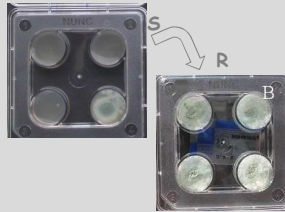


Antifungal resistance in moulds



Maiken Cavling Arendrup
maca@ssi.dk
Unit of Mycology
Statens Serum Institut
Denmark

Disclosures:

Research grants & Speaker: Astellas, Gilead, MSD & Pfizer;
Advisory board: MSD, Pcovery, Pfizer; Acted as consultant for: Alcomed, Astellas, Gilead & Pfizer
Chair(wo)man for EUCAST-AFST
Advisor for CLSI-AFST

Agenda

- Intrinsic susceptibility
 - *A. fumigatus* and beyond
- Acquired resistance mechanisms
 - In *Aspergillus*
- Size of the problem

Antifungals spectrum: Moulds

	<i>Aspergillus</i>			<i>Aspergillus</i> Cidal?	<i>Fusarium</i>	Zygo- mycetes
	<i>fumigatus</i>	<i>terreus</i>	<i>flavus</i>			
Amph. B	+	-	+/-	+	(+)	(+)
Anidulafungin	+	+	+	-	-	-
Caspofungin	+	+	+	-	-	-
Micafungin	+	+	+	-	-	-
Fluconazole	-	-	-	-	-	-
Itraconazole	+	+	+	-	-	-
Posaconazole	+	+	+	?	+/-	+/-
Voriconazole	+	+	+	+	+/-	-
5-FC	-	-	-	-	-	-
Terbinafine					+/-	

Aspergillus section *Fumigati*

- 10 anamorphs
 - *A. brevipes*
 - *A. duricaulis*
 - *A. fumigatiaffinis* *
 - *A. fumigatus*
 - *A. fummisynnematus*
 - *A. lentulus* *
 - *A. novofumigatus*
 - *A. turcosus*
 - *A. unilateralis*
 - *A. viridinutans* *
- 23 telemorphs (*Neosartorya*)
 - *N. assulata*
 - *N. aurata*
 - *N. aureola*
 - *N. australensis*
 - *N. coreana*
 - *N. denticulata*
 - *N. ferenczii*
 - *N. fennelliae*
 - *N. fischeri*
 - *N. galapagensis*
 - *N. glabra*
 - *N. hiratsukae*
 - *N. laciniosa*
 - *N. multiplicata*
 - *N. papuensis*
 - *N. pseudofischeri* *
 - *N. quadricincta*
 - *N. spinosa*
 - *N. stramenia*
 - *N. spathulata*
 - *N. tatenoi*
 - *N. udagawa* *
 - *N. warcupii*

Isolates in red have been isolated from humans, * resistant to one or more AFs
Samson in "Aspergillus fumigatus and Aspergillosis" 2008

Intrinsic and Primary resistance

Intrinsic: (✓) Primary: (✓)

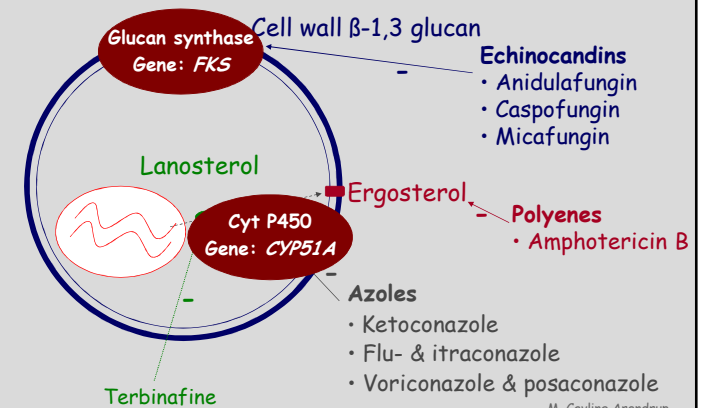
	AMB	Azoles	Echinocandins
<i>Aspergillus section fumigati</i>			
<i>A. fumigati</i> affinis	✓	✓	
<i>A. lentulus</i>	✓	✓	(✓)
<i>N. pseudofischeri</i>		✓	
<i>A. viridinutans</i>		✓	
<i>N. udagawae</i>	✓		

