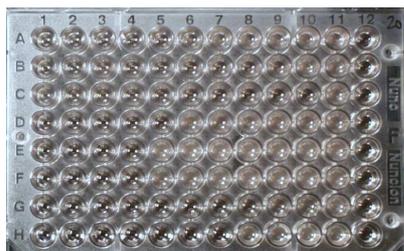


Aspergillus Resistance Trends & AFST



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Speaker honoraria: Astellas, Basilea, Gilead, MSD, Novartis, Pfizer & T2Candida
Chair(wo)man for EUCAST-AFST
Past advisory board (≤ 2014): MSD, Pcovery, Pfizer

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Agenda



❖ *Aspergillus*

- Intrinsic susceptibility pattern and epidemiology
- Acquired resistance in mechanisms
- Size of the problem

❖ *Aspergillus* AFST

- EUCAST E.Def 9.3
- Alternatives
 - Gradient strips (not licensed for mould testing)
 - Screening agar – upcoming EUCAST test

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Antifungals spectrum: Moulds



	<i>Aspergillus</i>				<i>Fusarium</i>	Mucorales	<i>Scedosporium</i>	
	<i>fumigatus</i>	<i>terreus</i>	<i>flavus</i>	<i>niger</i>			<i>apio.</i>	<i>proli.</i>
Amph. B	+	-	+/-	+	+/-	+/-	+/-	-
Anidula	+	+	+	+	-	-	-	-
Caspo	+	+	+	+	-	-	-	-
Mica	+	+	+	+	-	-	-	-
Fluco	-	-	-	-	-	-	-	-
Isavu	+	+	+	+	+/-	+/-	+	-
Itra	+	+	+	+/-	-	-	+/-	-
Posa	+	+	+	+	+/-	+/-	+	-
Vori	+	+	+	+	+/-	-	+	-
5-FC	-	-	-	-	-	-	-	-

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Antifungals spectrum: Moulds



	<i>Aspergillus</i>				<i>Fusarium</i>	Mucorales	<i>Scedosporium</i>	
	<i>fumigatus</i>	<i>terreus</i>	<i>flavus</i>	<i>niger</i>			<i>apio.</i>	<i>proli.</i>
Amph. B	+	-	+/-	+	+/-	+/-	+/-	-
Anidula	+	+	+	+	-	-	-	-
Caspo	+	+	+	+	-	-	-	-
Mica	+	+	+	+	-	-	-	-
Fluco	-	-	-	-	-	-	-	-
Isavu	+	+	+	+	+/-	+/-	+	-
Itra	+	+	+	+/-	-	-	+/-	-
Posa	+	+	+	+	+/-	+/-	+	-
Vori	+	+	+	+	+/-	-	+	-
5-FC	-	-	-	-	-	-	-	-

And
Cryptic *A. fumigatus* complex spp
&
Less common *Aspergillus* species
With intrinsic resistance
&
Azole Resistant *A. fumigatus*...

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Aspergillus section Fumigati



❖ 10 anamorphs

- *A. brevipes*
- *A. duricaulis*
- ***A. fumigatiaffinis*^R**
- ***A. fumigatus***
- ***A. fummisynnematus***
- ***A. lentulus*^R**
- *A. novofumigatus*
- *A. turcosus*
- *A. unilateralis*
- ***A. viridinutans*^R**

❖ 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*^R**
- *N. quadricincta*
- *N. spinosa*
- *N. stramenia*
- *N. spathulata*
- *N. tatenoi*
- ***N. udagawa*^R**
- *N. warcupii*

Isolates in red have been isolated from humans, ^R resistant to one or more Afs

A. fumigatus sensu stricto is the only thermotolerant section Fumigati species growing at 48-50 °C

Samson in "Aspergillus fumigatus and Aspergillosis" 2008

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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)	

<i>A. terreus</i> (and <i>A. alabamensis</i>)	✓		
<i>A. flavus</i>	✓	(✓)	(✓)
<i>A. versicolor</i> (and <i>A. sydowi</i>)	✓	(✓)	

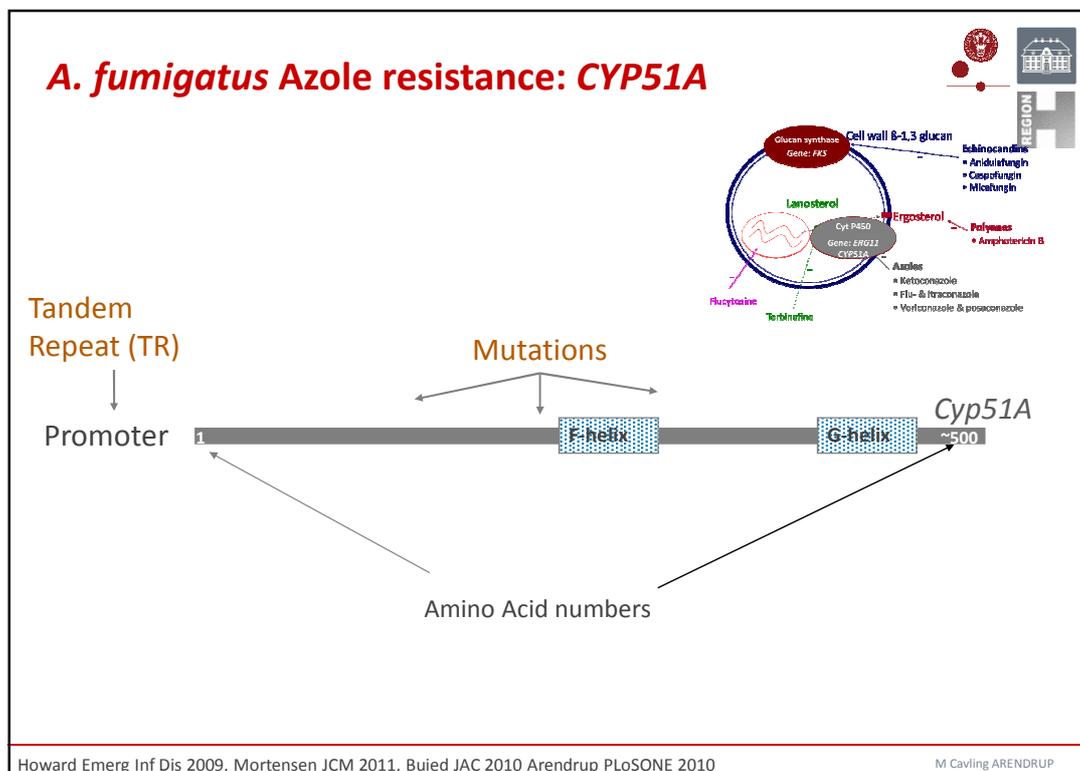
<i>A. calidoustus</i>		✓	(✓)
<i>A. allilaceus</i>	(✓)		(✓)

