p=0.057}$
Other *Candida*/yeast*	4 (6.8)	3 (5.1)^{NS}
Total	59	59
Intrinsic FLU resistance* **	16 (27.1)	35 (59.3)^{PP}

*** *C. glabrata, C. krusei, S. cerevisiae, C. guilliermondii, C. neoformans* (reduced FLU susceptibility)

^{P} Significant change from left to index column, ($p < 0.05$), calculated by Fischer's exact test.
^{PP}$p<0.01$. ^{NS} Non-significant change

Acquired FLU resistance in *C. glabrata*

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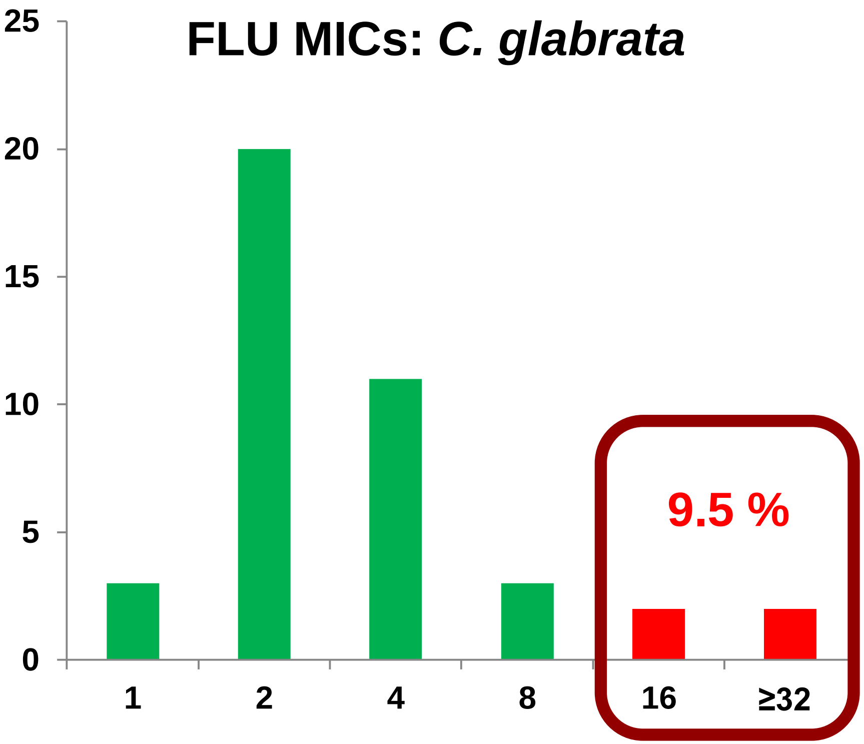
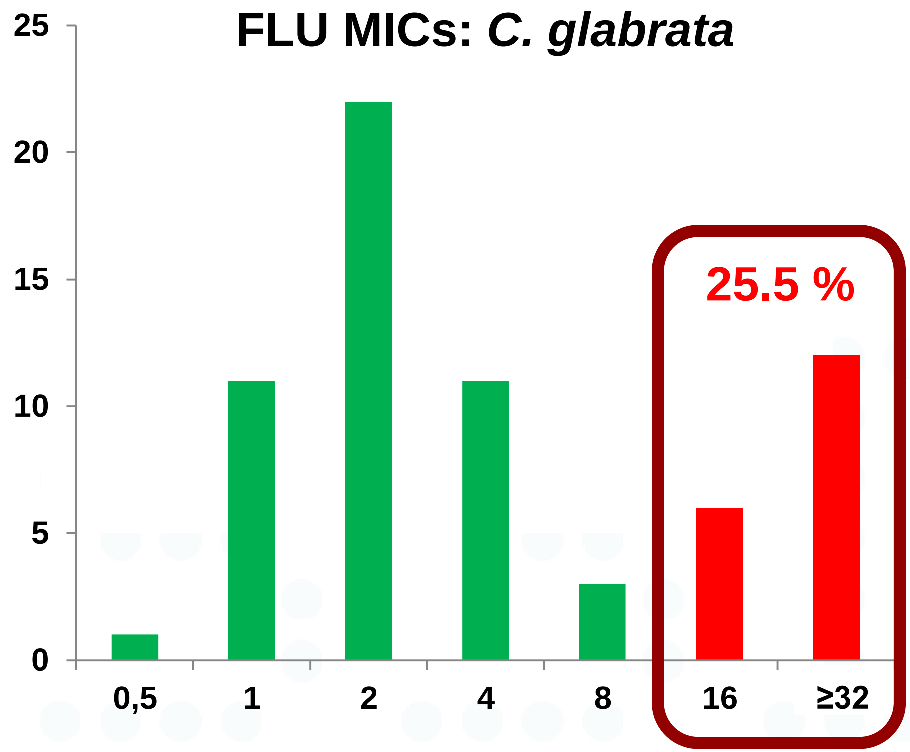
Patients treated with fluconazole harbouring *C. glabrata*



