RUTGERS
New Jersey Medical School

PHRI

Direct detection of resistance: candins vs. azoles

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TIMM
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Disclosures

Grant support and opinion leader panels for Merck, Pfizer, Astellas

Patent

- Assays for Resistance to Echinocandin-Class Drugs David S. Perlin et al
- Application number: 11/995,966
- Publication number:US 2010/0075302 A1
  Filing date: Jul 26, 2006

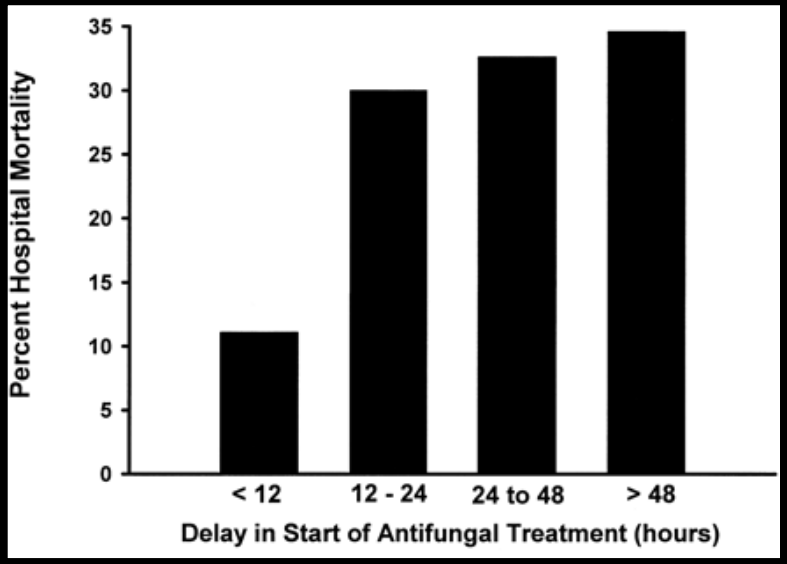
Why should molecular testing for drug resistance be considered?

- ✓ Rapidly assess ID with simulataneous assessment of primary and seconday resistance
  - Susceptibility testing is not timely: 48 h after initial identification (1-3 days). MDx takes hours or less
- ✓Multiple specimen types (blood, BAL, sputum, tissue, urine)
- ✓ Phenotypes may be complex (trailing edges, Eagle)
- ✓ Breakpoints may be low.. posing challenges for less experienced clinical labs
- ✓ Known resistance targets are easy to detect eliminating subjective lab-to-lab variation in some culture-based AFST
- ✓ Elevated MIC does not always translate to therapeutic failure

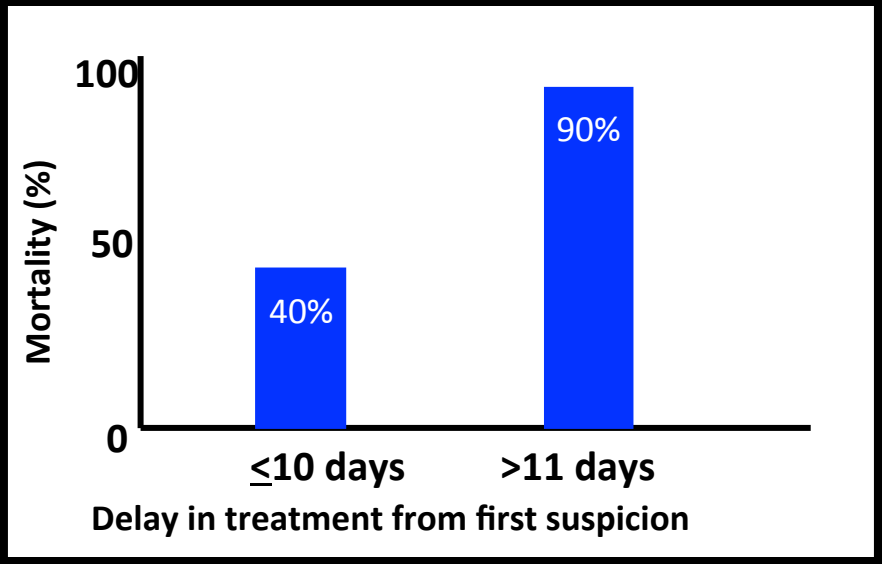
Advantages of Molecular Diagnostics for Clinical Assessment of Antifungal Drug Resistance, cont..

- ✓ Provides class and drug-specific resistance
- ✓ Useful for surveillance and epidemiology- clonal spread
- ✓ Specific mutations may provide information about drug-specific resistance and shifts
- ✓ Use to detect potential resistance in culture-Neg organisms
- ✓ Resistant infections diagnosed post-mortem

Promise: Rapid molecular detection promotes early and **appropriate** therapy that can reduce mortality.



Morell et al. AAC. 2005 49: 3640



Von Eiff et al, Respiration 1995;62:241

Relationship between hospital mortality and the timing of antifungal treatment.

A comprehensive clinical molecular drug resistance detection platform should provide….

- Genus and species (18S; 28S; ITS)
- 1^{o} resistant species (e.g. *C. glabrata, C. krusei, A. terreus*)
- 2^{o} acquired resistance mechanisms in otherwise susceptible organisms (e.g. Mutations in target site, TF)
- Internal amplification control (Extraction, Quantification)

What platform should be used?

- ✓Single v. multiple targets
- ✓Wt v. mutant targets (individual v. pooled)
- ✓Rapid assay turnaround time
- ✓Reliability and Accuracy (>95% sensitivity; specificity)

Amplification: PCR; digital PCR; LATE PCR; NASBA, other RNA or isothermal amplification

Detection: Real time detection: molecular beacons, TaqMan, Scorpion, HRMA, Mass Tag, microarray, gene chip, beads, HTSeq, Raman, luminex

Gold standard: DNA sequencing

All fungi produce a wide array of adaptive responses that induce *in vitro* elevated MICs but only those mechanisms conferring the potential for clinical failure should be profiled…..

Clinically-relevant validation is critical for the application of molecular technology.

Criteria for validated nucleic acid-based molecular resistance testing

Altered genetic sequence confers:

- Elevated MICs in standard testing
- Altered drug-target interaction (K_d; K_i)
- Resistance in animal models (PK/PD)
- Clinical failure

Molecular diagnostics are ideal for:

Echinocandin resistance in *Candida albicans* and *Candida glabrata*

Triazole resistance in *Aspergillus fumgatus*

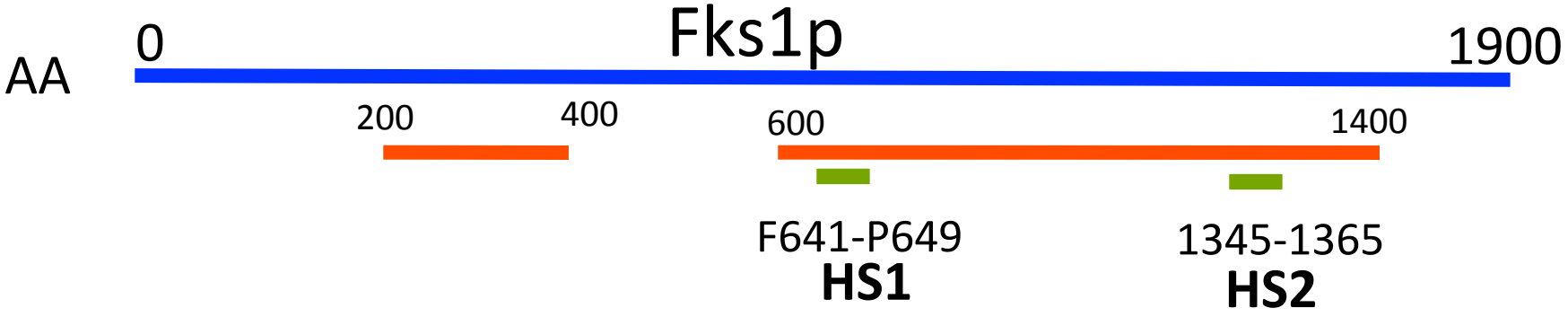
Echinocandin resistance is on the rise!