Alcazar-Fuoli AAC 2008; Perlin & Mellado in "Aspergillus fumigatus and Aspergillosis" 2008; Varga Eukariotc Cell 2008, Linden Med Mycol 2011^M Cavling ARENDRUP

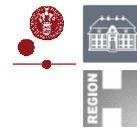
Acquired Resistance in *Aspergillus*

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 or P88L in <i>HapE</i>	✓	
Efflux pumps	ABC & MF		
	Found in DK isolates	* only laboratory engineered strains	

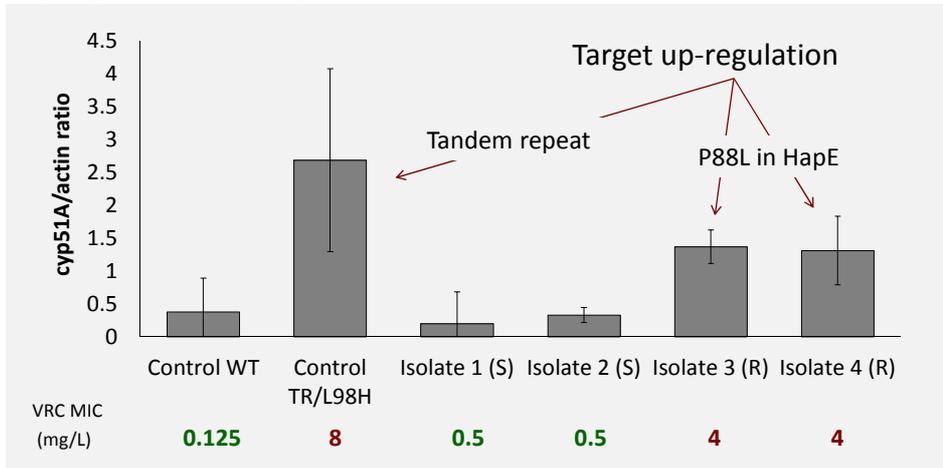
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Azole resistance target up-regulation



A. fumigatus from a CGD patient

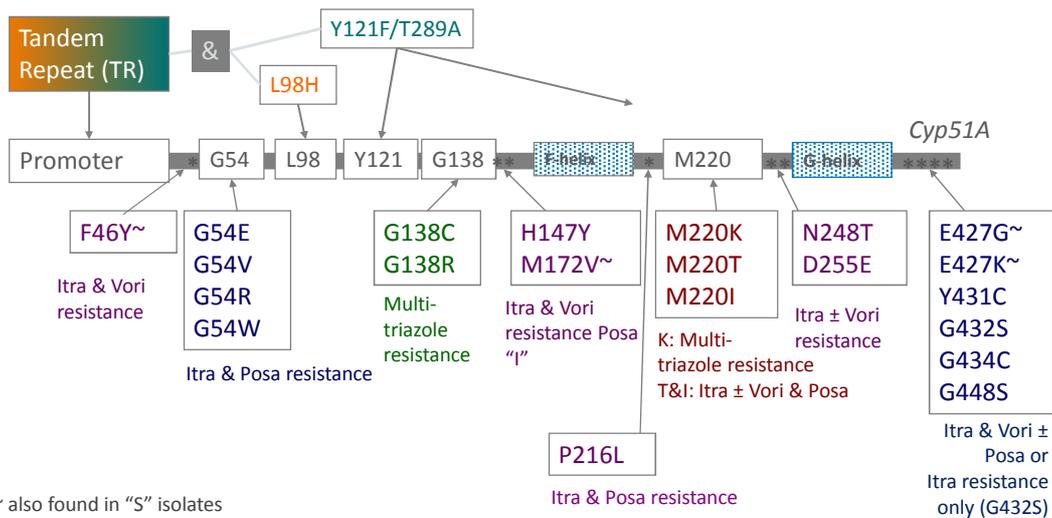


Arendrup PLoS ONE 2010, Camps PLoS ONE 2012.

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Aspergillus Azole resistance: *CYP51A*

Up-regulation of gene expression
Multi-triazole resistance or Vori- & Isavuconazole resistance



~ also found in "S" isolates

New mutations are continuously reported

Azole resistant isolates without *CYP51A* mutations have been reported

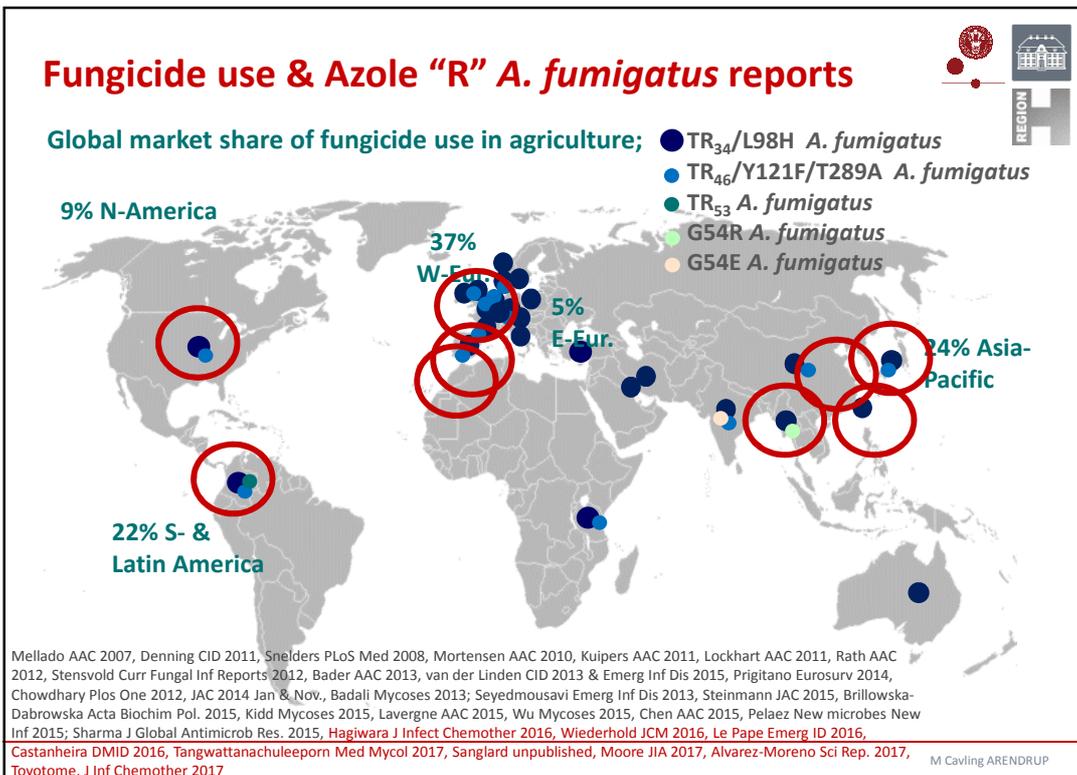
Howard Emerg Inf Dis 2009, Howard and Arendrup Med Mycology 2010, Stensvold Curr Fungal Inf Rep 2012