Geometric mean MIC = 3 mg/L



Geometric mean MIC = 4.17 mg/L

Acquired echinocandin resistance

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Species distribution in 65 patients treated with echinocandins

Species	Blood (%)	Swab (%)
C. albicans	20 (30.8)	13 (20)^{NS} ↓
C. glabrata	31 (47.7)	34 (52.3)^{NS}
C. krusei	3 (4.6)	4 (6.2)^{NS}
C. tropicalis	3 (4.6)	2 (3.1)^{NS}
C. parapsilosis	1 (1.5)	2 (3.1)^{NS}
S. cerevisiae	2 (3.1)	4 (6.2)^{NS}
Other *Candida*/yeast*	3 (4.6)	4 (6.2)^{NS}
Total	65	65
Intrinsic resistance***	1 (1.5)	3 (4.6)^{NS}

*** *C. parapsilosis, C. guilliermondii* (reduced echinocandin susceptibility)

^{P} Significant change from left to index column, ($p < 0.05$), calculated by Fischer's exact test. ^{PP}$p<0.01$. ^{NS} Non-significant change

Acquired echinocandin resistance

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Species distribution in <u>65 patients</u> treated with echinocandins

Species	Blood (%)	Swab (%)	No. of isolates with elevated anidulafungin MIC	FKS1	FKS2
C. albicans	20 (30.8)	13 (20)[NS]	2	**F641L, D648V**	
C. glabrata	31 (47.7)	34 (52.3)[NS]	3	WT	**F659L, S663P, F659-del**
C. krusei	3 (4.6)	4 (6.2)[NS]	1	WT	WT
C. tropicalis	3 (4.6)	2 (3.1)[NS]			
C. parapsilosis	1 (1.5)	2 (3.1)[NS]			
S. cerevisiae	2 (3.1)	4 (6.2)[NS]			
Other *Candida*/yeast*	3 (4.6)	4 (6.2)[NS]			
Total	65	65	**6 (9.2 %)**	NA	NA
Intrinsic resistance***	1 (1.5)	3 (4.6)[NS]	NA	NA	NA

*** *C. parapsilosis, C. guilliermondii* (reduced echinocandin susceptibility)

[P] Significant change from left to index column, ($p < 0.05$), calculated by Fischer's exact test. [PP] $p<0.01$. [NS] Non-significant change

Four messages to remember from Part I

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1. **Azole treatment shift species distribution → less susceptible species**

2. **Acquired FLU resistance in *C. glabrata* isolates upon azole exposure**

3. **> 9 % acquired echinocandin resistance**

4. **Identification and susceptibility testing crucial!**
    - Particularly in patients exposed to treatment

Transition to part II

What about selection of resistance *ex vivo* (in the environment)?

Few studies demonstrating resistant *Candida* found on fruit

ICAAC 2004
Washington, D.C.
Oct. 30 - Nov. 2, 2004

Crump, K. R. and T. D. Edlinds. 2004. Agricultural Fungicides May Select for Azole Antifungal Resistance in Pathogenic Candida. 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC 2004, Oct 30-Nov 2.M-1684.

Cross-Resistance to Medical and Agricultural Azole Drugs in Yeasts from the Oropharynx of Human Immunodeficiency Virus Patients and from Environmental Bavarian Vine Grapes

Antimicrobial Agents and Chemotherapy

Frank-Michael C. Müller, Andrea Staudigel, Stefanie Salvenmoser, Antje Tredup, Rudolf Miltenberger and Josef V. Herrmann
Antimicrob. Agents Chemother. **2007, 51(8):3014. DOI: 10.1128/AAC.00459-07.**
Published Ahead of Print 4 June 2007.

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Equivalently azole resistance in *A. fumigatus* is acquired by two routes

In the patient during long-term antifungal treatment

In the environment due to the use of fungicides

Mutations in the *CYP51A* gene induces resistance

TR34/L98H

Pan-azole resistance
Since 1998

Two major mechanisms found in the environment

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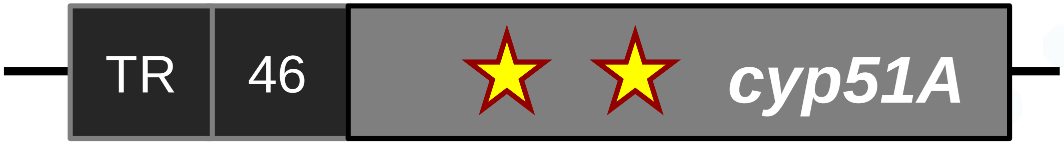
Mutations in the *CYP51A* gene induces resistance

TR34/L98H

Pan-azole resistance
Since 1998



TR46/Y121F/T289A

High voriconazole resistance
Since 2011

Both appear to originate from the environment but are increasingly found in clinical isolates

Azole resistant *A. fumigatus* in 4 Danish patients

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TABLE 1: Mould isolates, MICs, resistance genotypes and STR*Af* typing from the clinical isolates obtained from the four patients.