<i>A. terreus</i>	✓		
<i>A. ustus</i> (<i>A. calidoustus</i>)	✓	✓	✓

<i>A. flavus</i>	(✓)		(✓)
<i>A. allilaceus</i>	(✓)		(✓)

Alcazar-Fuoli AAC 2008; Perlin & Mellado in "Aspergillus fumigatus and Aspergillosis" 2008; Verweij Eukaryotic Cell 2008; M. Cavling Arendrup

Systemic Antifungals: Mode of Action



M. Cavling Arendrup

Acquired Resistance in *Aspergillus*

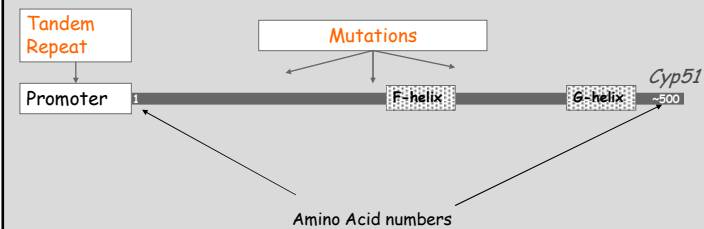
Compound	Azoles	Echinocandins	Amphotericin B
Target	P450 demethylase	Glucan synthase	Ergosterol
Target gene mutation	<i>CYP51A</i>	<i>FSK1</i> *	
Target up-regulation	<i>CYP51A+</i> Promotor	✓	
Efflux pumps	ABC & MF		

* only laboratory engineered strains

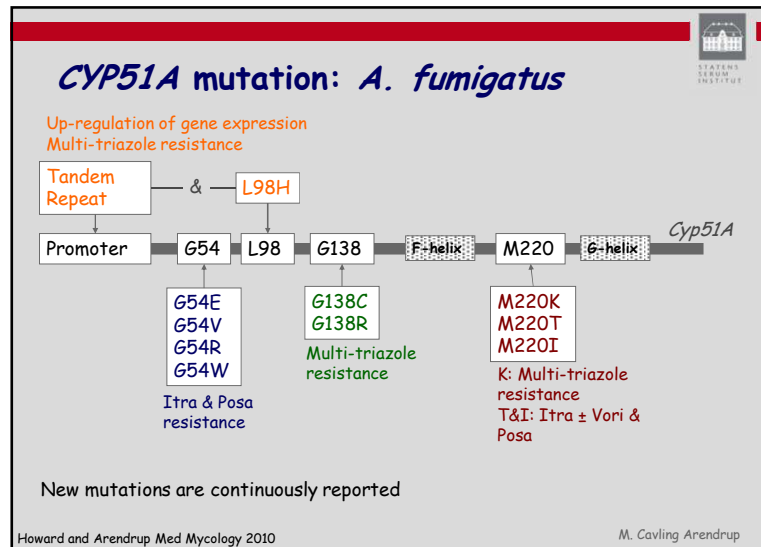
M. Cavling Arendrup

Azole resistance: Target gene

Three *Cyp51* genes: *CYP51A*, *CYP51B* and *CYP51C*



Howard Emerg Inf Dis 2009, Mortensen JCM 2011, Buied JAC 2010 Arendrup PLoS ONE 2010; M. Cavling Arendrup