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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



"Environmental" Azole resistant *A. fumigatus* in Europe

1st TR₃₄ pos sample

NL 1998

UK 1999

NO 2001

ES 2002-3

DK 2007

BE 2008

AU 2006-9

GE 2006-9

FR 2010-11

IT 2011-12

TU 2014

PL 2015

SW 2016

1st TR₄₆ pos sample

NL 2011

BE 2012

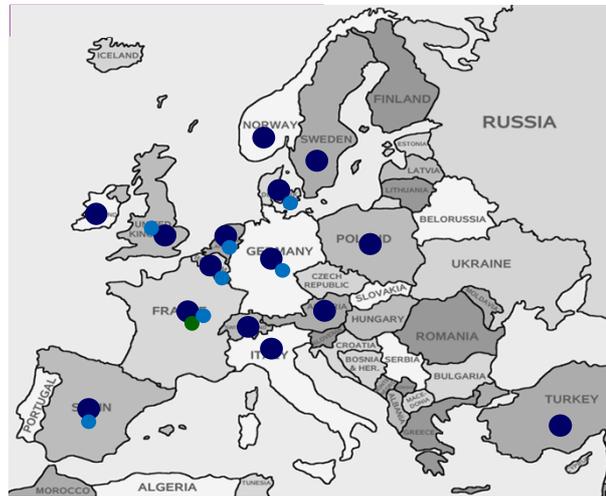
DK 2014

GE 2015

FR 2015

ES 2015

UK 2017



● TR₃₄/L98H detected
● TR₄₆/Y121F/T289A
● G432S

Denning CID 2011, Snelders PLoS Med 2008, Mortensen AAC 2010, Kuipers AAC 2011, Mellado AAC 2007, Rath AAC 2012, Stensvold Curr Fungal Inf Reports 2012, Bader AAC 2013, van der Linden CID 2013 & Emerg Inf Dis 2015, Prigitano Eurosurv 2014, Ozmerdiven ICAAC 2014, Steinmann JAC

2015, Brillowska-Dabrowska TIMM 2015, Pelaez New Microbes New Infect. 2015, Lavergne AAC 2015 Castanheira DMID 2016, unpublished DK data, Sanglard Personal Comm

Multi-azole resistant *A. fumigatus*

Site (no.) Sample ID.	EUCAST MIC (µg/ml)			Cyp51A
	ITC	VRC	POS	
Rigshospitalet (27)				
RH-13	>8	4	0.5-1	TR ₃₄ /L98H
Tivoli Gardens (23)	4/50 soil samples in Cph ~ 8%			
T-11	>8	4	0.5-2	TR ₃₄ /L98H
T-18	>8	4	0.5	TR ₃₄ /L98H
T-22	>8	2-4	0.25-0.5	TR ₃₄ /L98H
Control isolate				
CM-237	0.25	0.5	0.06	Wild type

Mortensen, AAC, 2010

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Case 1 (May-June 2012):



- ❖ A 77-year old woman
 - COPD
 - inhalation budesonide 400 µg*2/day
 - MPO-ANCA positive kidney disease (2008)
 - prednisolone 25 mg/day & mycophenolatmofetil 1 g/day
- ❖ Admitted for a diagnostic kidney biopsy
 - Bleeding, septic shock (*S. marcescens*), multi-organ failure and anuria → ICU and haemodialysis
 - Biopsy demonstrated Wegener's granulomatosis
 - plasmaferesis and methylprednisolonsuccinat 80 mg*1 i.v. were initiated day 5.

Case 1 in the ICU (~day 0)



- ❖ BAL (day 0) and 3 subsequent upper respiratory samples
 - Mould - not further investigated at the local laboratory.
- ❖ Clinically
 - lung infection necessitating pressure support.
- ❖ CT (day 3)
 - lobar pneumonia and diffuse infiltration.
- ❖ Tentative diagnosis:
 - Possible Invasive Pulmonary Aspergillosis (IPA)
- ❖ Voriconazole prescribed empirically (day 5)
 - 400 → 300 mg x 2 /day (weight 60 kg)

Case 1 - diagnostics



- ❖ Serum *Aspergillus* galactomannan (day 6)
 - 2.4 Positive (Cut off range: ≥ 0.5 for positive)
- ❖ BAL (day 7)
 - → Local lab → Mould → reference lab for ID and susceptibility
- ❖ Probable IPA → Voriconazole was maintained
- ❖ BAL (day 17)
 - *Aspergillus* GM: Positive 7.8*
 - → Local lab → Mould → reference lab for ID and susceptibility
- ❖ Poor clinical response! Considerations??

Astvad AAC 2014, *D'Haese JCM 2012

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Mycology



- ❖ Both moulds were *A. fumigatus*
- ❖ TDM (day 18): 1.09 mg/L (ref level 1-2 → 5.5 mg/L)

CASE	Day	Species	MIC ($\mu\text{g}/\text{mL}$) ¹⁾				
			POS	VOR	ITRA	AMB	CAS
2012	7	<i>A. fumigatus</i>	1	2	>16	0.5	0.064
	17	<i>A. fumigatus</i>	0.5	1	>16	0.5	0.064

MICs in bold indicate I/R classification

Voriconazole →
Ambisome 3 mg/L day 19

Day 29 the patient refused
further tx

Died day 30

Astvad AAC 2014

M Cavling ARENDRUP

Mycology

∴ *Cyp51A* sequencing and STRAf genotyping



CASE	Day	Species	MIC ($\mu\text{g}/\text{mL}$) ¹⁾					CYP51A genotype	STRAf (2A-2B-2C-3A-3B-3C-4A-4B-4C)
			POS	VOR	ITRA	AMB	CAS		
2012	7	<i>A. fumigatus</i>	1	2	>16	0.5	0.064	wt	18-19-8-26-10-21-9-9-5
	17	<i>A. fumigatus</i>	0.5	1	>16	0.5	0.064	TR ₃₄ /L98H+ S297T +F495I	14-10-9-30-9-6-8-10-20

??? !!! ???