CASE	Day	Species	MIC (µg/mL)*)					*CYP51A* profile	STR*Af* (2A-2B-2C-3A-3B-3C-4A-4B-4C)	
			POS	VOR	ITRA	AMB	CAS			
	7	*A. fumigatus*	0.06	1	0.25	0.5	0.064	wt	18-19-8-26-10-21-9-9-5	
1	7	*A. fumigatus*	1	4	>8	0.5	0.064	TR_{34}/L98H +S297T+F495I	14-10-9-30-9-6-8-10-20	✝
	17	*A. fumigatus*	0.5	1	>8	0.5	0.064	TR_{34}/L98H+ S297T +F495I	14-10-9-30-9-6-8-10-20	
	44	*A. fumigatus*	0.03	0.25	0.125	0.25	0.064	wt	14-20-11-34-9-7-8-10-12	
	90	*A. fumigatus*	0.5	4	>8	0.5	0.032	TR_{34}/L98H	25-10-12-79-9-9-8-10-11	
	90	*R. pusillus*	0.25	>4	0.5	0.5	>32	NA	NA	
2	106	*R. pusillus*	0.125	>4	0.25	0.5	NA	NA	NA	✝
	110	*R. pusillus*	0.125	>4	0.25	0.5	NA	NA	NA	
	117	*A. fumigatus*	≤0.03	0.5	0.25	1	0.064	wt	25-16-19-48-17-23-8-9-5	
	117	*R. pusillus*	0.25	>4	0.25	0.5	>32	NA	NA	
3	6	*A. fumigatus*	0.5	4	>8	0.25	0.064	TR_{34}/L98H	20-20-28-32-9-6-8-10-20	✝

Patient 3, azole naïve, patient 1 just two days of azole exposure.

Table from paper by Astvad, K. et al, submitted to AAC

Azole resistant *A. fumigatus* in 4 Danish patients

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TABLE 1: Mould isolates, MICs, resistance genotypes and STR*Af* typing from the clinical isolates obtained from the four patients.

CASE	Day	Species	MIC (µg/mL)*) POS	VOR	ITRA	AMB	CAS	*CYP51A* profile	STR*Af* (2A-2B-2C-3A-3B-3C-4A-4B-4C)	
1	7	*A. fumigatus*	0.06	1	0.25	0.5	0.064	wt	18-19-8-26-10-21-9-9-5	✝
	7	*A. fumigatus*	1	4	>8	0.5	0.064	TR_{34}/L98H +S297T+F495I	14-10-9-30-9-6-8-10-20	
	17	*A. fumigatus*	0.5	1	>8	0.5	0.064	TR_{34}/L98H+ S297T +F495I	14-10-9-30-9-6-8-10-20	
2	44	*A. fumigatus*	0.03	0.25	0.125	0.25	0.064	wt	14-20-11-34-9-7-8-10-12	✝
	90	*A. fumigatus*	0.5	4	>8	0.5	0.032	TR_{34}/L98H	25-10-12-79-9-9-8-10-11	
	90	*R. pusillus*	0.25	>4	0.5	0.5	>32	NA	NA	
	106	*R. pusillus*	0.125	>4	0.25	0.5	NA	NA	NA	
	110	*R. pusillus*	0.125	>4	0.25	0.5	NA	NA	NA	
	117	*A. fumigatus*	≤0.03	0.5	0.25	1	0.064	wt	25-16-19-48-17-23-8-9-5	
	117	*R. pusillus*	0.25	>4	0.25	0.5	>32	NA	NA	
3	6	*A. fumigatus*	0.5	4	>8	0.25	0.064	TR_{34}/L98H	20-20-28-32-9-6-8-10-20	✝
4	-7	*A. fumigatus*	0.06	0.5	0.125	0.75	0.094	wt	18-25-15-26-11-7-26-30-8	✝
	36	*A. fumigatus*	0.125	>4	0.25	0.75	0.032	TR_{46}/Y121F/T289A	26-21-16-32-9-10-8-14-10	
	58	*A. fumigatus*	0.25	>4	0.5	0.5	0.094	TR_{46}/Y121F/T289A	26-21-16-32-9-10-8-14-10	
	62	*A. fumigatus*	0.25	>4	0.5	0.5	0.064	TR_{46}/Y121F/T289A	26-21-16-32-9-10-8-14-10	