Table 1. Temporal trends in antifungal resistance of 313 isolates of *C. glabrata* from Duke Medical Center (submitted)

Year	No. Tested	Antifungal Agent	MIC (µg/ml)		% by category[b]
			Range	Mode	R
2001-02	61	Fluconazole	1->128	8	**18.0**
		Anidulafungin	0.0079-4	0.06	**4.9**
		Caspofungin	0.03->8	0.06	**3.3**
		Micafungin	0.015->8	0.03	**1.6**
2003-04	60	Fluconazole	1->128	8	20.0
		Anidulafungin	0.0079-4	0.06	**3.3**
		Caspofungin	0.03->8	0.06	**3.3**
		Micafungin	0.015->8	0.03	**3.3**
2005-06	55	Fluconazole	1->128	8	29.1
		Anidulafungin	0.0079-4	0.06	**1.8**
		Caspofungin	0.03->8	0.06	**1.8**
		Micafungin	0.015->8	0.03	**1.8**
2007-08	64	Fluconazole	1->128	8	26.2
		Anidulafungin	0.0079-4	0.06	**9.2**
		Caspofungin	0.03->8	0.06	**10.8**
		Micafungin	0.015->8	0.03	**9.2**
2009-10	73	Fluconazole	1->128	8	**30.1**
		Anidulafungin	0.0079-4	0.06	**12.3**
		Caspofungin	0.03->8	0.06	**13.7**
		Micafungin	0.015->8	0.03	**12.3**

Alexander, et al. CID 2013

Clinical Echinocandin Resistance in *Candida* spp: *FKS* Mechanism



Two regions (HS1 and HS2) of Fks1/2 are associated with resistance in *Candida*

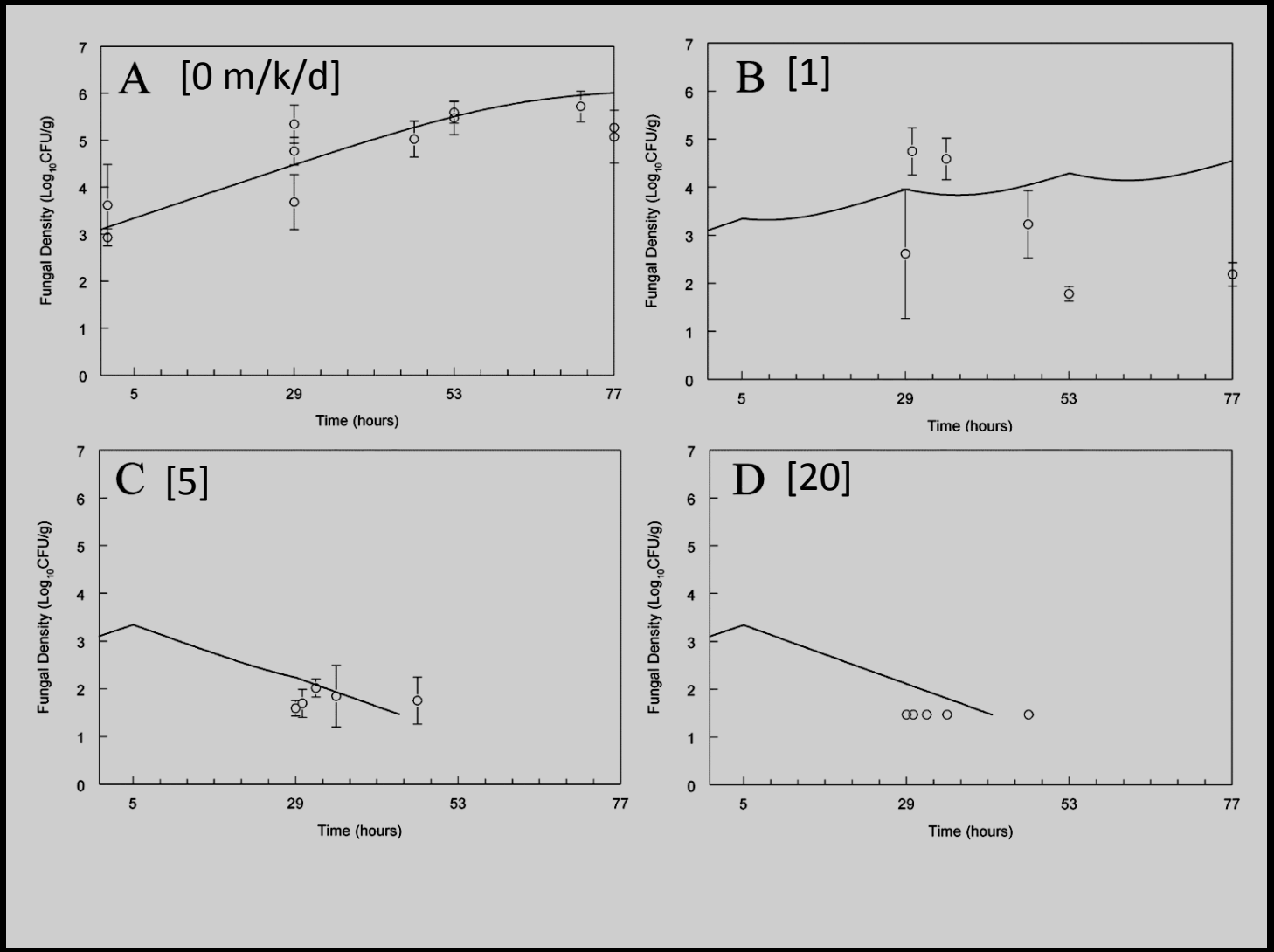
Prominent mutations confer cross-resistance to all echinocandin class drugs.

Association between *fks1* mutations, glucan synthase IC50 , ED99 values and clinical outcome

Patient	Isolate*	*Fks1* Change	MIC (μg/ml)	Glucan Synthesis IC_{50} (ng/ml)	Mouse Model (Burden) ED_{99} (mg/kg/day)
A	*C. albicans*/16998	None	0.5	0.56	< 0.06
A	*C. albicans*/18195	None	0.25	0.91	0.01
A	*C. albicans*16996	S645F	> 8	162	1.09
A	*C. albicans*/16997	S645P	> 8	1997	9.98