Astvad AAC 2014

M Cavling ARENDRUP

Mycology

∴ *Cyp51A* sequencing and STRAf genotyping



CASE	Day	Species	MIC ($\mu\text{g}/\text{mL}$) ¹⁾					CYP51A genotype	STRAf (2A-2B-2C-3A-3B-3C-4A-4B-4C)
			POS	VOR	ITRA	AMB	CAS		
2012	7	<i>A. fumigatus</i>	1	2	>16	0.5	0.064	wt	18-19-8-26-10-21-9-9-5
	17	<i>A. fumigatus</i>	0.5	1	>16	0.5	0.064	TR ₃₄ /L98H+ S297T +F495I	14-10-9-30-9-6-8-10-20

Re-cultured dilution series of the BAL day 7
 Picked 10 single colonies → susceptibility test
 Found 5 wild type and 5 resistant *A. fumigatus*

Astvad AAC 2014

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Mycology

- *Cyp51A* sequencing and STRAf genotyping of S and R strains

CASE	Day	Species	MIC ($\mu\text{g}/\text{mL}$) ¹⁾					CYP51A genotype	STRAf (2A-2B-2C-3A-3B-3C-4A-4B-4C)
			POS	VOR	ITRA	AMB	CAS		
	7	<i>A. fumigatus</i>	0.06	1	0.5	0.5	0.064	wt	18-19-8-26-10-21-9-9-5
2012	7	<i>A. fumigatus</i>	1	2	>16	0.5	0.064	TR ₃₄ /L98H +S297T+F495I	14-10-9-30-9-6-8-10-20
	17	<i>A. fumigatus</i>	0.5	1	>16	0.5	0.064	TR ₃₄ /L98H+ S297T +F495I	14-10-9-30-9-6-8-10-20

An azole naïve patient

Initial co infection with unrelated wild type "S" & environmental azole "R" strains

Astvad AAC 2014

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Environmental azole resistance - in DK *Aspergillus*

- 2007- 2012: TR₃₄/L98H found only in CF patients
- 2012 → TR₃₄/L98H in 3 non-CF patients 2 of whom = azole naïve

CASE	Day	Species	MIC ($\mu\text{g}/\text{mL}$) ¹⁾					CYP51A genotype	STRAf (2A-2B-2C-3A-3B-3C-4A-4B-4C)
			POS	VOR	ITRA	AMB	CAS		
	7	<i>A. fumigatus</i>	0.06	1	0.5	0.5	0.064	wt	18-19-8-26-10-21-9-9-5
2012	7	<i>A. fumigatus</i>	1	2	>8	0.5	0.064	TR ₃₄ /L98H +S297T+F495I	14-10-9-30-9-6-8-10-20
	17	<i>A. fumigatus</i>	0.5	1	>8	0.5	0.064	TR ₃₄ /L98H+S297T +F495I	14-10-9-30-9-6-8-10-20
2013	5	<i>A. fumigatus</i>	0.5	4	>8	0.25	0.064	TR ₃₄ /L98H	20-20-28-32-9-6-8-10-20
	44	<i>A. fumigatus</i>	0.03	0.25	0.25	0.25	0.064	wt	14-20-11-34-9-7-8-10-12
	90	<i>A. fumigatus</i>	0.5	4	>8	0.5	0.032	TR ₃₄ /L98H	25-10-12-79-9-8-10-11
	90	<i>R. pusillus</i>	0.25	>4	2	0.5	>32	NA	NA
2013	106	<i>R. pusillus</i>	0.125	>4	0.5	0.5	NA	NA	NA
	110	<i>R. pusillus</i>	0.125	>4	0.5	0.5	NA	NA	NA
	117	<i>A. fumigatus</i>	≤0.03	0.5	0.5	1	0.064	wt	25-16-19-48-17-23-8-9-5
	117	<i>R. pusillus</i>	0.25	>4	0.5	0.5	>32	NA	NA

Astvad AAC 2014

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Environmental azole resistance - in DK



2014

- TR₄₆/Y121F/T289A (LTX patient w agammaglobulinaemia)

CASE	Day	Species	MIC (µg/mL) ¹⁾					CYP51A genotype	STRAf (2A-2B-2C-3A-3B-3C-4A-4B-4C)
			POS	VOR	ITRA	AMB	CAS		
	0	<i>A. fumigatus</i>	0.06	0.5	0.25	0.75	0.125	wt	18-25-15-26-11-7-26-30-8
2014	43	<i>A. fumigatus</i>	0.125	>4	0.5	1	0.032	TR ₄₆ /Y121F/T289A	26-21-16-32-9-10-8-14-10
	62	<i>A. fumigatus</i>	0.25	>4	1	1	0.032	TR ₄₆ /Y121F/T289A	26-21-16-32-9-10-8-14-10

- 2/4 were azole naïve
- 3/4 polyfungal infection

All 4 died †

Astvad AAC 2014

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Aspergillus Azole resistance mechanisms in DK by 2017



A. fumigatus

CYP51A alterations

TR₃₄/L98H
 TR₄₆/Y121F/T289A
 TR₁₂₀/ + 10 AA alterations
 V101
 P216
 M220
 S297
 Y431
 Y491
 F495

Up-regulation
 P88L in HapE

No mechanism found yet



Tip of the iceberg?