CYP51A mutation: *A. fumigatus*

AA position	AA Substitution	ITRA	POSA	VORI
N 22	D	R	I	R
S 52	T	R	S	R
G 54	E, K, R, V, W	R	V	R
Q 88	H	R	V	R
TR-198	H	R	V	R
Y 101	F	R	I	R
Y 121	F	R	I	R
N 121	I	R	R	R
G 138	C, R	R	R	R
Q 141	H	R	R	R
H 147	Y	R	R	R
P 216	L	R	R	R
F 219	I	R	R	R
M 220	K, I, R, T, V, W	R	D	R
N 236	K, T, V	R	I	R
N 266	N	R	D	R
A 284	T	R	V	R
T 289	A	R	I	R
S 297	T	R	S	R
F 332	L	R	I	R
P 394	L	R	S	R
S 400	I	R	V	R
E 427	G	R	S	R
G 432	C	R	R	R
G 434	C	R	R	R
T 440	A	R	R	R
G 448	S	R	R	R
Y 491	H	R	R	R
F 495	I	R	R	R

R: resistant, I: intermediate, S: susceptible, V: variable susceptibility, D: decreased susceptibility

Howard 2009, Snelders 2008&10, Buiéd 2010, Mortensen 2010&11, Verweij 2010 Mellado 2007, Rodriguez-Tudela 2008, da Silva Ferreira 2004&5, Stensvold Current Fungal Infection Reports 2012 M. Cavling Arendrup

A. terreus: Azole R w CYP51A M217I

Sample-ID	MIC (mg/L)			CYP51A	Primer					Genotype
	Itraconazole ECOFF: 0.5	Posaconazole ECOFF: 0.25	Voriconazole ECOFF: 2		A.terr-2	NS7	P4	R108	CII	
MB-03-1	0.5	0.5	4	wt	t1	n1	p1	r1	c1	A
MB-04-1	0.5	0.5	4	wt	t1	n1	p1	r1	c1	A
MB-07-1	0.5	0.5	4	wt	t1	n1	p1	r1	c1	A
MB-07-2	2	0.5	4	M217I	t1	n1	p1	r1	c1	A
MB-07-3	2	0.25	4	M217I	t1	n1	p1	r1	c1	A
MB-07-4	0.5	0.25	4	wt	t1	n1	p1	r1	c1	A
MB-07-5	2	0.5	4	M217I	t1	n1	p1	r1	c1	A
MB-07-6	2	0.5	4	M217I	t1	n1	p1	r1	c1	A
MB-07-7	2	0.5	2	M217I	t1	n1	p1	r1	c1	A
MB-09-1	2	0.25	4	M217I	t1	n1	p1	r1	c1	A
MB-09-2	0.25	0.06	0.5	wt	t2	n2	p1	r1	c1	F
MB-09-3	1	0.125	1	M217I	t1	n1	p1	r1	c1	A
MB-09-4	1	0.25	1	M217I	t1	n1	p1	r1	c1	A
MB-09-5	1	0.5	2	M217I	t9	n1	p1	r1	c1	B
MB-09-6	2	0.5	1	M217I	t1	n1	p1	r1	c1	A
MB-09-7	2	0.25	2	M217I	t1	n1	p1	r1	c1	A
MB-09-8	1	0.25	2	M217I	t1	n1	p1	r1	c1	A
MB-09-9	1	0.5	4	M217I	t1	n1	p1	r1	c1	A
MB-11-1	2	0.5	2	M217I	t1	n1	p1	r1	c1	A
MB-11-2	2	0.5	2	M217I	t1	n1	p1	r1	c1	A
UKNEQUAS-97	0.125	0.06	0.5	wt	t3	n3	p3	r3	c1	C
UKNEQUAS-99	0.125	0.06	0.5	wt	t3	n3	p3	r3	c1	C
OUH-04	0.06	<0.03	0.25	Not done	t8	n4	p2	r2	c2	D
Estbjerg-05	0.125	0.06	0.5	wt	t6	n1	p5	r4	c1	H
Skejby-05	0.06	0.06	0.25	wt	t7	n1	p7	r1	c1	G
RH-08	0.125	0.06	0.5	A221V	t5	n5	p6	r5	c1	E