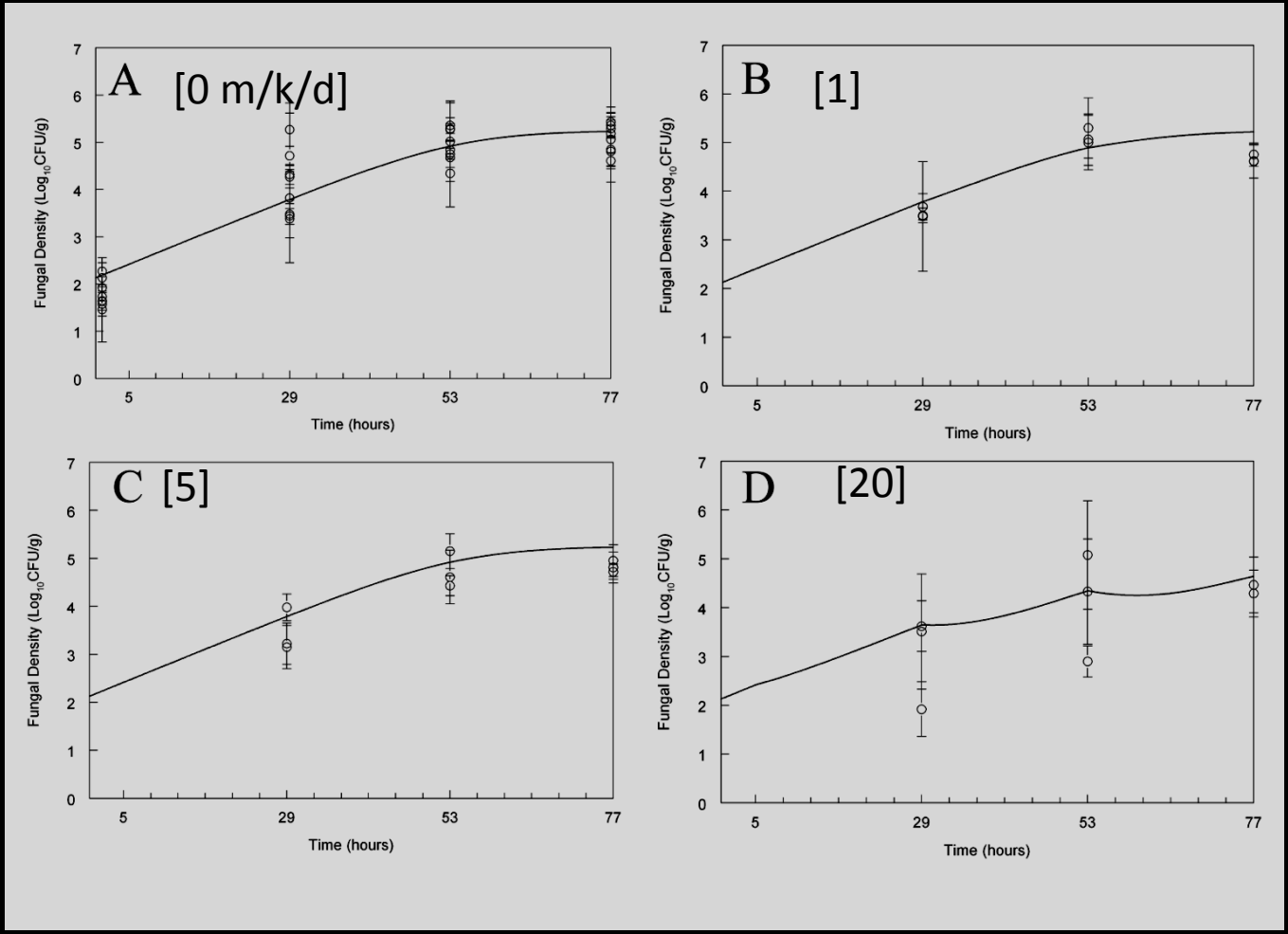
Park et al. 2005 AAC

PD study of Micafungin with wild type *C. albicans*



Slater et al. 2011 AAC

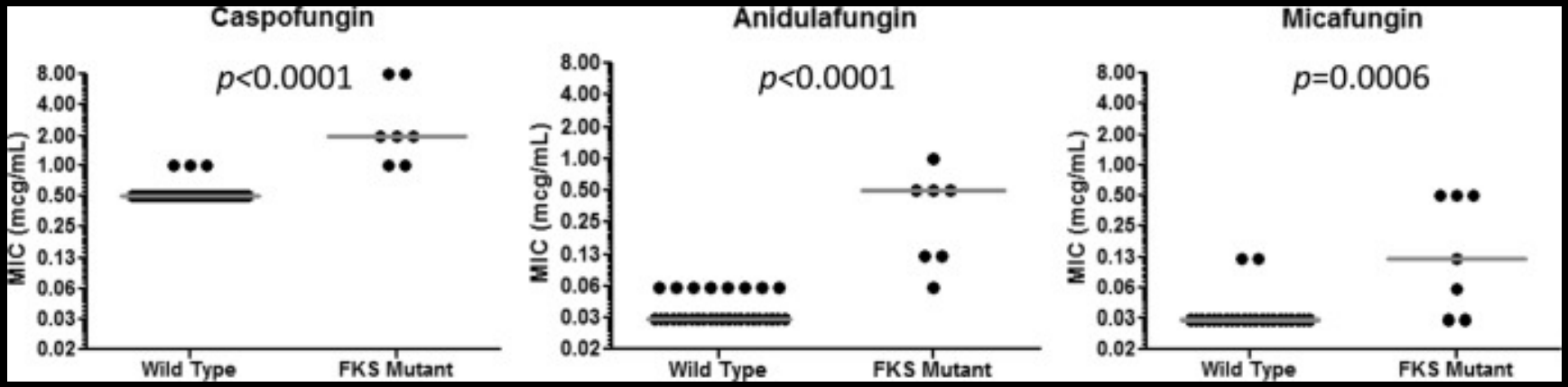
PD study of Micafungin with *fks*-S645F *C. albicans* mutant

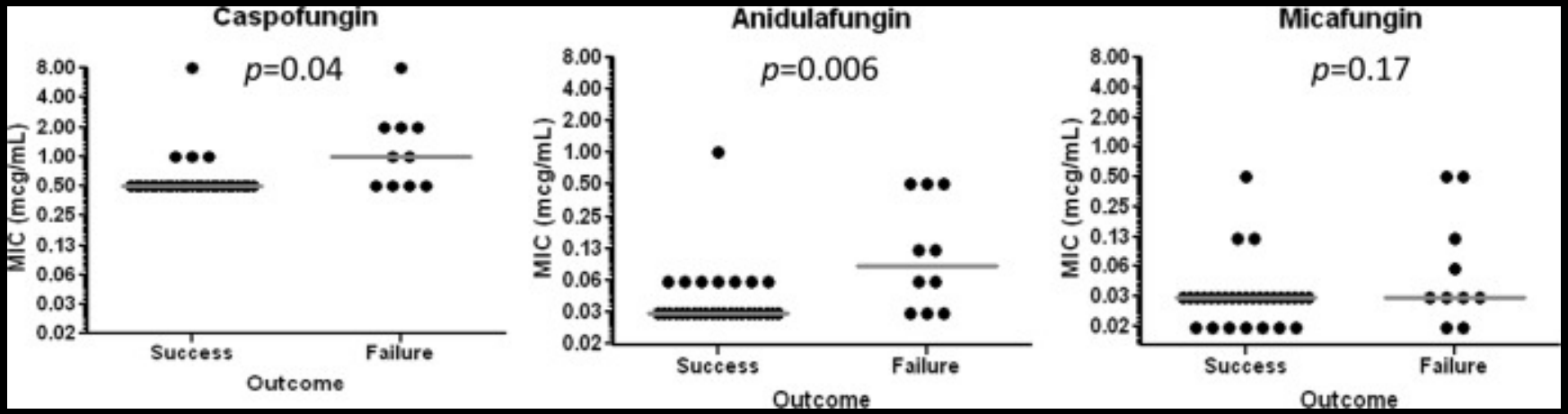


Untreatable!!

Slater et al. 2011. AAC

The presence of *FKS* mutation rather than MIC is an independent risk factor for failure of echinocandin therapy among patients with invasive candidiasis due to *Candida glabrata*.





Shields et al. AAC 2012

Amino acid substitutions within the Fks proteins from *Candida spp.* isolates resulting in clinical failures

Species	GenBank accession no.	Fks protein(s) (amino acid substitution[s])
C. albicans	XM_716336	**Fks1p (F641S),[c] Fks1p (S645Y), Fks1p (S645F), Fks1p (S645P),[b] Fks1p (S645F and R1361R/H), Fks1p (D648Y), Fks1p (P649H)**
C. dubliniensis	GQ342611	**Fks1p (S645P)**
C. glabrata	XM_446406 and XM_448401	**Fks1p (F625S), Fks1p (S629P), Fks1p (D632G), Fks2p (F659V), Fks2p (F659S),[c] Fks2p (S663P), Fks2p (S663F), Fks2p (D666G), Fks2p (D666E), Fks2p (P667T)**
C. krusei	EF426563	**Fks1p (R1361G), Fks1p (F655F/C), Fks1p (L658W and L701M), Fks1p (D700M),[d] Fks1p (L701M)[d]**
C. tropicalis	EU676168	**Fks1p (F76S), Fks1p (S80P),[b] Fks1p (V213I and V265I)[d]**