A. terreus

CYP51A alterations

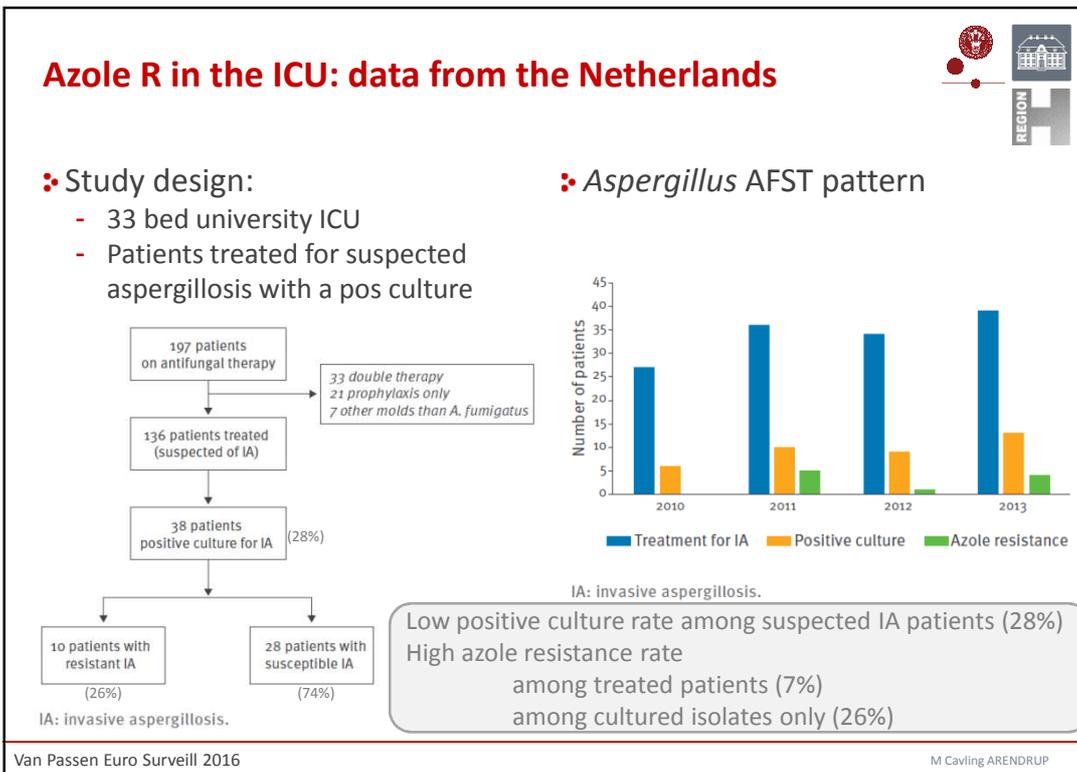
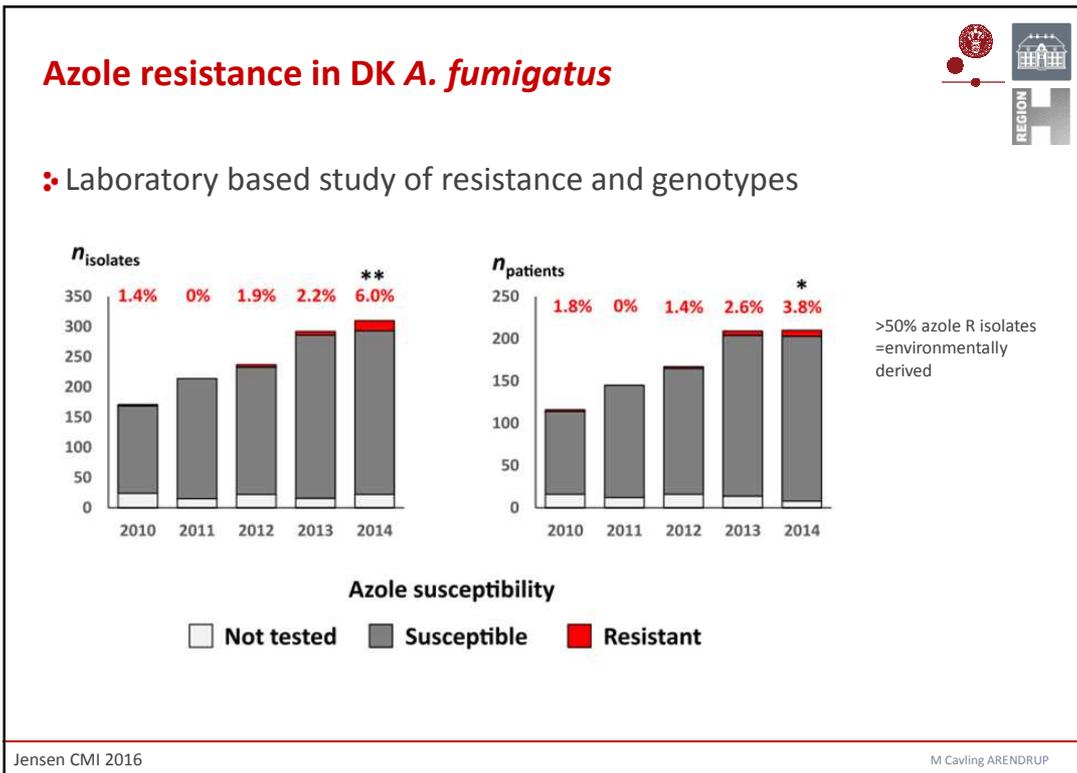
M217

Up-regulation

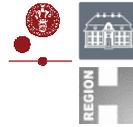
MRD2

Mortensen JCM 2011, EJCMI 2011, Arendrup PlosOne 2010 and JID 2012, Camps PLoS ONE 2012; Astvad AAC 2014

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EUCAST E.Def 9.3 Mould AFST Method



Conidia forming moulds E.Def 9.3

1-2.5 x 10⁵ cells/mL

Avoid clumps (water with 0.1% Tween 20; ± filter step 11 µm pore diameter)
Standardisation by conidia counting/spectrophotometre

2-fold drug dilutions in RPMI-2% glucose buffered w MOPS

48 h → adequate growth

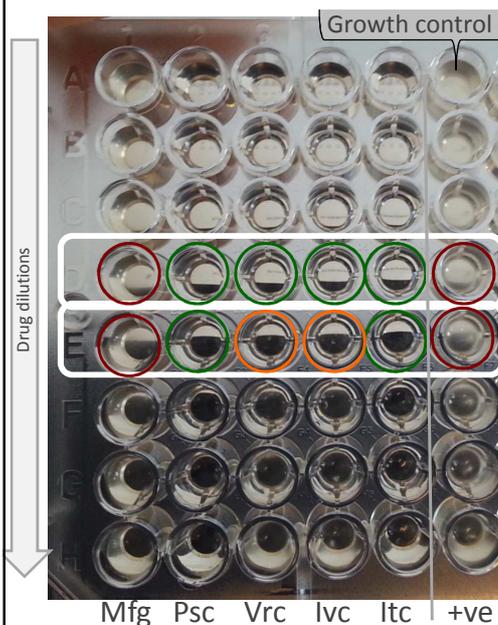
24h may be sufficient for some mucorales,
some moulds require 72h)