Arendrup, JID 2012 M. Cavling Arendrup

Azole R *A. terreus*: CYP51A M217I

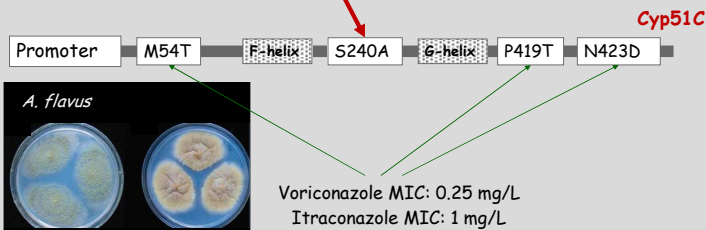
	EUCAST Azole MICs			Ampho. B MICs
	Itraconazole (ECOFF: 0.5)	Posaconazole (ECOFF: 0.25)	Voriconazole (ECOFF: 2)	EUCAST (48h)
Controls (6)	0.06-0.125 (0.125)	<0.03-0.06 (0.06)	0.25-0.5 (0.5)	2-4 (2)
MB wt-F (1)	0.25	0.06	0.5	4
MB wt-A (4)	0.5 (0.5)	0.25-0.5 (0.5)	4 (4)	0.5-4 (2)
MB M217I-A (14)	1-2 (2)	0.125-0.5 (0.5)	1-4 (2)	2-4 (2)

Arendrup, JID 2012 M. Cavling Arendrup

A. flavus: Azole R w Cyp51C S240A

■ AML pt with IPA failing 2 months(+) voriconazole

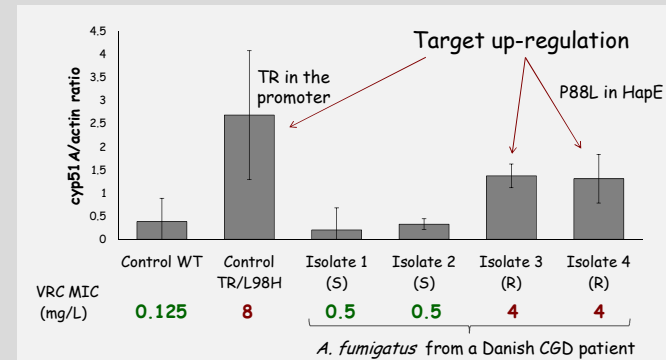
- Voriconazole MIC: 8 mg/L (EUCAST BP: S: ≤ 1 mg/L)
- Itraconazole MIC: 2 mg/L (EUCAST BP: S: ≤ 1 mg/L)



Liu AAC 2012

M. Cavling Arendrup

Resistance 2: target gene up-regulation



Arendrup PLoS ONE 2010, Camps in preparation.

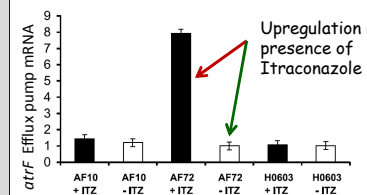
M. Cavling Arendrup

Resistance 3: Efflux pumps

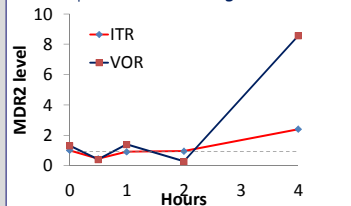
■ Two families

- ATP-Binding Cassette transporters (ABC)
- Major Facilitator (MF) transporters

ABC: *atrF* ↑ in ITR resistant *A. fumigatus*



MDR2 ↑ in VOR MIC 4 mg/L *A. terreus*



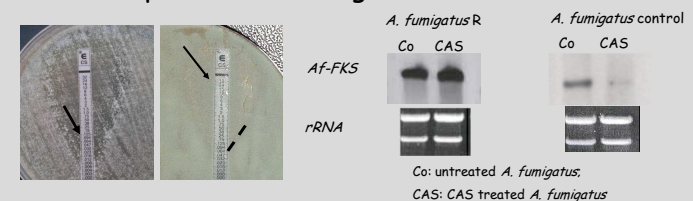
Slaven Fungal Genetics and Biology 2002, Arendrup-Perlin unpublished data. M. Cavling Arendrup