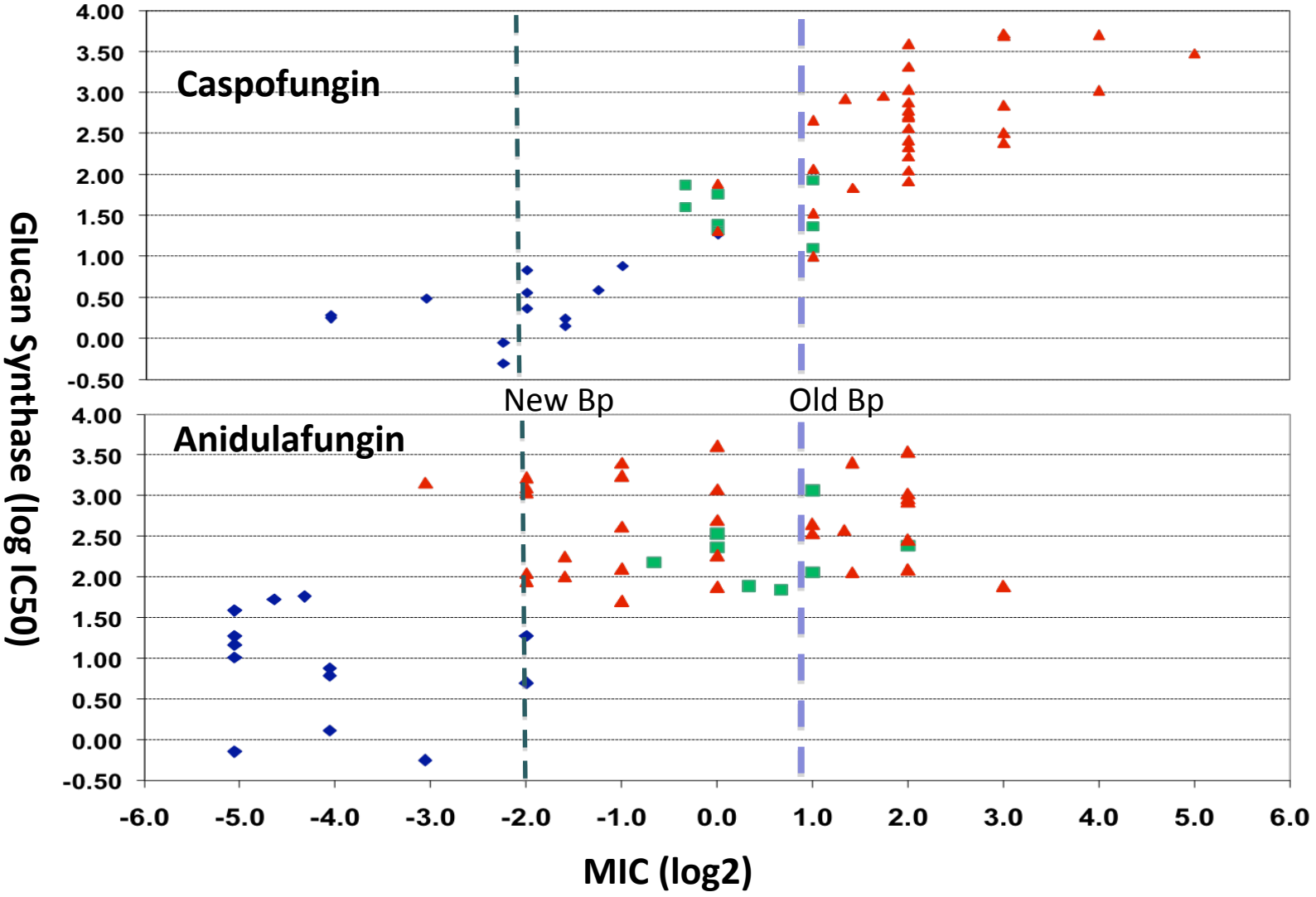
Not all elevated MICs are the same

MIC	FKS Genoptype	Glucan Synthase	*P* successful Clinical Outcome
Low	WT	Sensitive	Good
High	WT	Sensitive	Good
High	mutant	weakly resistant	Good
High	mutant	moderately resistant	Mixed
High	mutant	strongly resistant	Poor

S645, F641 > L642, T643, L644, A645, L646, R647, D648 > P649

>70% failures

Initial CLSI breakpoint MIC >2 mg/l incompletely covered strains with fks mutations



Garcia-Effron et al. 2009

Revised MIC breakpoint based on data from PK/PD, kinetic studies, and MIC/ECV testing.

MIC (μg/ml)
Candida spp.

	S	I	R
CFG, AFG, MFG	**<0.25,**	**0.5,**	**>1**
MFG (*C. glabrata*)	**<0.16,**	**0.25,**	**>0.5**

Captures most *fks* mutants

Pfaller et al. 2011 MDR

How do we ensure that isolates containing *fks* mutations conferring resistance are captured by MIC?

- What methodology: EUCAST or CLSI?
- Medium supplments: serum?
- What drugs is the best surrogate? Anidulafungin, micafungin
- What about variability with caspofungin AFST?

Detection of of high MIC isolates with *fks* mutations can be complicated

Antimicrob Agents Chemother. 2010 54(1):426-39.

Echinocandin susceptibility testing of Candida species: comparison of EUCAST EDef 7.1, CLSI M27-A3, Etest, disk diffusion, and agar dilution methods with RPMI and isosensitest media.

Arendrup MC, Garcia-Effron G, Lass-Flörl C, Lopez AG, Rodriguez-Tudela JL, Cuenca-Estrella M, **Perlin DS**.

Abstract

This study **compared 9 susceptibility testing methods and 12 endpoints for anidulafungin, caspofungin, and micafungin** with the same collection of blinded **FKS hot spot mutant (n = 29) and wild-type isolates (n = 94)**. .....

. **The methods with the lowest number of errors (given as VMEs/MEs) across the three echinocandins were CLSI (12%/1%), agar dilution with RPMI-2G medium (14%/0%), and Etest with RPMI-2G medium (8%/3%). The fewest errors overall were observed for anidulafungin (4%/1% for EUCAST, 4%/3% for CLSI, and 3%/9% for Etest with RPMI-2G)**..... F

Caspofungin and variability

EUCAST MIC_{50} is highlighted in colour

	No. of *C. albicans* isolates at the individual EUCAST MICs (mg/L)												No. Tested
	0.002	0.004	0.007	0.015	0.03	0.06	0.12	0.25	0.5	1	2	≥ 4	
CFG													
Lab 1	ND	ND	ND	20	30	**35**	45						130
Lab 2	ND	ND	ND	ND	ND	123	162	**209**	102	9		1	606
Lab 3	ND	ND	ND	ND	ND		10	161	**219**	8	5	1	404
Lab 4	ND	ND	ND	2	105	**264**	182	54	11	3	1	4	626
Lab 5	ND	ND	6	12	**12**	5	3						38
Lab 6	ND	ND	ND	ND	**25**	5	1						31
Lab 7	ND	ND	ND	ND	1	2	14	330	**373**		1		721
MFG													
Lab 1	ND	ND	ND	ND	107								107
Lab 2	ND	**121**	2										123
Lab 3	ND	34	**19**	12	30	4	1						100
Lab 4	ND	**78**	19	2		1							100
Lab 5	ND	ND	ND	**520**	35	2	1			2			560
Lab 6	4	87	**252**	239	4	4							590
Lab 7	ND	ND	**87**	2									89

Unacceptable variation for caspofungin

→EUCAST abstained from setting Breakpoints

Espinel-Ingroff AAC in press, www.EUCAST.org

Courtesy M. Arendrup

Antimicrob Agents Chemother. 2013 Sep 9

Interlaboratory variability of caspofungin MICs for Candida spp. using CLSI and EUCAST methods: Should the clinical laboratory be testing this agent?

Espinel-Ingroff A, Arendrup MC, Pfaller MA et al.

Abstract

Although CLSI clinical breakpoints (CBPs) are available for interpreting echinocandin MICs for Candida spp., epidemiologic cutoff values (ECVs) based on collective MIC data from multiple laboratories have not been defined. While collating CLSI caspofungin MICs for 145 to 11,550 Candida isolates from 17 laboratories (Brazil, Canada, Europe, Mexico, Peru and the United States), we observed an extraordinary amount of modal variability (wide ranges) among laboratories as well as truncated and bimodal MIC distributions. The species-specific modes across different laboratories ranged from: 0.016-0.5 µg/ml for C. albicans and C. tropicalis; 0.031-0.5 µg/ml for C. glabrata; 0.063-1 µg/ml for C. krusei; variability was also similar among C. dubliniensis and C. lusitaniae. The exceptions were C. parapsilosis and C. guilliermondii MIC distributions, where most modes were within one twofold dilution of each other. These findings were consistent with available EUCAST data (403 to 2,556 MICs) for C. albicans, C. glabrata, C. krusei, and C. tropicalis. Although many factors (caspofungin powder source, stock solution solvent, powder storage time length and temperature, and MIC determination testing parameters) were examined as a potential cause of such unprecedented variability, a single specific cause was not identified.

Therefore, it seems highly likely that the use of the CLSI species-specific caspofungin CBPs could lead to reporting an excessive number of wild-type [WT] (e.g., C. glabrata and C. krusei) as either non-WT or resistant isolates.

Until this problem is resolved, routine testing or reporting of CLSI caspofungin MICs for Candida is not recommended; micafungin or anidulafungin data could be used instead.