Visual endpoint reading

- amphotericin & azoles = no growth endpoint (MIC)
- echinocandins = aberrant growth (MEC)

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Visual reading Azoles & AMB: Full inhibition endpoint



Notes

- Place a black paper on a white table
- Hold the plate elevated above the table
- Examine sharpness of the line through the wells
- Last well with a sharp line ~ the MIC

M Cavling ARENDRUP

Visual reading Azoles & AMB: Full inhibition endpoint

Drug dilutions

Growth control column

Mfg Psc Vrc lvc ltc +ve

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EUCAST ECOFFs for *Aspergillus* species

❖ Epidemiological Cut Off values

- Red indicate the ECOFF is greater than for *A. fumigatus*

AF compound	<i>Aspergillus</i>				
	<i>flavus</i>	<i>fumigatus</i>	<i>nidulans</i>	<i>niger</i>	<i>terreus</i>
Amphotericin	4	1	ND	1	4
Isavuconazole	2	2	0.25	4	1
Itraconazole	1	1	1	4	0.5
Posaconazole	0.5	0.25	0.5	0.5	0.25
Voriconazole	2	1	1	2	2

- MIC > ECOFF :
 - Acquired resistance (and potentially clinical failure)?
 - Incorrect species ID?

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EUCAST *Aspergillus* BPs 2012



⚡ Clinical breakpoints

- BPs indicated as: $S \leq x / R > y$

AF compound	<i>Aspergillus</i>				
	<i>flavus</i>	<i>fumigatus</i>	<i>nidulans</i>	<i>niger</i>	<i>terreus</i>
Amphotericin	IE*	1/2	Note	1/2	Poor Target
Isavuconazole	IE	<u>1</u> /1	0.25/0.25	IE*	1/1
Itraconazole	1/2	1/2	1/2	IE*	1/2
Posaconazole	IE*	<u>0.125</u> /0.25**	IE*	IE*	Note
Voriconazole	Note	1/2	Note	Note	Note

- * MICs are higher than for *A. fumigatus*, there is Insufficient Evidence this species is a good target for the compound in question
- ** provided sufficient levels can be achieved
- Note: the MICs are similar to *A. fumigatus* but insufficient clinical data

Arendrup CMI 2012; Hope CMI 2013; www.eucast.org

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EUCAST *Aspergillus* BPs 2012



⚡ Clinical breakpoints

- BPs indicated as: $S \leq x / R > y$

AF compound	<i>flavus</i>	<i>fumigatus</i>
	Amphotericin	IE*
Isavuconazole	IE	<u>1</u> /1
Itraconazole	1/2	1/2
Posaconazole	IE*	<u>0.125</u> /0.25**
Voriconazole	Note	1/2

- * MICs are higher than for *A. fumigatus*, there is Insufficient Evidence this species is a good target for the compound in question
- ** provided sufficient levels can be achieved
- Note: the MICs are similar to *A. fumigatus* but insufficient clinical data

Isavuconazole & posaconazole
“S” breakpoint is 1 dilution
below ECOFF!



Some wild type will be mis-
classified as I/R



Recommendation:

- repeat testing including itraconazole & voriconazole;
- consider referral to ref lab & *CYP51A* sequencing if still “non-S” to one of these azoles

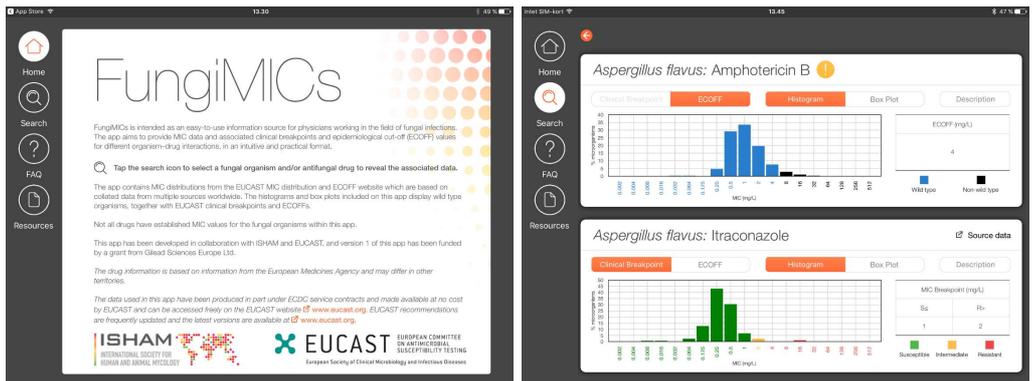
Arendrup CMI 2012; Hope CMI 2013; www.eucast.org

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FungiMICs

❖ A EUCAST – ISHAM joint effort

- App for tablets and smart phones
- Translating an MIC → S, I, R og wild type non-wild type



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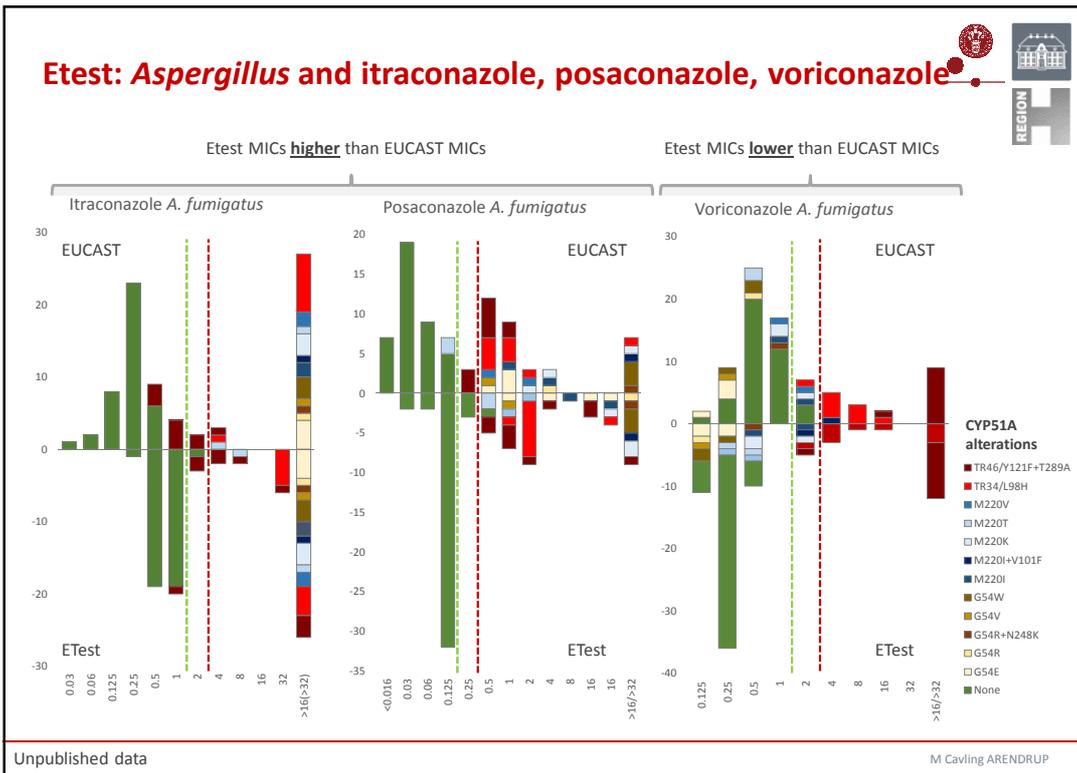
Etest: Aspergillus azoles