Aspergillus Echinocandin resistance

■ Mutations in *FKS1* gene coding glucan synthase

- S678Y laboratory generated mutant
- S678P laboratory generated mutant

■ Over-expression of the gene



Gardiner Med Mycol 2005; Rocha AAC 2007; Arendrup AAC 2008

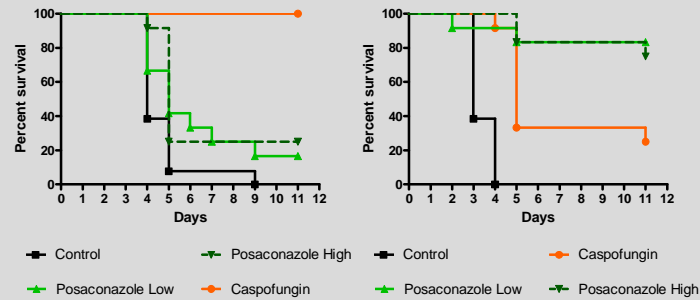
M. Cavling Arendrup

Aspergillus resistance - in vivo efficacy

Mice inoculated with *A. fumigatus* and subsequently treated for 10 days

Pan-Azole R isolate (Pos > 4; M220K)

Caspofungin R isolate (up-regulation)



Arendrup AAC 2008

M. Cavling Arendrup

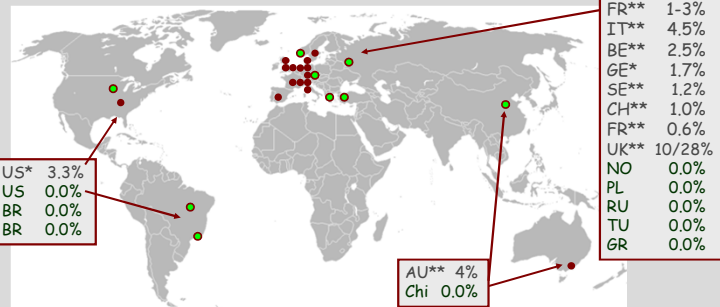
Agenda

- Intrinsic susceptibility
 - *A. fumigatus* and beyond
- Acquired resistance mechanisms
 - In *Aspergillus*
- Size of the problem

M. Cavling Arendrup

Aspergillus SCARE study prelim. data

- Azole resistant *A. fumigatus* 2009-10



Study coordinators: Verweij, van der Linden & Arendrup

M. Cavling Arendrup

Azole Resistant CNS aspergillosis

Patient	Specimen Type	Cyp51A alterations			MIC, mg/L				
		TR	Codon 98	Other	AmB	ITZ	VCZ	POS	CAS
1. 42-y HIV	Brain biopsy	+	L98H	-	0.25	>16	8	0.5	0.25
2. 64-y Cancer	Brain biopsy	+	L98H	-	1	>16	16	0.5	0.5
3. 11-y ALL	Lung biopsy	+	L98H	-	0.5	>16	16	2	0.5
Control	Sputum	-	-	-	0.25	0.25	0.25	0.125	0.25
Control	Sputum	-	-	-	0.5	0.125	0.5	0.063	0.25
Control	Sputum	-	-	-	0.25	0.25	0.25	0.063	0.5

- ✓ Pt 1: Voriconazole → ambisome
- ✓ Pt 2: Voriconazole → voriconazole + caspofungin
- ✓ Pt 3: Voriconazole → voriconazole + caspofungin → ambisome + caspofungin
- ✓ All died