Eliminate biological variability

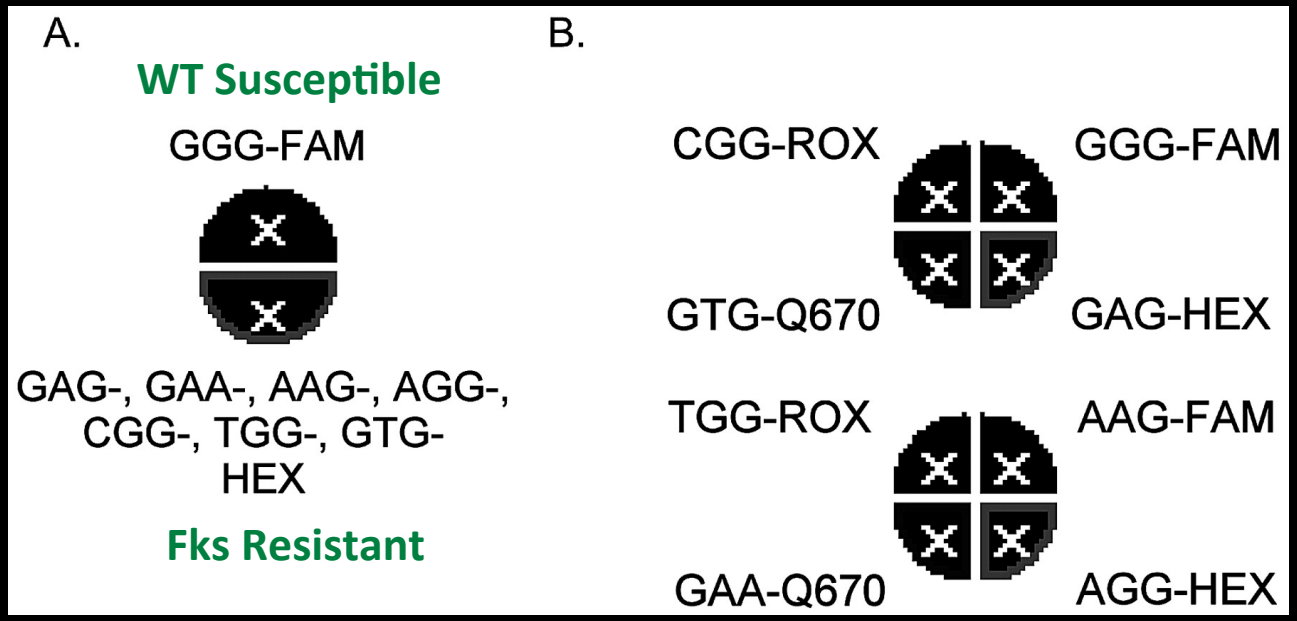
Molecular detection of *FKS*-mediated echinocandin resistance overcomes confounding issues of AFST variability.

It can be applied in any clinical lab with molecular capability

AAC 2006; 50:2058-63.

Assessing resistance to the echinocandin antifungal drug caspofungin in *Candida albicans* by profiling mutations in *FKS1*.

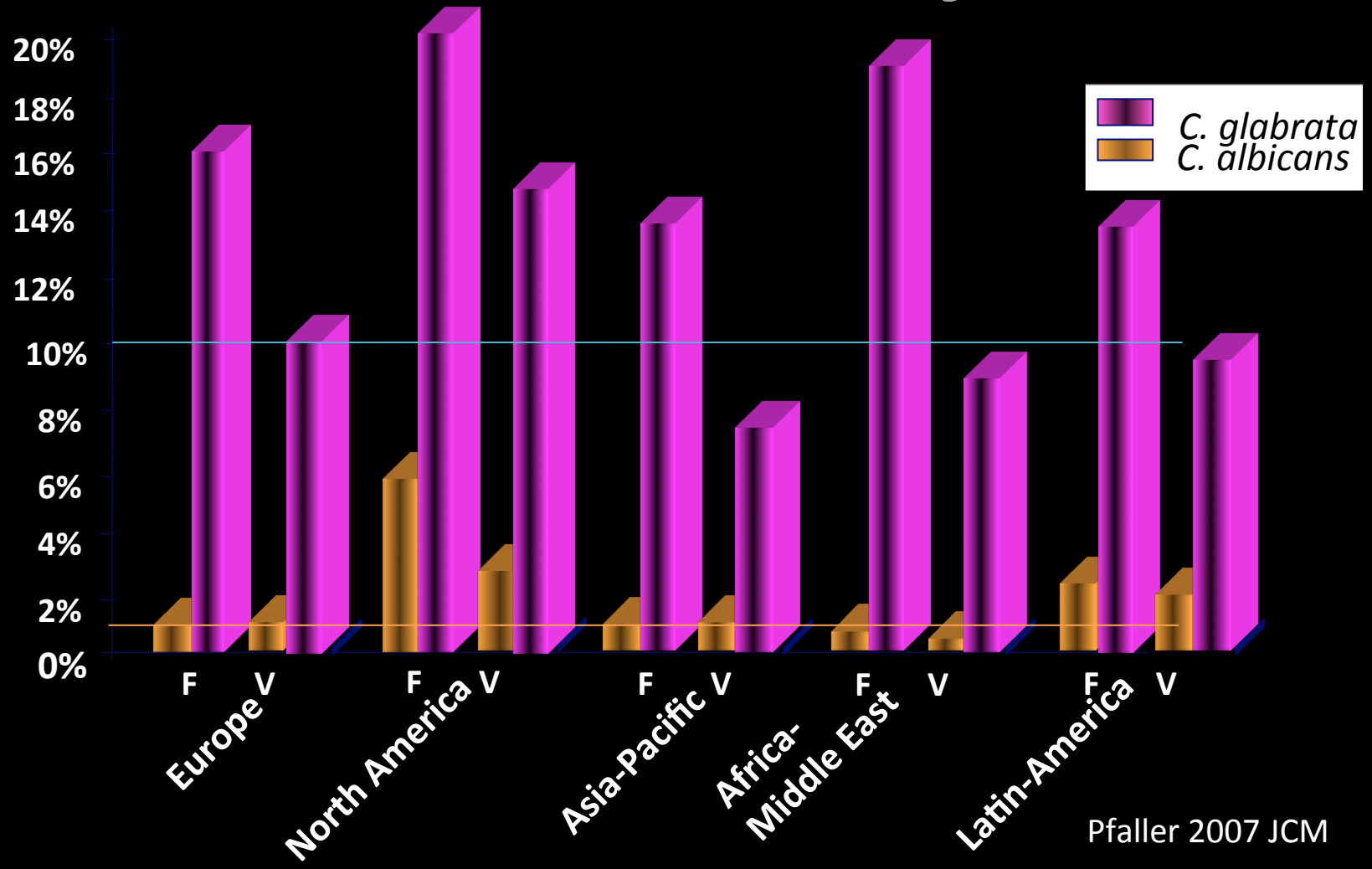
Balashov SV, Park S, Perlin DS.



Single or multiplex detection of resistance targets

Azole Resistance in *Candida spp.*

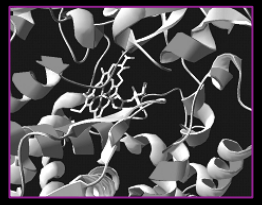
Global variation in resistance to fluconazole and voriconazole for clinical isolates of *C. albicans* and *C. glabrata*



Pfaller 2007 JCM

Mission Impossible: Profile all known azole resistance mechanisms?

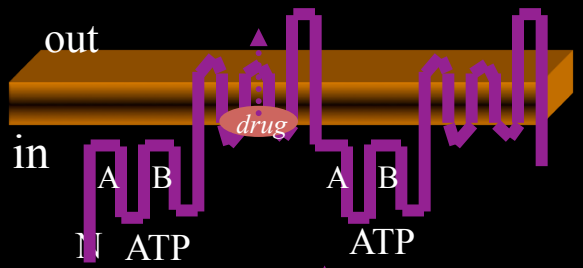
I. Alteration of Drug Target



Erg11 *Candida* spp.
Erg5 Candida spp.
Erg11 *Cr. neoformans*
Cyp51A *Aspergillus* spp.