❖ Upcoming EFISG/ESCMID guidelines OBS DRAFT – may change!!!

- Recommendation regarding clinically relevant *Aspergillus*

Intervention	SoR	QoE	Comment
EUCAST ref. method + BP	A	II	Applicable to all <i>Aspergillus</i> spp.
CLSI ref. method + ECVs	B	II	No clinical BPs
Etest	C	III	Low to moderate / divergent correlation with CLSI in different studies No very major errors, but major errors in general when evaluated in mycologically experienced labs. Experience and validation in house required. Confirmation by reference test recommended

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Liophilchem strip: *Aspergillus* and isavuconazole

• Day 2 reading preferred

- Easier to read
- Lower inter-reader variation

Day 1

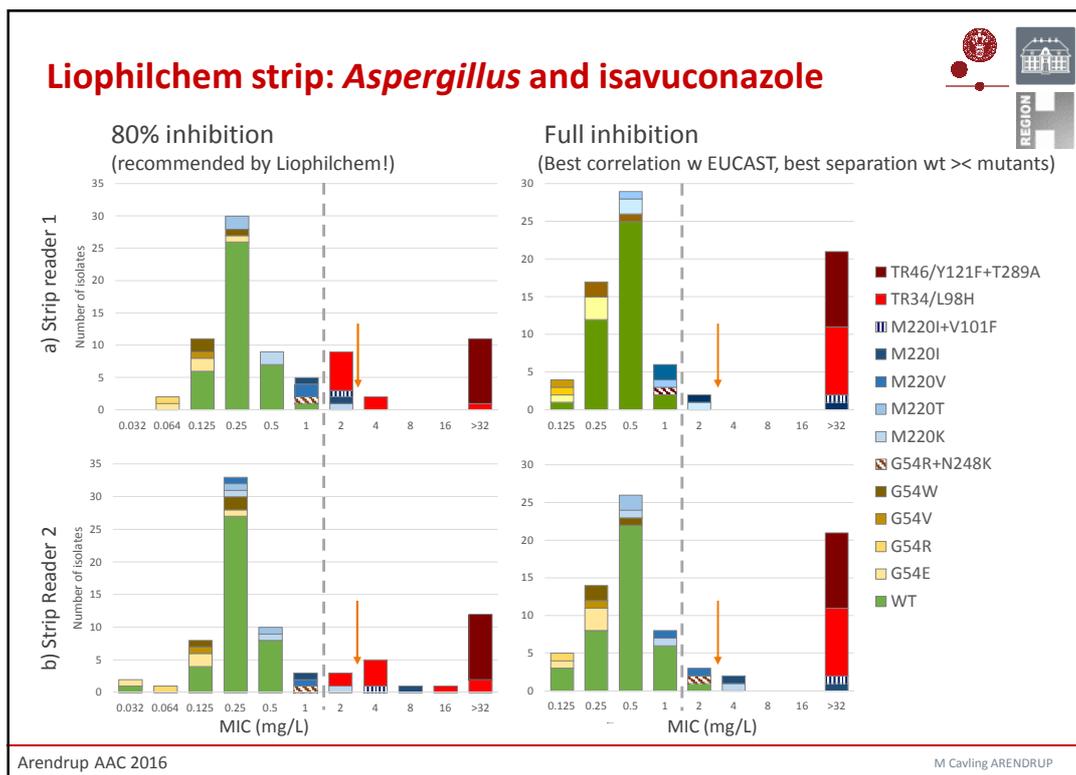
Fuzzy zones and non confluent growth

Day 2

80% endpoint: 0.19 mg/L 0.38 mg/L

Full inhibition endpoint: 0.25 mg/L 0.5 mg/L

Arendrup AAC 2016
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Etest: *Aspergillus* azoles

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- Recommendation regarding clinically relevant *Aspergillus*

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Intervention	SoR	QoE	Comment
Screening test followed by MIC test	A	III	MIC testing as soon as the strain is isolated and without waiting for species ID In geographical areas where azole resistance has been found
MIC testing of multiple colonies (up to 5)	B	III	Multiple genotypes, i.e. azole-susceptible and azole-resistant, may be present

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Azole resistance Screening Agar

```

graph TD
    A[Touch the colonies with a moist swab] --> B[Inoculate w 25 µl MacFarland 0.5-2]
    B --> C[Incubate 37°C for 2 days]
    C --> D[Read if sufficient growth in the control well]
    E[Touch the surface of the agar gently] -.-> A
    E -.-> C
  
```

Susceptible WT

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Azole resistance Screening Agar

⚙️ **Notes**

- 2(+) days of incubation important
- Designed for *A. fumigatus*
- Heavy inoculum → growth on azole well despite no resistance
- Meant for screening only → at least 10% S strains will grow!
- Check correct agar height- evaporation → low sensitivity

Day 1

Day 2

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Multicentre evaluation azole screening agars



- ❖ 3 laboratories, 2 observers/lab; 2 brands of plates
- ❖ *A. fumigatus* blinded strain collection
 - 40 *CYP51A* wild-type clinical *A. fumigatus* isolates
 - 39 azole resistant *Cyp51A* mutant
 - G54 (10): G54E (4), G54R (1), G54R+N248K, (1), G54V (1), G54W (3)
 - M220 (10): M220I (2), M220I+V101F (1), M220K (3), M220T (2), M220V (2)
 - TR₃₄/L98H (9)
 - TR₄₆/Y121F/T289A (10)
- ❖ Test the “Pick 5 colonies” recommendation
 - Touch 4 wt colonies and 1 R colony for inoculum prep
 - Wt+G54 & wt + TR₃₄/L98H