All three azole naïve

van der Linden CID 2009

M. Cavling Arendrup

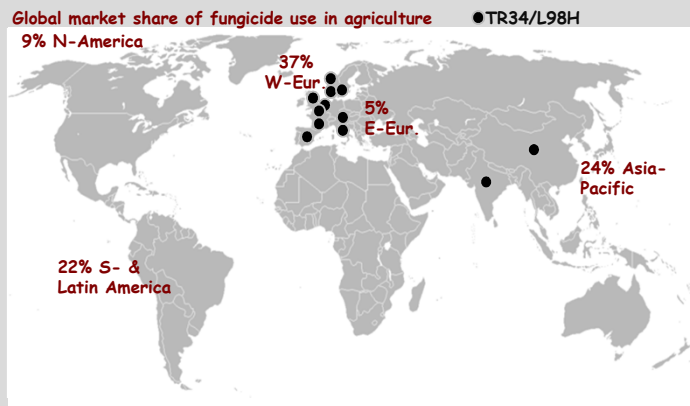
Multi-azole resistant *A. fumigatus*

Site (no.) Sample ID.	EUCAST MIC (µg/ml)			<i>Cyp51A</i>
	ITC	VRC	POS	
Rigshospitalet (27) RH-13	>8	4	0.5-1	TR ₃₄ /L98H
Tivoli Gardens (23) T-11	4/50 soil samples in Cph ~ 8%			TR ₃₄ /L98H
T-18				TR ₃₄ /L98H
T-22				TR ₃₄ /L98H
Control isolate CM-237	0.25	0.5	0.06	Wild type

Mortensen, AAC, 2010

M. Cavling Arendrup

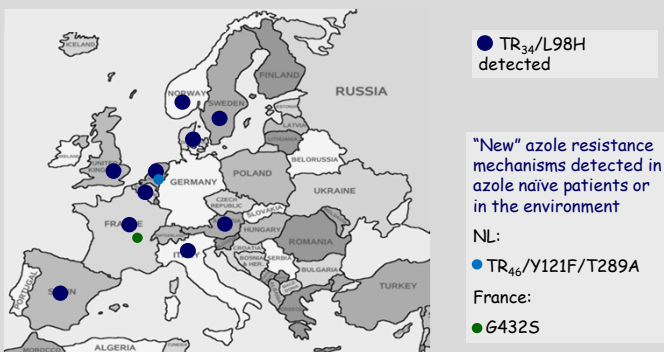
Fungicide use & TR₃₄/L98H reports



Stensvold Current Fungal Infection Reports 2012

M. Cavling Arendrup

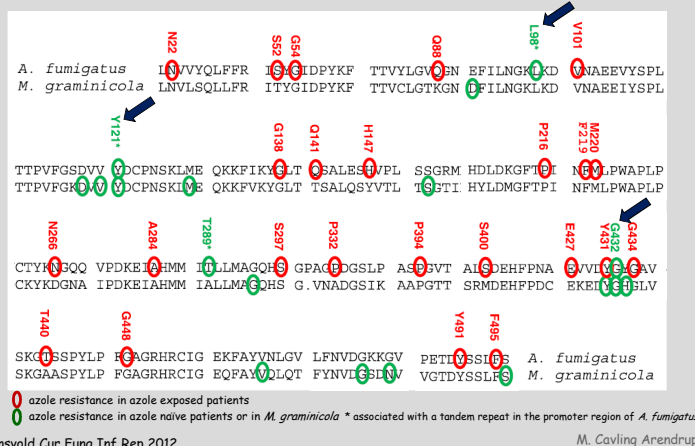
Azole resistant *A. fumigatus* in azole naïve patients in Europe



Scare Study, Denning CID 2011, Snelders PLoS Med 2008, Mortensen AAC 2010, Verweij ICAAC 2010, Mellado AAC 2007