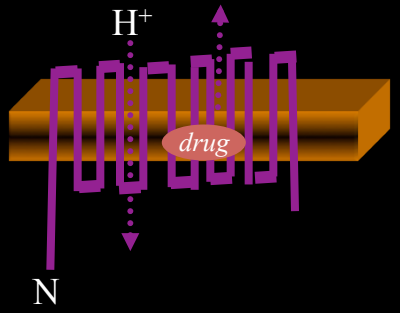
II. Over-expression of Efflux Transporters

ABC



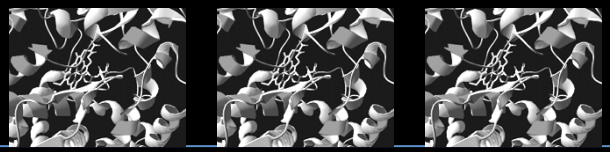
Cdr1, *C. albicans*
Cdr1 *C. glabrata*
Pdh1 *C. glabrata*
Mdr1,2,4 *A. fumigatus*
Afr1 *C. neoformans*

MFS



Mdr1 *C. albicans*
Flu1 *C. albicans*
Mcm1 *C. albicans*
Mdr3 *A. fumigatus*

III. Over-expression of Target Site



Erg11 *Candida spp.*
Cyp51A *A. fumigatus*

IV. Chromosomal duplications

C. albicans

V. Transcription factors

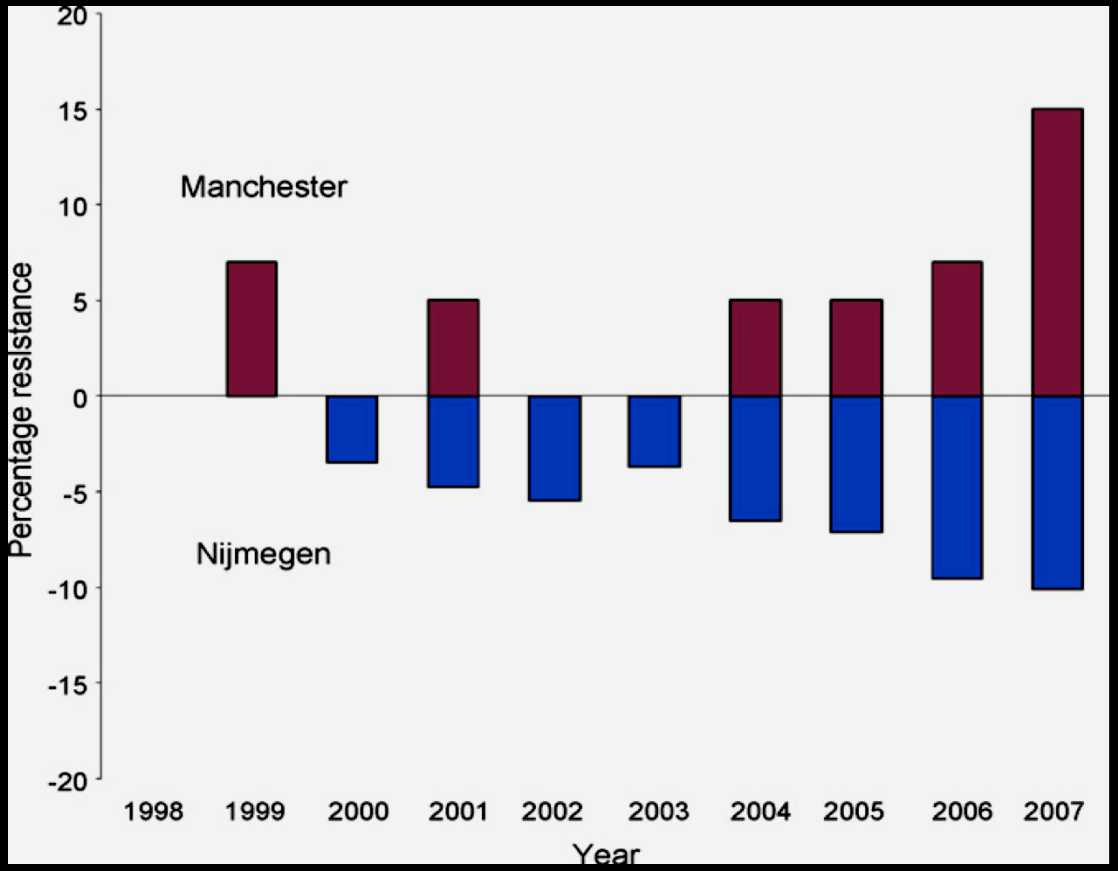
Drug pumps
Tac1, Mrr1, cdr1, cgd
Erg 11
Upc2p C. albicans
Aspergillus?

Molecular profiling of azole resistance in *Candida spp.*

C. albicans (Complex)
Multiple mechansims (Target (Erg11), pumps overEx, TFs) and interpretations can be difficult (e.g. overexpression of target, ABC transporter)

C. glabrata (Feasible)
Pump overEx (profile TF)
Needs validation of TF mutations

Aspergillus fumgiatus: The problem of triazole drug resistance.



Percentage of patients with azole-resistant *A. fumigatus* strains in Manchester, UK, and Nijmegen, the Netherlands (1998–2007)

Is it suitable for molecular profiling?

Verweij 2009 DRU

Amino acid substitutions in the target Cyp51A is the principal mechanism conferring triazole resistance

Cyp51A Locus	Amino acid substitutions	MIC, mg/L ITRA	VORI	POSA
TR34 -L98	H	>8	8	1–2
TR46 -Y121/T289	F/A	>4	>16	1
F46	Y	>8	2–4	0.125–0.5
G54	E, R, V	>8	0.125–1	1–>8
H147§	Y	>8	>8	0.5
M172	V	>8	2–4	0.125–0.5
P216	L	>8	1	1
F219	C	>8	1	1
M220	K, T	>8	1–4	0.5–>8
N248	T	>8	2	0.25
D255	E	>8	2	0.25
E427	G, K	>8	2–4	0.125–0.5
Y431	C	>8	4	1
G434	C	>8	4	1
G448	S	>8	>8	0.5–1

Environmental

Acquired during therapy

Adapted from Howard and Arendrup (111) and Bader et al. (18)

^TR#: Tandem repeat promoter (# bases).

A prominent relationship exists between mutations in *Cyp51A* causing high MIC and probability for breakthrough infections

Multiple-Triazole–Resistant *Aspergillus fumigatus*

Table 1. Characteristics of Nine Patients from Whom *A. fumigatus* Resistant to Multiple Triazoles Was Cultured.

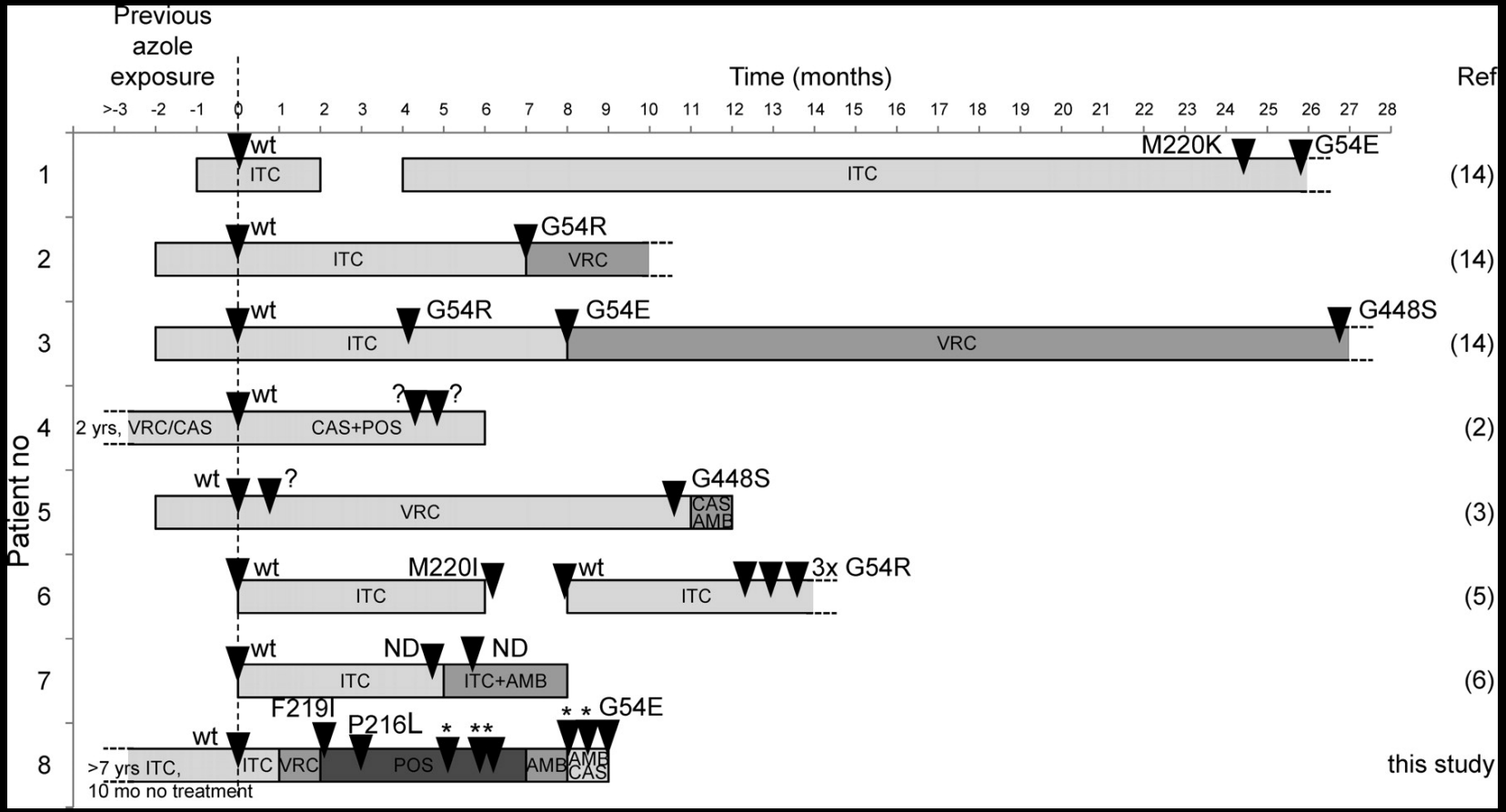
Sex	Yr of Age	Underlying Disease	Date of Isolation	Site of Isolation	Disease Classification*	Previous Azole Exposure	Treatment	Outcome
Male†	15	X-linked chronic granulomatous disease	April 4, 2002	Sputum	Breakthrough invasive pulmonary aspergillosis, proven	Prophylaxis with itraconazole (for 6 yr)	Voriconazole (high-dose)	Survived
Male	73	None	Dec. 3, 2003	Ear swab	Invasive aspergillosis of mastoid cavity, proven	None	Surgery and topical therapy	Survived
Male	16	Hyper-IgE syndrome	Nov. 19, 2004	Bronchoalveolar-lavage fluid	Breakthrough invasive pulmonary aspergillosis, proven	Treatment with voriconazole (for 2 yr)	Surgery and posaconazole	Survived
Female	76	Pulmonary fibrosis	June 26, 2005	Sputum	Invasive pulmonary aspergillosis, possible	None	Voriconazole	Survived
Male	31	Chronic granulomatous disease	Nov. 1, 2005	Lung aspirate	Breakthrough invasive pulmonary aspergillosis, probable	Prophylaxis with itraconazole (for >10 yr)	Caspofungin and posaconazole	Survived
Female	68	Acute myeloid leukemia	Feb. 14, 2006	Bronchoalveolar-lavage fluid	Disseminated invasive aspergillosis, probable	None	Voriconazole	Died
Female	62	Chronic obstructive pulmonary disease	April 5, 2006	Bronchoalveolar-lavage fluid	Invasive pulmonary aspergillosis, possible	None	Voriconazole, amphotericin B, and posaconazole	Survived
Male	19	Chronic granulomatous disease	April 15, 2006	Bone	Breakthrough aspergillus osteomyelitis, proven	Prophylaxis with itraconazole (for >2 yr)	Voriconazole, caspofungin, and posaconazole	Survived
Male	45	Acute myeloid leukemia and allogeneic hematopoietic stem-cell transplantation	May 11, 2006	Nose swab	Breakthrough aspergillus sinusitis, proven	Prophylaxis with itraconazole (for 4 wk)	Posaconazole	Died