Meletiadias ECCMID 2017 poster P1750, ms and technical note in preparation

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Multicentre evaluation azole screening agars



- ❖ Sensitivity & specificity based on presence of *CYP51A* mutations & growth in >1 azole containing agar well

Centre	VIPcheck Plates Observer 1		In house Plates Observer 1		VIPcheck Plates Observer 2		In house Plates Observer 2	
	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.
ITC/VRC/PSC								
Overall	99%	99%	99%	98%	99%	100%	99%	98%
ITC/VRC								
Overall	99%	99%	99%	98%	99%	100%	98%	98%
ITC/PSC								
Overall	88%	99%	88%	98%	87%	100%	89%	98%
VRC/PSC								
Overall	85%	99%	85%	100%	89%	100%	88%	100%

Meletiadias ECCMID 2017 poster P1750, ms and technical note in preparation

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Multicentre evaluation azole screening agars



- Sensitivity & specificity based on presence of *CYP51A* mutations & growth in >1 azole containing agar well

Centre	VIPcheck Plates		In house Plates		VIPcheck Plates		In house Plates	
	Observer 1		Observer 1		Observer 2		Observer 2	
	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.
ITC/VRC/PSC	<ul style="list-style-type: none"> ➤ Excellent overall performance for detection of most common mutant <i>A. fumigatus</i> ➤ 4-well Plate (ITC/VRC/PSC/Drug free) sensitivity 99% specificity 98-100% ➤ Similar performance without PSC 							
Overall	85%	99%	85%	100%	89%	100%	88%	100%
ITC								
ITC/VRC								
Overall								
VRC/PSC								
Overall								

Meletiadias ECCMID 2017 poster P1750, ms and technical note in preparation

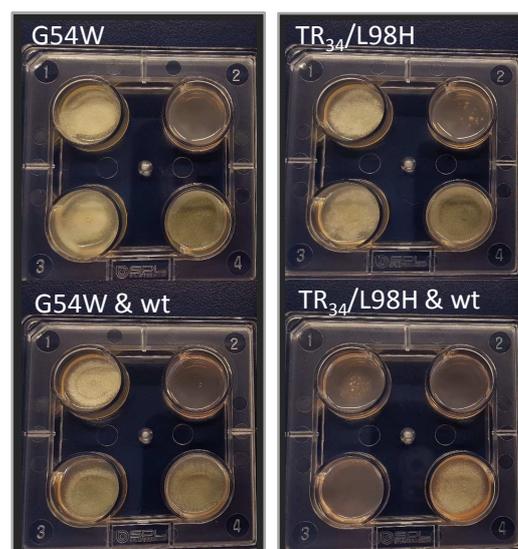
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Multicentre evaluation azole screening agars



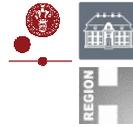
“Pick 5 colonies” test

- Either
 - 1 swab (2 laboratories)
 - 4 touches in wt colony
 - 1 touch in mutant colony
 - 5 swabs (1 laboratories)
 - 4 touches in wt colony
 - 1 touch in mutant colony
- Prepare inoculum McFarland 0.5
- 25 µl per well
- 48 h



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Multicentre evaluation azole screening agars



Test of the "Pick 5 colonies" strategy (4 wt and 1 R)

	TR ₃₄ /L98H + wt				TR34/L98H				G54W + wt				G54W			
	ITC	VRC	PSC	Growth control	ITC	VRC	PSC	Growth control	ITC	VRC	PSC	Growth control	ITC	VRC	PSC	Growth control
Overall pos rate																
Lab 1: wt → R	83%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Lab 2: wt → R	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Lab 3: R → wt	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Overall pos rate ITC+VRC																
Lab 1: wt → R	83%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Lab 2: wt → R	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Lab 3: R → wt	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

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Multicentre evaluation azole screening agars



Test of the "Pick 5 colonies" strategy (4 wt and 1 R)

	TR ₃₄ /L98H + wt				G54W + wt				G54W			
	ITC	VRC	PSC	Growth control	ITC	VRC	PSC	Growth control	ITC	VRC	PSC	Growth control
Overall												
Lab 1: wt → R	83%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Lab 2: wt → R	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Lab 3: R → wt	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

➤ Weaker growth when wt and mutant colonies are mixed → even the weakest growth should be taken as positive
 ➤ 4-well Plate (ITC/VRC/PSC/Drug free) sensitivity 83-100% specificity 100%
 ➤ Still much better than just picking 1 colony for AFST

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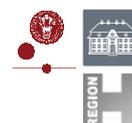
Conclusion Epidemiology and AFST



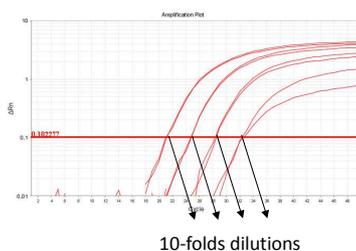
- ⚡ ↑↑↑ Azole resistance in *Aspergillus*
- ⚡ Epidemiology varies
 - DK rate 3.8% incidence rate (incident cases only)
 - ICU in NL: 26% culture pos patients treated for w suspected aspergillosis
- ⚡ Epidemiological surveillance important
 - Probably only the tip of the iceberg is diagnosed
- ⚡ Susceptibility testing clinically relevant *Aspergillus*
 - Recommended by ESCMID/EFISG
 - Easy to implement screening test available
 - Resistance should be confirmed at ref labs

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Strategies for GM antigen positive culture negative cases?



- ⚡ Optimal culture conditions to minimise false negativity
 - Sufficient volume
 - Include Sabouraud or similar selective agar
 - Use three dot inoculation to help discrimination of contamination
 - Incubate at 30 °C for 5-7 days
- ⚡ Consider specific Asp PCR including resistance markers
 - In house and AsperGenius kit available – targets:
 - *Aspergillus*
 - *A. fumigatus*
 - TR₃₄/L98H
 - TR₄₆/Y121F/T289A



Spiked BAL samples
Limit of detection

10 – 30 CFU/mL or 1-3 copies/μL

100% analytic specificity

20 *Aspergillus* spp
18 non-*Aspergillus* spp

The list is continuously expanding

Fraczek Mycoses 2014, Jensen ECCMID P0968 2017

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Acknowledgements EUCAST AFST



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 J Mouton (EUCAST-SC)
 F Barchiesi (NAC, Italy)
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2016-17

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**Thank you for
 your attention**