M. Cavling Arendrup

CYP51 codons involved in azole R



Stensvold Cur Fung Inf Rep 2012

M. Cavling Arendrup

Azole resistant Asp: In vs. Ex vivo Origin

Date	ITC	AMB	cyp51A alteration	2A	2B	2C	3A	3B	3C	4A	4B	4C
Patient 4												
17.07.2007	1	0.5	Not found	18	23	16	35	13	18	15	9	10
05.12.2007	0.5	0.5	Not found	18	23	16	35	13	18	15	9	10
09.07.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	9	10
16.07.2009	>4	0.25	M220I + V101F	18	23	16	35	13	18	15	12	10
21.07.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	12 & 9	10
10.08.2009	>4	0.25	M220I + V101F	18	23	16	35	13	18	15	9	10
07.09.2009	>4	1	M220I + V101F	18	23	16	35	13	18	15	12	10
23.10.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	12	10
24.11.2009	>4	1	M220I + V101F	18	23	16	35	13	18	15	9	10
26.11.2009	0.5	1	Not found	18	23	16	35	13	18	15	9	10
14.12.2009	>4	0.5	M220I + V101F	18	23	16	35	13	18	15	9	10
Patient 5												
01.08.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
16.11.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
20.11.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
06.12.2007	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
12.05.2009	>4	0.5	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
28.07.2009	0.25	0.5	Not found	23	24	15	34	12	19	13	9	5
31.08.2009	>4	1	TR+L98H+S297T+F495I	14	20	8	40	9	11	8	10	20
13.11.2009	0.25	0.5	Not found	13	20	9	34	9	10	10	10	19
24.11.2009	>4	0.5	TR+L98H	23	10	9	10	10	6	8	10	20

Mortensen JCM 2011

M. Cavling Arendrup

4.5% *Aspergillus* Azole resistance in DK

A. fumigatus

CYP51A alterations

TR34/L98H

V101

M220

S297

Y491

F495

Up-regulation

P88L in HapE

No mechanism found yet



A. terreus

CYP51A alterations

M217

Up-regulation

MRD2

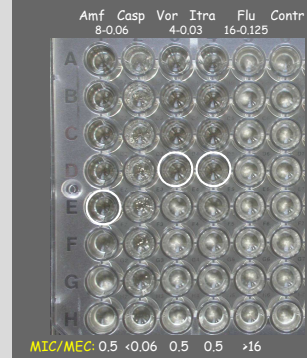
Tip of the iceberg?

Mortensen JCM 2011, EJCMI 2011, Arendrup PlosOne. 2010 and JID 2012; Camps in prep

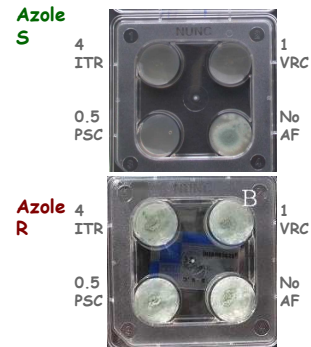
M. Cavling Arendrup

Susceptibility testing recommended!

EUCAST EDEF 9.1 for the ref lab



Azole screening agar for the routine lab



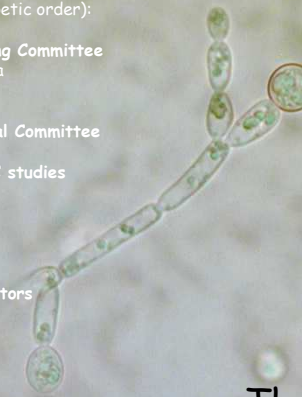
M. Cavling Arendrup

Acknowledgements
(in alphabetic order):

The EUCAST Steering Committee
M Cuenca-Estrella
W Hope
C Lass-Flörl
The EUCAST General Committee

Collaborators on MIC studies
SJ Howard
DS Perlin
M Pfaller
P Verweij

The Danish Collaborators
RH Jensen
HK Johansen
KL Mortensen
T Pressler
M Skov
R Stensvold



Thank you for
your attention