* Diseases were classified according to consensus criteria defined by the European Organisation for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group.
† Information about this patient is from Warris et al.[3]

Factors: Inherent resistance dominated by a single mechanism Leu98/TR

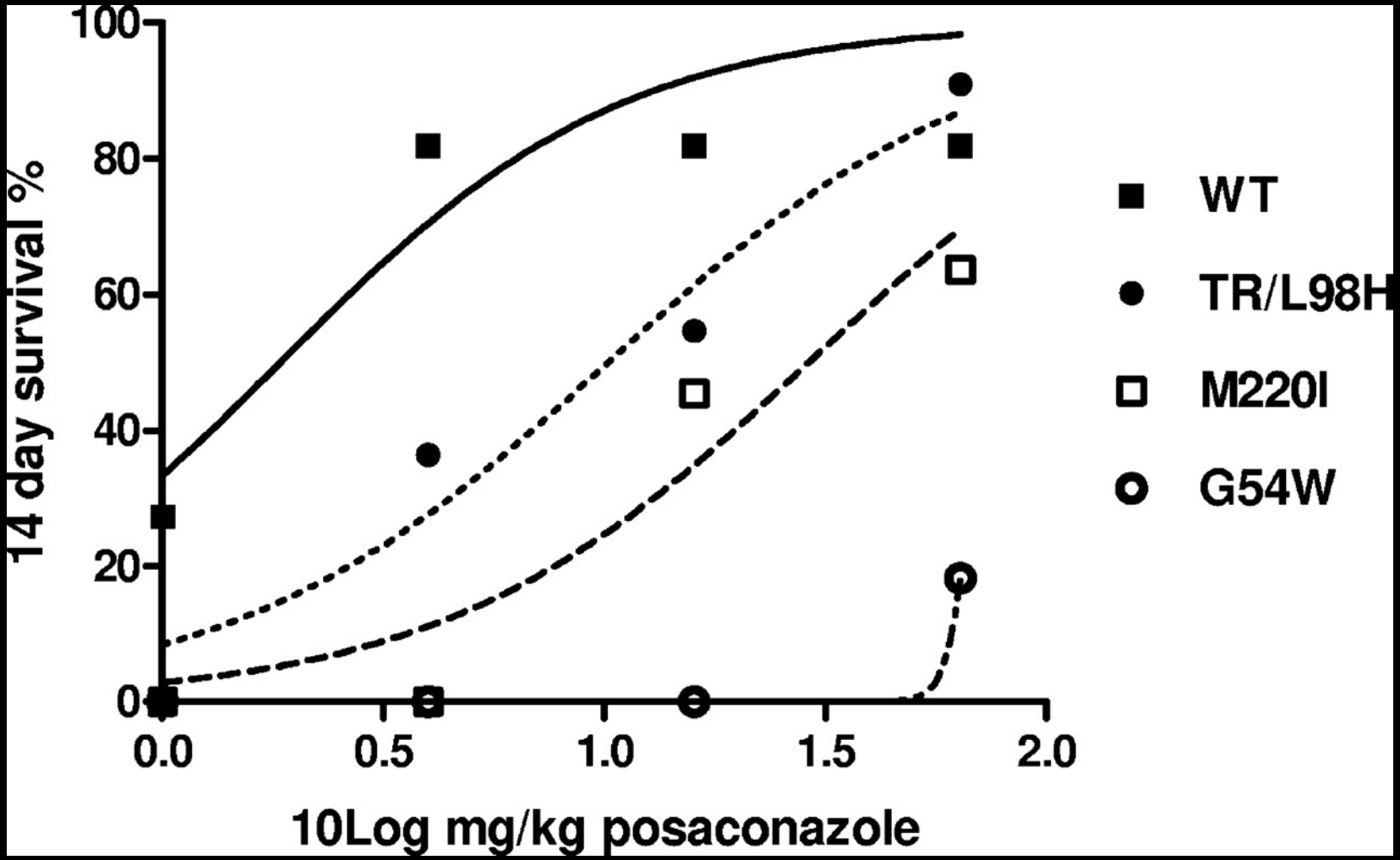
Verweij et al. 2007 NEJM

Reported cases of acquired azole resistance in *A. fumigatus* from breakthrough infections