NA: Not appropriate, *R. pusillus* is intrinsically resistant and thus caspofungin susceptibility testing was not deemed relevant. STR*Af* and *CYP51A*

Patient 4, the first case in Denmark with the resistance genotype **TR46/Y121F/T289A**

Table from paper by Astvad, K. et al, submitted to AAC

Azole resistant isolates spread across the environment

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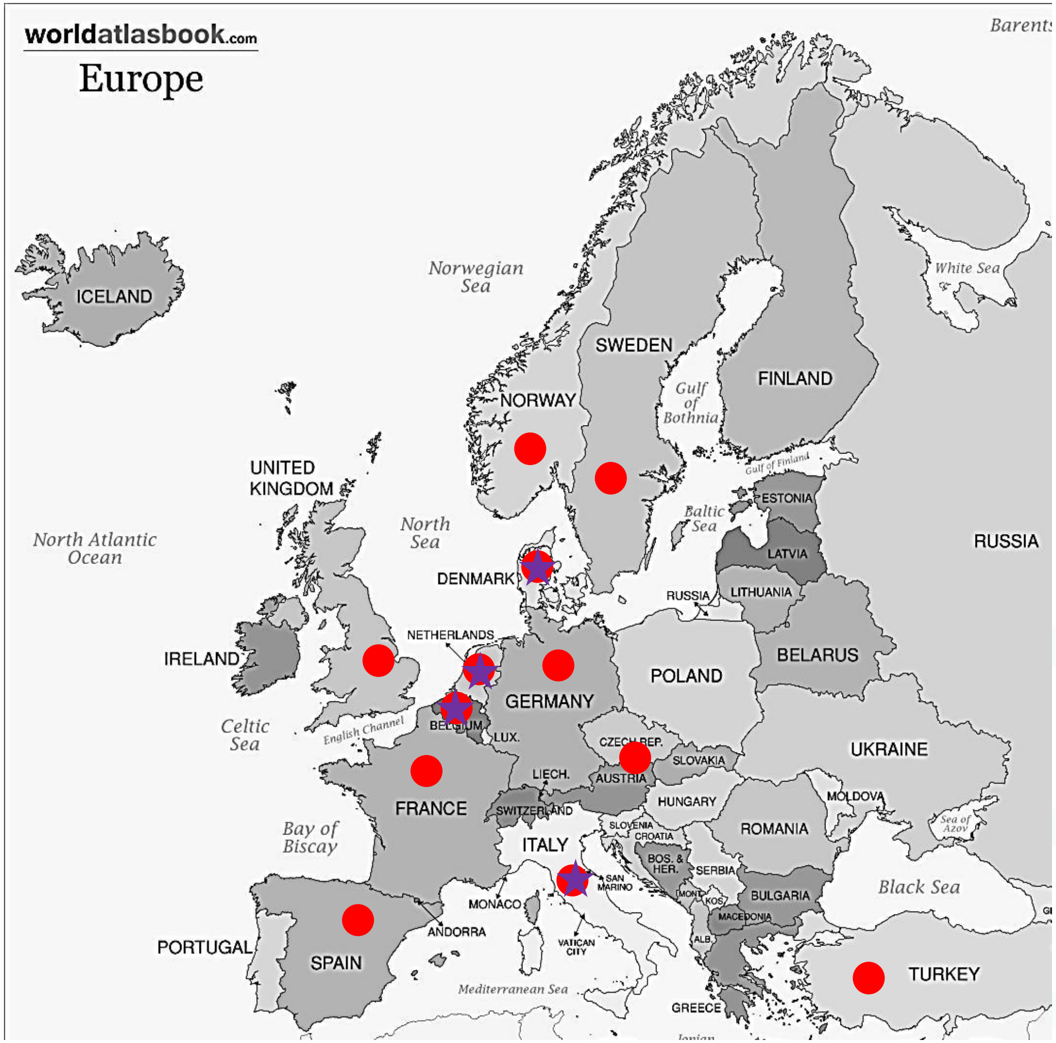

● **TR34/L98H detected**

+ Iran, Kuwait, China, India, US and Australia

★ **TR46/Y121F/T289A detected**

+ India

<u>In Denmark</u>

Prevalence of resistance in clinical isolates probably < 5 %

Final comment

Resistance in both *Aspergillus* and *Candida* is present

Prevalence may be low

Identification and susceptibility testing crucial!

Acknowledgements

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