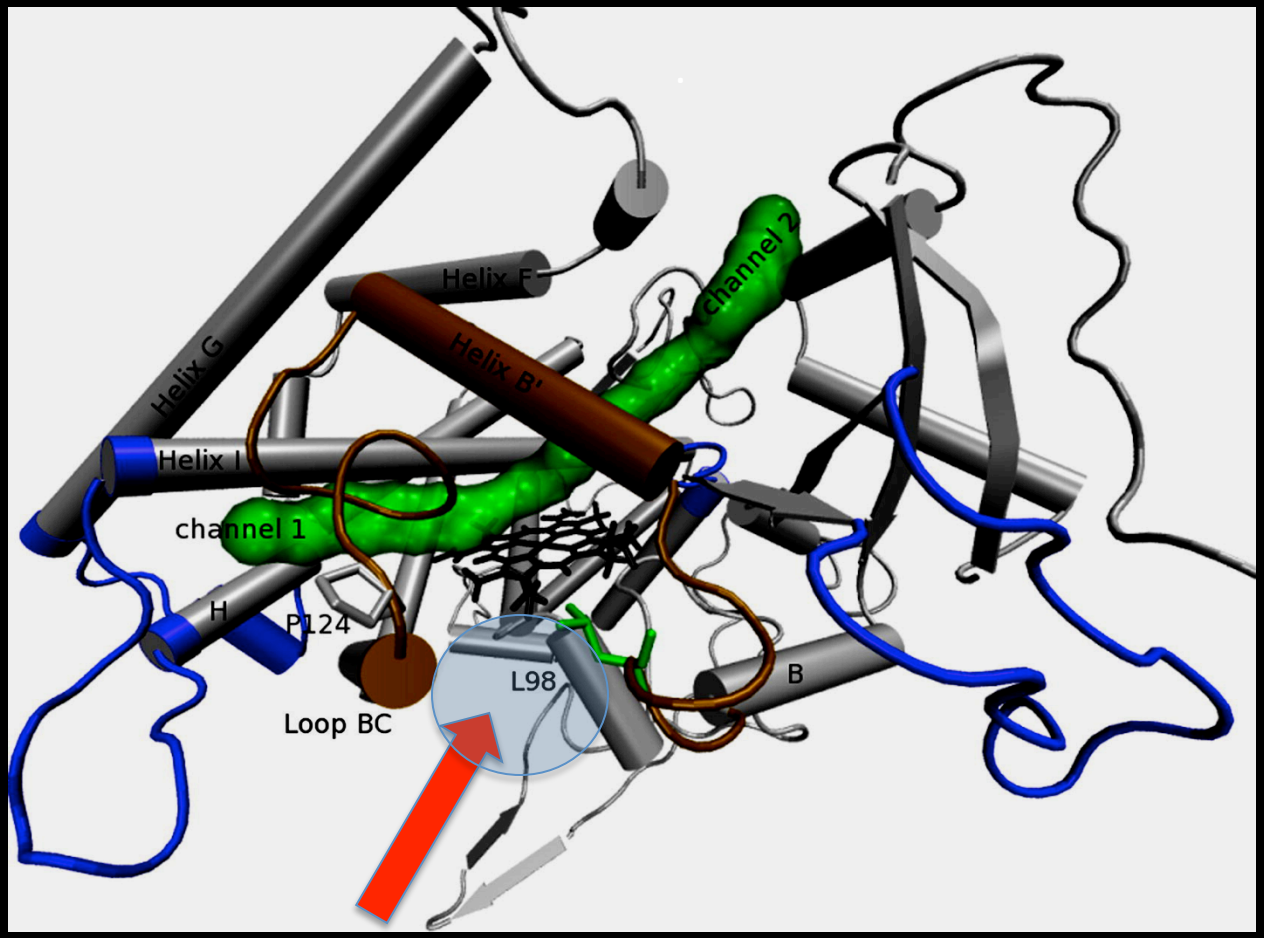
Camps S M T et al. AAC 2012

PD Study: 14-day survival as a function of posaconazole dose for *A. fumigatus* isolates with different Cyp51A genotypes



Mavridou E et al AAC 2010

Model of CYP51A with Leu98 highlighted.



Heme cofactor is in black, the two channels are filled with green

Snelders et al. 2010 AAC

Well characterized *Cyp51A* mutations associated with triazole resistance are ideal for molecular profiling

JCM 2010, 48:1478-1480

Rapid Diagnosis of Azole-Resistant Aspergillosis by Direct PCR Using Tissue Specimens

Jan W. M. van der Linden, Eveline Snelders, Jan P. Arends, Simon M. Daenen, Willem J. G. Melchers, and Paul E. Verweij

ABSTRACT

We report the use of PCR techniques on a formalin-fixed and paraffin-embedded tissue specimen for direct detection of one dominant azole resistance mechanism in a case of disseminated invasive aspergillosis. Rapid detection of mutations associated with azole resistance directly in tissue significantly reduces diagnostic delay.

Sensitivity of MDx allows for Detection of Resistance in Cryptic Infections

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High-frequency Triazole Resistance Found In Nonculturable *Aspergillus fumigatus* from Lungs of Patients with Chronic Fungal Disease

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Molecular probing improves detection sensitivity with a simultaneous assessment of drug resistance.

Table 1

Aspergillus culture, qPCR and *A. fumigatus* resistance mutation detection in 4 study populations.

Laboratory result	ABPA	CPA	IPA	Normals
Culture positive for *Aspergillus* spp.	0/19	7/42 (16.7%)	20/22 (90.9%)	0/11
Culture positive for *A. fumigatus*	0/19	7/42 (16.7%)	10/22 (45.5%)	0/11
qRT PCR positive for *Aspergillus* spp	15/19 (78.9%)	30/42 (71.4%)	21/22 (95.5%)	4/11 (36.4%)
A. fumigatus CYP51A mutation detected directly from qPCR positive sample	6/8 (75%)	12/24 (50%)	NT[a]	NT[a]

ABPA = allergic bronchopulmonary aspergillosis; CPA = chronic pulmonary aspergillosis; IPA = invasive pulmonary aspergillosis

a = Not tested (insufficient sample remaining)

Denning et al. 2011 CID

Azole resistance in *Aspergillus fumigatus* from bronchoalveolar lavage fluids of patients with chronic diseases

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Objectives: We explored the *A. fumigatus* azole resistance profiles in bronchoalveolar lavage (BAL) fluid samples from Danish patients examined for aspergillosis.

Methods: A total of 94 BALs from 87 patients were evaluated by galactomannan (GM) test and *A. fumigatus CYP51A* profiling by PCR.

Results: *Aspergillus* spp. were isolated from 27/48 (56.3%) cultured samples, including 23 *A. fumigatus* with one resistant strain (4.3%). Samples were classified into GM positive (≥ 3.0), intermediate (0.5 - < 3.0), and negative (< 0.5) groups, where *CYP51A* PCR was positive in 81.8% (36/44), 56.3% (18/32), and 38.9% (7/18) samples, respectively.

Nine *CYP51A* PCR positive samples (9/61, 14.8%) were found to have mutations resulting in amino acid substitutions.

Conclusions: Azole resistance in *A. fumigatus* can be cryptic and may go undiagnosed. The combination of improved culture/susceptibility test and the direct molecular detection of resistance marker will facilitate prompt institution of appropriate antifungal therapy.

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TABLE 4. Mutations in the *cyp51A* gene of *A. fumigatus* detected directly from BAL fluids

No. of samples	Amino acid substitutions caused by non-synonymous mutations	Polymorphisms caused by synonymous mutations	Culture/azole susceptibility [a]
Samples carrying single or multiple non-synonymous mutations			
1	N425S		*A. fumigatus*/susceptible
1	M172V [b], F46Y [b]		*A. fumigatus*/susceptible
1	L206P, L210P	G89G	Negative/NA
1	K67E	G89G	*Penicillium* species/ POS=1, VRC=4, ITC>4
1	Y107C	D70D, G89G	Negative/NA
1	**M220V** [c]	G89G	*A. fumigatus*/susceptible
1	N33D, K80E	G89G	ND/NA
1	F41S, E66G	D70D, G89G	ND/NA
1	**P216L** [d]	G89G	Negative/NA
Samples carrying synonymous mutations only			
1		G89G, F495F	ND/NA
4		D70D, G89G	3 ND+1 negative/NA
1		V44V, G89G	Negative/NA
1		G89G, F165F	*A. fumigatus*/susceptible
38		G89G	One resistant *A. fumigatus* isolate POS=1, VRC=2, ITC >4

[a] ND: culture not done; NA: not available; POS, VRC and ITC denote posaconazole, voriconazole, and itraconazole, respectively.
[b] Mutations found previously [5, 21].
[c] Mutation confirmed to be associated with azole resistance in *A. fumigatus* [40].
[d] Mutation confirmed to be associated with azole resistance in *A. fumigatus* [15].
[c] and [d] are shown in bold due to their confirmed association with azole resistance in *A. fumigatus*.

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Is molecular detection of triazole resistance in *Aspergillus* sufficient to detect drug resistance?

Depends on geography

✓ **YES** Single mechanism that predominates

(e.g. TR/L98)-Netherlands

✓ **NO** Non-Cyp51A mechansims prevalent

- 20-50% of isolates do not show any known resistance mechanism- Manchester

Use as adjunct to MIC

Hurdles for acceptance of nucleic acid-based molecular resistance testing

- Does not cover mechanism-independent resistance
- Regulatory (FDA, State)
  - Clinical validation v. "gold standard"
- Manufacture
- QC/QA (Proficiency testing)
- Equipment/Cost per assay
- Technician training (Sample prep)
- Reporting (R or S v. specific mech)
- Capture (>90%?)
- High sensitivity in non-sterile specimens may overstate "R"
- Cultural acceptance (catch-up with viral and bacterial world)

Perspective

Molecular diagnostics (MDx) are ideal for rapid detection of echinocandin resistance in *Candida* spp. and triazole resistance in *Aspergillus*

MDx can be used to detect cryptic fungal species with simultaneous detection of drug resistance

Clinical studies are needed to better assess the value of molecular detection of drug resistance for patient management

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